ISSN 0513-1634

THE STATE NIKITA BOTANICAL GARDENS

BULLETIN SNBG

NUMBER 118

Yalta 2016

12+

THE STATE NIKITA BOTANICAL GARDENS

BULLETIN SNBG

Number 118

Yalta 2016

CONTENTS

DENDROLOGY

UDK 635.054:57.017(477.47)

GROWTH AND DEVELOPMENT PECULIARITIES OF *SABAL MINOR* (JACQ.) PERS. IN NIKITA BOTANICAL GARDENS

Aleksandr Pavlovich Maksimov, Yury Vladimirovich Plugatar, Gennady Yuryevich Spotar, Vera Mikhailovna Novikova

Nikita Botanical Gardens – National Scientific Centtre, the city of Yalta
298648, the Republic of Crimea, the city of Yalta, vil.Nikita
cubric@mail.ru

Introduction

Palms in landscaping of South Coast of the Crimea (SCC) are rather important for today. These plants are possible to improve ornamentality and aesthetic value of resort landscaping, especially if biological characteristics of a definite cultivar correspond growing conditions, when all growing capacities of any exotic plant can be revealed completely. Trying to find out reasons and factors that favor effective growth and development of *Sabal minor* (Jacq.) Pers. possesses scientific novelty and practical value in terms of the region. This cultivar has got a long 200-years history of introduction testing in Arboretum of Nikita Botanical Gardens (NBG) and now it is considered as quite winter-resistant one. But our inspection of SCC landscaping and 30-years observations of *Sabal minor* experimental plants revealed some specimens died as growing conditions didn`t respond to their biological requirements. Based on analysis of all environmental factors which anyhow impact on plants, we developed agrotechnical recommendations of *Sabal minor* successful cultivation on SCC. The second urgent task was to investigate biology and reproductive capacity of this cultivar being introduced. Study of blossoming process, flower pollination, fruiting, seed quality and their growing biological characteristics will make it possible to obtain seeds and cultivate plants out of local reproduction seeds. Wider introduction of *Sabal minor* on SCC allowing for its biological requirements and growing conditions will enhance its ornamental properties. New approaches in landscape architecture aimed at use of *Sabal minor* in arrangement of green spaces will strengthen ornamental value and aesthetic view of landscaping.

Objects and methods of the research

Objects of our investigations were collection plants of *Sabal minor* in Primorsky park of NBG Arboretum (clump 148 and 154), introduction of 1913 and 1914, and in the Lower park (clump 107), introduction of 1984. Besides all revealed plants of this cultivar either on SCC or within Sevastopol were observed.

The purpose of this work is 1) to reveal reasons why some plants of *Sabal minor* die on SCC applying comparative analysis of climatic data of habitat and area of introduction and to develop recommendations aimed at its successful cultivation; 2) to research peculiarities of *Sabal minor* growth and development on SCC and find out factors that have negative effect on plant vegetative and generative spheres; 3) to study seed efficiency of plants, determine quality, quantity and size of obtained seeds, their real and potential capacity.

The principal task was to study biology of seed germination and develop recommendations for *Sabal minor* seed propagation with further adoption at landscaping of SCC and Sevastopol.

Methods of the research: comparative and analytical methods applying climadiagrams by way of Walter and Leith; visual phenological observations using common methods; observation method of damages caused by frosts during severe winters applying our own working - 6-point scale of palm frosting-up, where 0 – no damages; 1 – tops of leaf segments are damaged; 2 – a half of leaf plate is damaged; 3 – leaf plate is damaged till the point of segment crossing (rachis); 4 – the whole leaf plate and a part of footstalk are damaged; 5 – all crown leaves are damaged, but roots and formative tissue of perennating and dormant buds are kept viable and a plant is capable to recover; 6 – all vital organs are damaged and a plant dies [1, 2].

Climate of *Sabal minor* native habit, the USA northern border, and introduction regions on the Black Sea Coast of Russia is illustrated by climadiagrams, made by method of Walter and Leith, with applications (fig.1). They present a fundamental difference of climate types, what allows to develop agrotechnology of *Sabal minor* cultivation in any introduction regions [3, 9].

Symbols that explain climadiagrams: **a** – a settlement, altitude of observations above the sea level (in brackets), in the second line it`s an index of meteorological point and its coordinates; **b** – average annual temperature (°C) and an average amount of precipitations (mm); **c** – period of observations (in square brackets), years; **d** – a curve of an average monthly amount of precipitation (thick line); **e** – a curve of an average monthly temperature (thin line); **f** – a curve of an average temperature minimum (dashed line); **g** – a curve of the absolute temperature minimum (dotted line); **h** – an absolute minimum since the beginning of the XX century, °C.

Curves of temperature and precipitations parameters are correlated, that is 10°C corresponds 20 mm of precipitations. If a precipitation curve is below the curve of an average monthly temperature, space between these lines is dotted (dry season). If the precipitation curve is above, space is dashed (humid season). Precipitations of 100 mm has a ratio of 1:10 and are blackened. Unfavorable cold periods are pointed on abscissa with different spaces for each month: colored – average monthly minimum is below 0°C; dashed – absolute minimum is below 0°C.

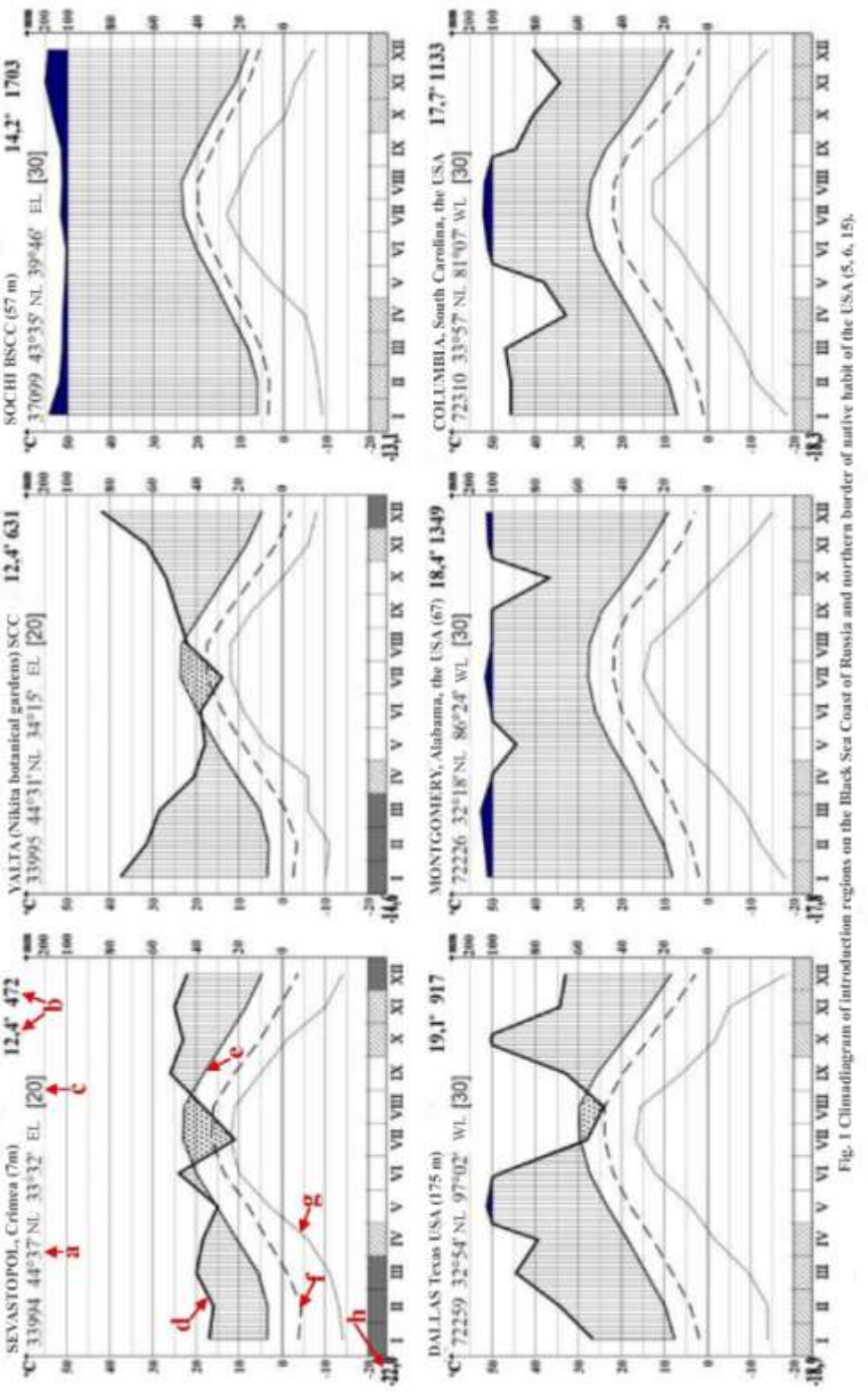


Fig. 1 Climadiagram of introduction regions on the Black Sea Coast of Russia and northern border of native habit of the USA (5, 6, 15).

During dry vegetative period we observed drought damages according to plant growth and crown state. Biometrical investigations included balance of new and died crown leaves, quantity and length of developed flower stacks, quantity and quality of obtained seeds, real product (percentage from potential crop capacity of inflorescences, that is from the total amount of set ovules in the inflorescence). All measurements were carried out using the caliper, linear scale and tape measure. Study results of seed quality, plumpness of endosperm, embryo and external covers gain special scientific value. Longitudinal sections and seed preparation aimed at embryo isolation to fix it, were conducted using scalpel. Seed germination of *Sabal minor* was researched in laboratory on moistened filter paper using Petri dish.

Results and discussion

Sabal minor is a shrubby palm with underground stem, sometimes with overground stem, but not large. Root leaves are fan-shaped, hard, glaucous and green, depending upon conditions they are capable to reach 30 sm - 1,5-2m. Footstalk is equal to leaf plate length or longer a bit. Footstalk edges are bare, sharp, scion on the end of footstalk situated before leaf plate ranges from 2-3 till 4-7 sm, with turned up edge. Leaf plates of 70-100 sm are sectioned radially into folded linear segments, about 40 units. The segments are shortly sectioned and pointed to the end, but not sharp, width ranges from 3 till 6 sm; section of the middle segments is longer than section of the edge ones. A number of inflorescences on one palm varies from 1 till 3, they have complicated and paniculate structure, they are upright, branchy at the top, length gets 2-2,5 sm, width – 2-3 sm at the foot. Flowers are actinomorphous 3,5-5,2 sm across diameter. A fruit is a global drupe with thin seed vessel, 7-10 mm across diameter, brown and black or rather black, glossy. Seeds are rounded 5-7 mm across diameter, brown, flattened a bit (fig.2).

Natural areal of *Sabal minor* is in the USA from the central Florida up to the eastern part of Northern Caroline, in west from Oklahoma till the central Texas (fig.3). At the same time unrelated areas were found out in the Eastern Sierra Madre, Nuevo Leon, Mexico. This cultivar of *Sabal* is the most spread in the USA than others. It prefers marsh and damp soil, along river and reservoir banks, sometimes it forms dense growth; it can be found in drier areas as well, for instance on the slopes of dry hills in Texas. *Sabal minor* plants that grow in Louisiana *(Sabal minor* (Jacq.) Pers.*)'louisiana',* have got rather big and developed stems, it was considered as a separated cultivar. According to current classification it is one of *Sabal minor* forms [4,7,8]. In countries with subtropical climate it is cultivated as an ornamental plant [10, 11, 12].

Sabal minor is believed as one of the most frost-resistant palms in the world [9, 13]. Having compared climadiagrams (see fig.1, fig.3) of points, situated on the northern border of *Sabal minor* growing area in the USA, it`s possible to conclude that temperature regime is almost the same: absolute minimum since the beginning of XX century ranges from (-18,9)°C in Dallas till (-17,8)°C in Montgomery; average minimum of the coldest month (January) isn`t below 0°C with parameters from 0,9°C in Columbia till 2,1°C in Montgomery. Higher temperature in Montgomery is caused by more southern position than other points according to growth area.

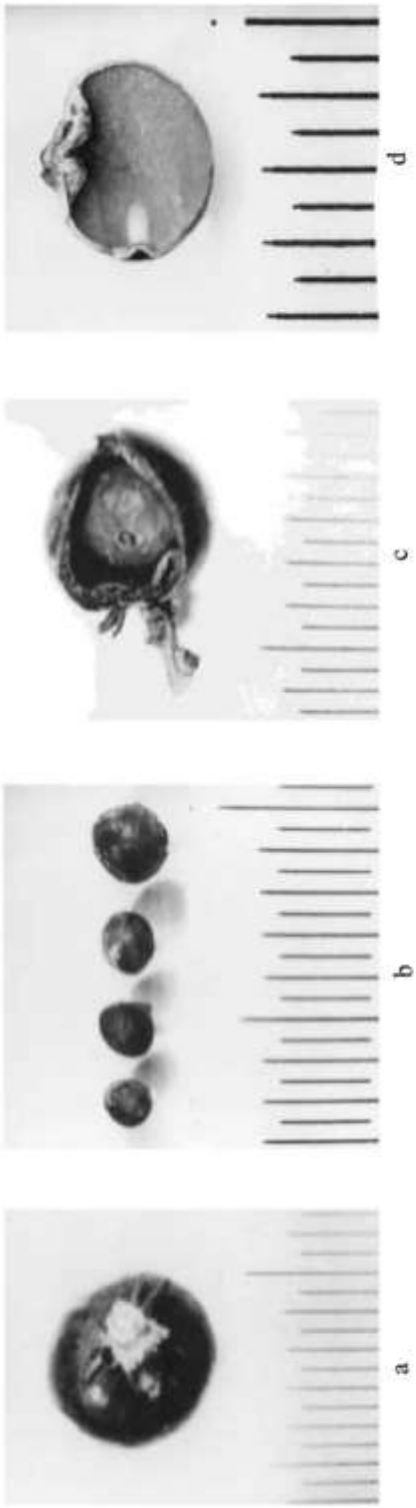


Fig. 2 Sabal minor fruits: a) general view; b) size variations; c) pericarp and seed; d) seed longitudinal section

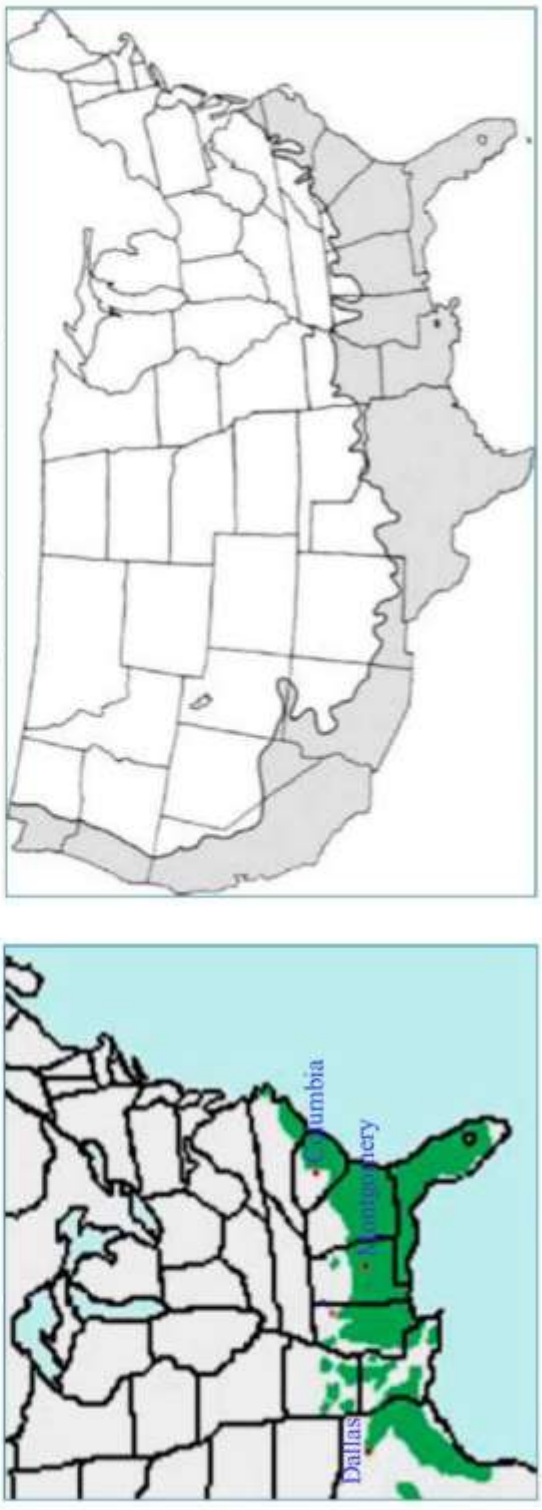


Fig.3 Natural habit in the USA [14] and potential range of plantations [11].

Comparing temperature parameters in the Crimea, Yalta (Nikita Botanical Gardens) and Sevastopol, it`s clear that average annual temperature is the same: 12,4°C, though difference is noticeable at average minimum temperature of the coldest month (February): (-4,4)°C in Sevastopol and (-3,4)°C in Yalta, especially it concerns absolute minimum parameters since the beginning of XX century: (-22,0) °C for Sevastopol and (-14,6) °C for Yalta (Nikita Botanical Gardens.

Such difference at absolute minimum is caused by geographical position of Sevastopol that is absence of barrier that could protect it from cold fronts, in comparison with SCC; this factor gains a great importance for species composition of vegetation. The following should be noted that difference of altitude above the sea level 200 m) reduced considerably the gap between temperature parameters of both points of observation.

Climate of Sochi is milder and more humid 1,5-1,9 times in comparison with the USA points. Average minimum of February makes 3,3°C allowing for absolute minimum of 13,1°C below zero.

Amount of precipitation in study points of the Crimea is much less (1,5-2,9 times) than on the border of *Sabal minor* areal in the USA. Absolute minimum of temperature in Yalta (Nikita Botanical Gardens) are considerably smoothed, but parameters of Sevastopol exceeded parameters of the USA. Only in the city of Okolona, Mississippi what is situated to north, in the region of growth areal border, absolute minimum was fixed – (-21,1) °C, that is possible to compare with Sevastopol - (-22) °C having average minimum of January of 0,3°C. Average minimum of the coldest month in comparison with Columbia for Nikita Botanical Gardens is 4,3°C lower and 5,3°C lower for Sevastopol. Therefore on Sevastopol coast minimum winter temperature is lower, but possible to compare with northern border of the USA areal.

Northern border of growth areal in the USA is situated in the zone of frost-resistance USDA 8a, what corresponds to the range of average annual minimum temperatures (-9,4) - (-12,2) °C. Reference [11] notes that potential range of plantation is from 7b till 11 of frost-resistance zone USDA (see fig.3), that is before the average annual minimum temperature (-15) °C. According to climadiagram of Sevastopol (see fig.1) absolute minimum for 20 years of observation was no less than (-14) °C. It should be noticed that it concerns coastal region of Sevastopol only.

For the first time *Sabal minor* was introduced in NBG by Ch.Ch. Steven in 1814, taken from greenhouse of dukes Razumovskys` botanical garden in Gorenki. S.G. Saakov [7, 8, 9] notes that specimen died and the reasons being unknown. He didn`t point the year of death and the plant age. 100 years later *Sabal minor* was introduced in Nikita Botanical Gardens repeatedly, in 1913 from botanical garden of Sukhumi and in 1914 from St.-Petersburg botanical garden. Majority of plants were planted in Primorsky park of NBG Arboretum on clumps 148 and 154, and some of them were left in NBG greenhouse №2 to have an opportunity to preserve cultivar as it is, if specimens planted into open ground died. Setting palm garden in the lower park of NBG Arboretum (clump 107) in 1984, 5-years seedlings of *Sabal minor* were planted; they were introduced from Sochi. Having inspection of SCC parks in 1985, single specimen of *Sabal minor* was found in Simeiz only. By the beginning of 2000s in landscaping of private areas on either SCC or in Sevastopol they started to plant not only usual fruit-bearing and exotic trees, but palms as well. In 2003 repeated inspection of SCC and Sevastopol parks was carried out; considerable amount of *Sabal minor* was found. Since 1990 the single specimen growing in Simeiz, mentioned above, was also under observation. Since 2003 specimens, planted in Sevastopol were regularly observed, and especially thoroughly during sever winter of 2005/2006 and 2014/2015.

In Primorsky park, Simeiz, there are plants with typical form, grey and green leaves, while Lower Park, Sevastopol, presents *Sabal minor* f. Louisiana with blue leaves.

Primorsky park of NBG has got three fruit-bearing *Sabal minor* plants of more than 100 years old , which don`t reach (crown leaves) even 1,5 m high. A number of crown leaves ranges from 5 to 7 on average. Annually 1-3 new leaves appear and the same number of leaves dies; in this way habit characteristics of the same specimen in 1984 tended to keep unchangeable in comparison with 2014. It`s possible to explain by rubble soil of clumps, that block development of the underground stem and insufficient irrigation reduces growth of vegetative and reproductive plant organs, what sometimes causes chlorosis. Besides outside pneumatophore of the end of U-shaped stem transforms into dry bunch of mass what resembles dried bottom of leave stalks. Practically plant reduces organ that supplies with air like unnecessary one, if it doesn`t grow within marshes. And *Sabal minor* is a helophyte, which should be planted only on projected horizontal areas with sufficient moistening or even if to irrigate enough during vegetative period.

Before planting, soil of projected area is to be prepared properly, at the same time it should be loose without rubble fractions, better loamy. On SCC the best ratio of soil ingredients for *Sabal minor* is the following: 4 parts of chernozem soil, 2 parts of bank sand, 1 part of peat and 1 part of rotted dung. Following these scheme will make it possible to develop full-grown large underground stem with cone-shaped outside pneumatophore, vigorous leaf crown, sufficient number of full-grown reproductive organs and produce germinated seeds, enough for mass cultivation in nurseries with the purpose of wide *Sabal minor* introduction in landscaping on SCC.

Data of long-term phenological observations shows that beginning of growing process after forced dormant season in winter occurs on April the 12-16th and lasts till November the 27th – December the 16th. True dormancy isn`t common for this cultivar. Vegetation period of *Sabal minor* is limited by negative temperature either during autumn-winter or early-spring period and makes 229-233 days on average. Formation of a flower-bearing stem out of the central bunch of leaves starts with growing of the main inflorescence arrow from May the 18th and lasts till June the 11th. As a rule, 5-7 days later arrows of elemental inflorescences differentiate out of the main inflorescence arrow and in 1-3 days flowers appear. High blossoming occurs from June the 10th till 30th and lasts up to June the 21 – July the 8th. Common blossoming period of *Sabal minor* makes about 2 weeks, but sometimes it is 4-7 days more or less. Though according to long-term data blossoming terms are considerably increased and range since May the 24th till June the 26th, what is more than a month flowering period (fig.4). Seed setting during blossoming happens during 1-3 days after flower formation. Order of flower pollination by insects depends upon their formation term and happens 1-3 days later since it is available for pollination. Pollinated or non-pollinated flowers of the main and elemental inflorescences fall out in 2-4 days after formation.

Fig. 4 *Sabal minor* in blossom and its flowers. Primorsky park, NBG

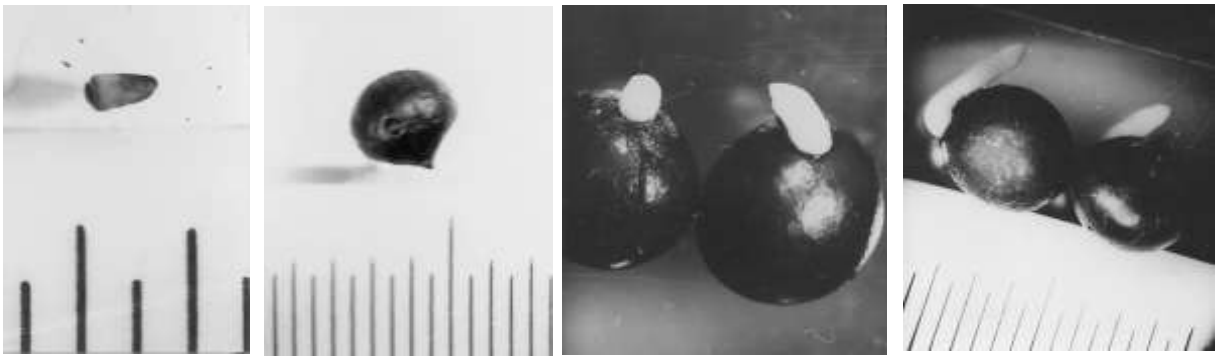
Fig. 5 Fruictiferous *Sabal minor* and its fruit-bearing shoot. Primorsky park, NBG.

The fall makes 27-36% of the total amount of the generated ovules in the inflorescence.

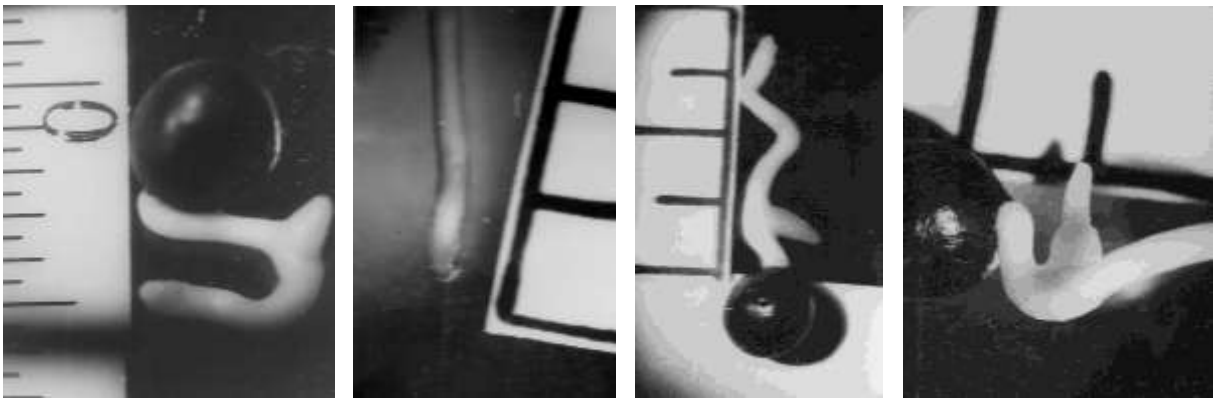
Falling of set seeds happens on their setting and lasts for a week and makes 14-23%. Sum of dead flowers and rejection of some fruits makes 41-59%. Total amount of ovules per one inflorescence has been registered for some years and ranges from 1897 till 2785. In conclusion there are from 1000 till 2000 seeds per one main and 7-11 elemental inflorescences. Actual crop capacity of one inflorescence makes 986-2005 fruits. That is further fruit falling caused by different factors in terms of their ripening ranges from 11 till 13%. Ripening of left set seeds on the main and elemental inflorescences occurs as a rule till the end of *Sabal minor* vegetative period. Coefficient of crop capacity, ratio of real seed productivity and potential one, makes 52-72%. By the end of period seeds ripen completely under conditions of SCC (see fig.2, fig.5).

Quality of seeds, annually yielded in Primorsky park of NBG Arboretum was determined by method of flotation; in this way viable seeds makes more than 90% while unvital – less than 10% of the total amount. Size of *Sabal minor* fruits and seeds harvested from the experimental plants of NBG are 1/3 less in comparison with plants that grow in natural areal and have got the following parameters: fruit diameter with pericarp is x=7,29 mm; Sx=0,10 mm; V=13,25%, without pericarp x=4,08 mm; Sx=0,03 mm; V=7,59%. Probably it is caused by individual inherited peculiarities or unfavorable soil and climatic factors in the introduction region (stoniness and insufficient soil moistening). Such an ecological heterogeneity of seeds often reveals in case of introduction, that is changing cultivation conditions [3]. Further introduction of *Sabal minor* into landscaping on SCC, having conformity of growing conditions to biological requirements of cultivar, will favor investigation of these problems.

Sprouting of *Sabal minor* seeds without pericarp in Petri dishes on moistened filtered paper, was carried out on windowsill having complete solar illumination and indoor temperature (+16, 19°). On the third day some seeds has got germ of future root (according to classification by I.G. Serebryakov [4] this is a shoot of the first order). Process of shoot lengthening of the first order lasts for 6-11 days within all group of seeds (100 units). Some seeds aren`t capable to germinate (3-4%) in spite of that further preparation determined high quality of all structures. In 1-2 days pileorhiza appears on the germ, what protects growth of apical meristems from new underground conditions, supposed by genetics. Having reached 1-2 sm long, germ forms “heel” what develops future stem (second-order shoot), later it transforms into perennial bud with formation of leaf crown and terminal inflorescences, and first-order shoot forms all structures of the underground part of plant. In this way growth velocity of apical meristems with negative geotropism is twice more than those with positive direction. Later it provides development of underground U-shaped stem (fig.7) with further full-grown powerful cone-shaped overground pneumatophore.



a **b** **c** **d**



e **f** **g** **h**

Fig. 6 Peculiarities of *Sabal minor* germination: a) seed embryo; b) location of embryo; c) sprouted seeds; d) root germ; e) phase of germ development; f) root and pileorhiza; g) "heel" formation – future stem; h) stem move out of "heel".

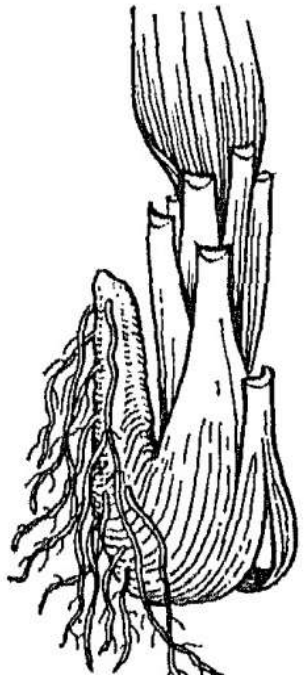


Fig. 7 underground part of a young plant

Fig 8. *Sabal minor* in Arboretum of Sochi

Figure 8 presents plantations of *Sabal minor* in Sochi, where climatic conditions suggest sufficient moistening and not so severe frost periods.

Conclusions

Sabal minor is a quite perspective cultivar for SCC, but slowly-growing one. The principal advantage of these plants is a high winter-resistance. This parameter is the most important limiting factor, that restricts capacity of many palms cultivation on South Coast of the Crimea. Proper soil and high level of agrotechnology are possible to make *Sabal minor* cultivation even in subarid introduction conditions of SCC and ChPK on the same level as within natural areal. Planting pits for seedlings of *Sabal minor* has got the following size: 2 x 2 x 2 m; ground is totally substituted into more favourable mixture (4 parts of chernozem, 2 parts of bank sand, 1 part of turf and 1 part of rot manure). Only flatted area are used for

plantations, because in this way systems of surface and root irrigation are possible to apply. Mulching of circles next to the stem with sawdust (leaf-bearing and defoliation breeds of trees 15-20 sm thick) reduces or even removes any signs of chlorosis. High level of agrotechnology is to supply valuable development of the underground stem, causes increasing of number and size of leaves, inflorescences, flowers and seeds. Vegetative period of subarid regions on SCC and BSCC is rather enough for full-growth and plant development, according to data of phenological investigations. A number of seeds in NBG is rather high and makes 91-96% of the total amount of seeds, marked out by floatation method. Though self-sawing of *Sabal minor* in study regions wasn`t fixed. Cultivation of *Sabal minor* without cover during period of extreme negative temperature on SCC is possible from Laspi on south-west till Sudak on north-east, while on the BSCC from Adler on south-east to Gelendzhyk on north-west. In more severe climatic conditions in the Crimea (Sevastopol, Feodosiya) and on the Black Sea of Krasnodar krai (Novorossiysk, Anapa) *Sabal minor* could be used in landscaping, but limited number of plants, in warmer and more protected from cold winds areas with short-term cover for period of extreme negative temperature.

References

1. *Maksimov A.P., Vazhov V.I., Antyufeyev V.V.* Morozostoikost palm na Yuzhnom beregu Kryma // Trudy Gos.Nikitskogo botan.sada. Yalta – 1988. – T. 106. – S. 63-75.

2. *Maksimov A.P., Novikova V.M.* Rezultaty introduktsionnogo ispytaniya Sabal minor (Kacq.) Pers. na Yuzhnom beregu Kryma. Tezisy dokladov VIII syezda Ukrainskogo botanicheskogo obshchestva (UBO). – Naukova dumka. – 1987. – S. 229.

3. *Maksimov A.P.* Rezultaty introduktsii palm *(Arecaceae* C.H. Schultz*)* na Yuzhnom beregu Kryma // Gos. Nikitsky botanichesky sad. Yalta. – 1989. – S. 24 Deponirovana v VINITI 17.07.1989 g. № 4735 – V – 89.

4. Metodicheskiye ukazaniya po semenovedeniyu inrodutsentov. – AN SSSR, Glavny botanichesky sad. – M.: 1980. – 64 s.

5. Pogoda i klimat.2004-2016. - http://www.pogodaiklimat.ru/

6. *Prudok A.I., Adamenko T.I.* Agroklimatichesky spravochnik po Avtonomnoy respublike Krym (1986-2005 g.g.) – Simferopol: TsGM v ARK, 2011. – 341 s.

7. *Saakov S.G.* Itogi introduktsii palm na territorii SSSR // Trudy Botan.instituta An SSSR. L. – 1952. – Ser. 6. Vyp.2. – S. 16-75.

8. *Saakov S.G.* Palmy i ih kultury v SSSR. – M. – L., 1954. – 320 s.

9. *Saakov S.G., Shypchinsky N.V., Pilipenko F.S.* Palmae Juss. – Palmy. V kn.: Derevya i kustarniki SSSR / pod red. S.Ya.Sokoova. – M.-L., 1951. – T. 2. – S. 56-85.

10. *Bomhard M.L.* Palm trees in the United States. – Agris. Inform. Bull/ U.S.D.A., vol. 22, 1963. – P. 1 – 28.

11. *Gilman E. F.* Sabal minor Bluestem Palmetto, Dwarf Palmetto // series of the Environmental Horticulture Department, UF/IFAS Extension. – 1999. – FPS518. – C. 1-2.

12. *McCurrach J.C.* Palms of the world. – New York. – 1960. – 290 p.

13. *Mowry H.* Native and exotic palms of Florida. – Bull. Florida Agric. Exp. Sta. - №228, 1931. – P. 1 – 71.

14. The Department of Forest Resources and Environmental Conservation, Virginia Tech. 2015 г. – http://dendro.cnre.vt.edu/dendrology/syllabus/factsheet.cfm?ID=351

15. Weatherbase. 1999-2016. – http://www.weatherbase.com/

The article was received at editors 12.04.2016

Maksimov A.P., Plugatar Yu.V., Spotar G.Yu., Novikova V.M. Growth and development peculiarities of *Sabal minor* (Jacq.) Pers. in Nikita Botanical Gardens // Bull. Nikit. Botan. Gard. – 2016. – № 118. – P. 5-16.

The article presents history of *Sabal minor* (Jacq.) Pers. introduction in Nikita Botanical Gardens and distribution of this cultivar along South Coast of the Crimea. It contains data of phenological observations and average quantitative biometric parameters of leaf growth and dying out during vegetative period. Reasons and factors causing irregular blooming and fruiting were determined in terms of the research that is poor rubbly soils and insufficient irrigation. Ornamentality of *Sabal minor* blooming and fruiting is also illustrated here. The article includes data of morphology and anatomy of seeds, embryo and endosperm. Process of seed germination and germ differentiation on root and stem part were traced back as well. At the same time the article contains recommendations in agrotechnology of *Sabal minor* cultivation under conditions of South coast of the Crimea.

Key words: *Sabal minor (Jacq.) Pers., description, distribution, phenology, blooming, fruiting, morphology, anatomy, seed germination, cultivation, South coast of the Crimea*

OIL-BEARING AND MEDICINE PLANTS

UDK 581.192:633.812

VARIABILITY AND INHERITANCE OF ESSENTIAL OIL CONTENT WITHIN ALLOTRIPLOID LAVANDULA HYBRIDS

Valery Dmitriyevich Rabotyagov, Olga Vladimirovna Mitrophanova

Nikita Botanical Gardens – National Scientific Centre, the city of Yalta
298648, the Republic of Crimea, the city of Yalta, vil.Nikita
onlabor@yandex.ru

Introduction

Selection of lavandin, perspective for manufacture as oil-bearing crop, is associated with breeding of interspecific hybrids F_1 on diploid level, crossing result of *Lavandula angustifolia* and *L. latifolia.* These hybrids are of great interest as they are characterized by heterosis [1, 7-10]. Lack of theoretical basis at matching of breeding pairs makes difficult directed selection and synthesis of hybrids with set properties. We consider, formation of hybrid genotype applying interspecific hybridization with induced polyploidic forms [3-5] is a quite perspective direction. Previously we need to know regularities of inheritance in such crossing combinations. Decision of this problem involved induction of amphidiploidic forms and directed crossings with detailed analysis of obtained generation to develop theoretical approaches aimed at matching of breeding pairs for crossing and prognostication of its results.

Objects and methods of the research

Initial breeding pairs were introduced by the following chemotypes: *Lavandula officinalis* sort Record with mass fraction of essential oil 2,1% per green weight or 5,8% per absolute dry product, sort Prima with mass fraction of essential oil 1,8% and 5,2% per dry product, Belyanka was chosen as a specimen with mass fraction of essential oil 1,6 per green weight and 4,65% per dry weight. Amphidiploid № 48 was used as a parent form with mass fraction of essential oil 2,5% per green weight or 6,7% per absolute dry weight. Interspecific crossing were carried out between amphidiploids and three sorts of *Lavandula officinalis,* hybrids, as a study results, were investigated to find out essential oil content.

Artificial hybridization was applied to get allotriploidic hybrid F_1 of lavender. Morning is the most convenient time for this method. Technique of interspecific crossings involves: castration before flower opening (phase “pupa”), corolla and its stamens were

moved away by pincer. At the same time other flower buds and flowers in blossom were moved away as well. Top of the bell was cut a bit (1/3) to release stigma. After castration flower stalk with inflorescence was wrapped around with cotton and covered with parchment insulator. Pollination was carried out on the second day-third day with pollen of ripened anther of the parent plant [5, 6]. All plants, as a result of interspecific crossings were investigated to find out mass fraction of essential oil in green weight that was carried out owing to hydrodistillation of inflorescences with Clevenger apparatus, the data was converted into absolute dry mass. Experimental results were statistically processed [2].

Results and discussions

Induced amphidiploids and diploid sorts of *Lavandula officinalis* were used in interspecific hybridization aimed at breeding of lavender hybrids with high concentration of essential oil. According to researches genetic system of amphidiploid lavender forms is characterized by high stability and capable to reproduce due to seed way. In this way only zygotes develop, that were formed owing to merging of gametes with 48 chromosomes and genomes of parent cultivars. Amphidiploid was chosen as a parent plant.

Hybrid genomes, as a result of crossing of *Lavandula officinalis* amphidiploid and diploid, have got somatic number of chromosomes 2n=72, including 2 genomes of *Lavandula angustifolia* (AA) and one genome of *L. latifolia* (genome composition AA-L); they are considered as allotriploides. Their formation is presented in the scheme below:

♀ Amphidiploid	X	♂ Diploid
2n=96, AALL		2n=48, AA
MI 24_{II} *L. angustifolia*		24_{II} *L. angustifolia*
$+24_{II}$ *L. latifolia*		
gametes AL (n=48)		*A* (n=24)

↓

2n=72, AAL

Crossing combination: Amphidiploid x Record, Amphidiploid x Prima, Amphidiploid x Belyanka

Analysis of essential oil content revealed that initial forms – sorts Record, Prima and Belyanka are contrasting according to essential oil content. Belyanka has got the lowest average parameter, but amphidiploids № 48.8 presents the highest one. Interval of variation is strictly differentiated (table 1), degree of this parameter changeability is insignificant. It should be noted that initial forms considerably differ either by average or extreme characters. The most significant difference was registered according to its minimal content (table 1).

Hybridization of amphidiploid and diploid sorts and all its combinations combinations causes increasing of interval and degree of essential oil content changeability, where heterotic forms are possible to reveal. In this way parameter of heterotic effect is determined by its content of parent form. So, applying high-oil sort Record as a parent plant, heterotic effect makes 196%, while in case with Belyanka it is 117%.

Table 1

Concentration of essential oil of parent forms and hybrids F_1 (% per absolute green weight)

Sort, clone, hybrid	Average concentration x±Sx	Variation limits	Coefficient of variation
L. angustifolia – Record	5,8±0,2	5,4-6,2	4,2±0,4
L. angustifolia - Prima	5,2±0,2	4,8-5,5	5,6±0,6
L. angustifolia - Belyanka	4,65±0,2	3,5-5,2	5,1±0,6
Amphidiploid №48	6,7±0,9	6,4-6,9	5,6±0,7
Amphidiploid x Belyanka	7,1±1,2	3,75-9,5	35,3±4,8
Amphidiploid x Prima	7,5±1,1	4,5-9,5	30,0±3,4
Amphidiploid x Record	7,8±1,2	5,0-10,5	23,5±2,8

Therefore we can fix influence of parent form on heterotic effect. Comparison investigation of crossing combinations revealed that percentage of plants with heterotic effect gets 70% maximum in crossing combination (Amphidiploid x Record), but in combination Amphidiploid x Prima it reaches only 50%, while minimum parameter was registered in combination Amphidipploid x Belyanka – 25,7% (table 2).

Table 2

Inheritance character of essential oil content of interspecific hybrids F_1 (% from the total amount of plants in each crossing combination)

Type of inheritance	Crossing combination		
	Amphidiploid 48 x Record	Amphidiploid 48 x Prima	Amphidiploid 48 x Belyanka
Exceeded parameters of the best parent plant	70,0	50,0	25,7
Equal to parameters of the best parent plant	13,3	18,0	34,3
Intermediate	10,0	14,3	31,4
Equal to parameters of the worst parent plant	5,0	10,7	5,6
Lower than parameters of the worst parent plant (depression)	1,7	7,0	3,0

Investigations clearly revealed essential oil content of parent forms effects on possibility of hybrids with heterosis (see table 2). Than higher essential oil concentration than more heterotic hybrids as a result. But in the third crossing combination a number of hybrids with essential oil content, like best parent form has, increased significantly: accordingly 34,3%, while in the third crossing combination it makes 18%, in the first – 13%.

High essential oil capacity of hybrids in crossing combination (Amphidiploid x Record) was probably grounded by genetic characteristics of parent forms; in particular they possess wide spectrum of changeability of essential oil concentration in case of self-pollination, what indicates their heterozygosis. Heterozygous character of initial hybrids determines effect of heterosis, what improves sampling opportunity of hybrids with high essential oil concentration.

Figure 1 presents comparison of different crossing combinations concerning essential oil content. There is distribution of allotriploid hybrids according to essential oil mass fraction. Distribution curve of hybrids in crossing combination Amphidiploid x Belyanka is regular. Variation interval of essential oil mass fraction is shifted to both sides with the lowest concentration (3,75%) as a result depression signs and to the side with the high concentration (9,5% per absolute green weight). Hybrids are concentrated in the interval with essential oil content from 4,5% to 7% and makes 71,4% from the total amount of plants. A

number of plants with essential oil content from 7,0 till 8,25% makes 11,4% and only one hybrid possesses essential oil concentration 9,4%.

In the crossing combination (Amphidiploid x Prima) distribution curve is regular as well, but shifted to the side with high essential oil concentration. Variation interval of content ranges from 4,5% till 9,5%. The principal mass of lavender allotriploid hybrids is located in the interval from 5,75 till 8,25% and makes 80% of the total amount of hybrids. It should be noted in this crossing combination hybrids with high essential oil concentration were successfully resulted in the range from 8,25% till 9,5%, what improves sampling of hybrids with high essential oil concentration (fig.1).

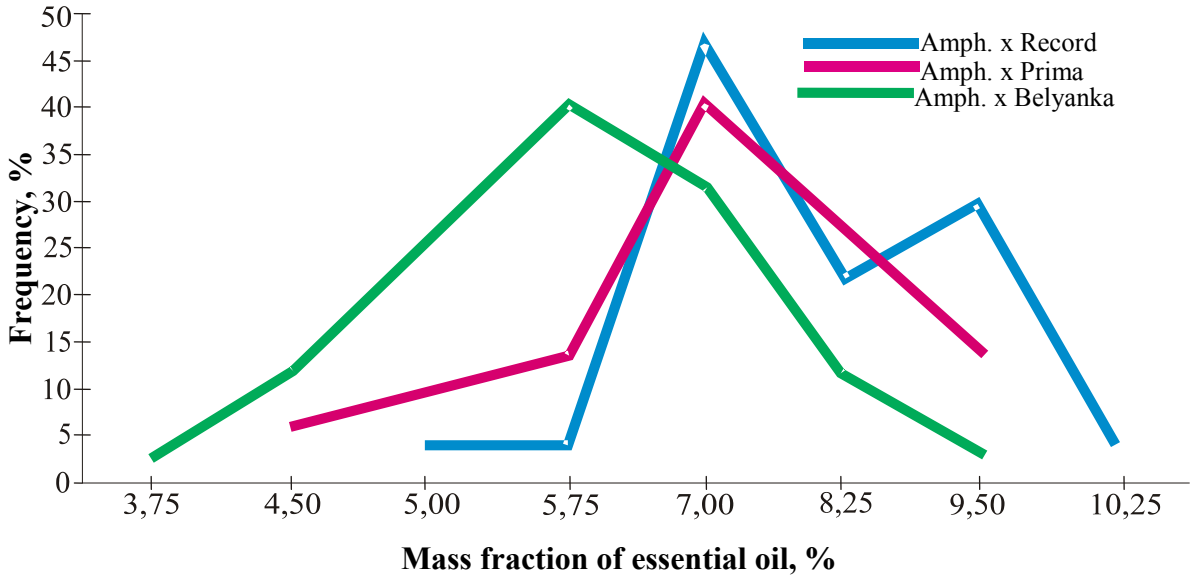


Fig. Distribution of interspecific hybrids F_1 according to essential oil concentration in different crossing combinations

The most successful sampling of breeding pairs aimed at selection of allotriploid hybrids with high essential oil mass fraction was Amphidiploid 48.8 x Record). The way of hybrids distribution by essential oil content is presented at figure. In this way variation interval extends to the side of higher parameters. Hybrids in the variation interval of essential oil mass fraction from 8, 25% till 10,25% are of great interest for breeding and makes 25% of the total amount. There is a high possibility of heterotic hybrids. In this crossing combination hybrids with the highest content of essential oil (10,0% and 10,25% per absolutely dry product) were successfully synthesized, what is of great interest for breeding.

In crossings of allotriploids and *Lavandula officinalis* (sorts Record and Prima) there is an opportunity to synthesize considerable heterotic effect with high possibilities to sample allotriploids with high essential oil content in hybrid generation.

Conclusions

Investigation of breeding pairs ability to be combined, aimed at selection of hybrids with high essential oil content revealed, it demands directed interspecific crossings within combination of amphidiploids with *L. angustifolia*, sort Record, what will make it possible to synthesize allotriploids with two genomes of *L. angustifolia* and one genome of *L. latifolia* up to 3,6% with essential oil mass fraction of 9,9% and 10,25%.

The work was granted by the Russian Scientific Foundation (project № 14-500079).

References

1. *Akimov Yu.A., Rabotyagov V.D.* Vnutrividovaya i vnutriklonovaya izmenchivost efirnogo masla u *L. angustifolia* i *L. latifolia* pri semennom razmnozhenii // Rast.resursy. – 1987. - № 3. – S. 417.

2. *Dospekhov V.A.* Metodika poevogo opyta. – M.: kolos, 1975. – 424s.

3. *Rabotyagov V.D.* Problema synteza lavandina. // Tr. Gos. Nikitskogo botan.sada. – 1983. – T. 91. S. 92.

4. *Rabotyagov V.D.* Preodoleniye sterilnosti u lavandina (*L. angustifolia* i *L. latifolia*) // Tsytologiya i genetika. – 1975. – T.9. - №5. – S. 443.

5. *Rabotyagov V.D., Akimov Yu.A.* Nasledovaniya soderzhaniya i sostava efirnogo masla pri mezhvidivoy hybridizatsii lavandy // Genetika. – 1986. – T. 22. № 7. – S.1163.

6. *Shoferistov Ye.G., Rabotyagov V.D., Mashanov V.I.* Organogenez i biologiya tsveteniya lavandy i lavandina // Botan.journ. – 1977. – T. 62. – S. 1479.

7. *Mala V.D., Beck A*. et al. La selection du Lavandin // Riv. Ital. 1981. V.63. №3. P.163.

8. *Martin C., Zola A*. Etude sur de nouvelles varieties de Lavandin // Riv. Ital. 1973.

9. *Paris R., Dillemann W.* Le Problime du Lavandin et les lois de L^Hybridation // La France et ses Parfums, 1959.№13. P.14.

10. *Tucker A.O., Hensen K.L.W*. The cultivars of Lavander and Lavandin (Labiata / e) // Baileya. 1985. V.22. №4. P.168.

The article was received at editors 18.11.2015

Rabotyagov V.D., Mitrophanova O.V. **Variability and inheritance of essential oil content within allotriploid Lavadula hybrids //** Bull. Nikit. Botan. Gard. – 2016. – № 118. – P. 16-20.

The article concerns the problem of breeding pairs fitting according to synthetic selection of allotriploid Lavandula hybrids with two genomes (*Lavandula angustifolia* Mill) and one (*L. latifolia* Medic) having high concentration of essential oil. There are comparison data of essential oil mass fraction in synthesized allotriploids as a result of crossing of introduced amphidiploid and Lavandula officinalis L. cultivars. Mechanisms of mass fraction variability and inheritance of hybrids – results of different crossing combinations – are discussed in terms of the research as well. Heterotic interspecific hybrids with 10,0% and 10,25% from the total dry material were obtained in course of the study.

Key words: *Lavandula; allotriploid; amphidiploids; heterosis; essential oil mass fraction; crossing combination.*

UDK 665.52:582.929.4

COMPONENT COMPOSITION OF *ELSHOLTZIA STAUNTONII* ESSENTIAL OIL, ROZOVOYE OBLAKO CULTIVAR

Lyudmila Anatolyevna Khlypenko, Taisiya Ivanovna Oryol

Nikita Botanical Gardens – National Scientific Centre, the city of Yalta
298648, the Republic of Crimea, the city of Yalta, urb.vil.Nikita
taisiyaorel@yandex.ru

Introduction

Breeding of high-productive sorts of essential oil-bearing plants and their introduction into industry extend assortment of essential oils, the principal components for perfume and cosmetic industry, alcoholic beverage and non-alcoholic drinks, a number of medicines.

The habitat of *Elsholtzia stauntonii* Benth. is North China. In Nikita Botanical Gardens (NBG) it has been investigated as a crop since 1967 [3]. Essential oil of *Elsholtzia stauntonii* Benth. possesses bright antimicrobial action concerning *Staphylococcus aureus* 209P, antibacterial action towards *Escherichia coli* M-17, *Proteus vulgaris*. It was highly rated by specialists (4,5 points) in the field of perfume and cosmetics products [2]. Its essential oil is one of the ingredients of aromatizers, made on the basis of domestic raw materials [3].

Use of essential oils in food industry as natural aromatizers and preservatives demands accurate study of their component composition.

The aim of this research is to identify EO component composition of *Elsholtzia stauntonii* Benth., Rozovoye Oblako cultivar.

Objects and methods of the research

Object of the research is essential oil of *Elsholtzia stauntonii* Benth., Rozovoye Oblako cultivar, that was extracted during mass blooming out of overground parts of the plant which grows on introduction and collection area of NBG (the second decade of September). Mass fraction of essential oil was determined due to method of hydrodistillation by Ginsberg out of raw material [1]. Component composition of essential oil was studied applying chromatograph Agilent Technology 6890N with mass-spectrum detector 5973N. Chromatographic column – capillary HP-1 (30 m long); inside diameter – 0,25 mm. Injector temperature is 250°C. Carrier gas is helium, stream velocity – 1,0 sm^3/min. Transfer from gas chromatograph to mass spectrometric detector was heated till 230°C. Source temperature was kept up at 200°C. Electronic ionization was carried out at 70 eV with mass range m/z from 29 till 450. Identification of essential oil components was guided by comparison results obtained in terms of chromatograph of chemical substances mass spectra of study mixtures with publishing data of mass spectra NIST05-WILEY2007 (about 500000 of mass spectra) [5].

Results and discussion

Elsholtzia stauntonii Benth is a semi-shrub of *Lamiaceae* family. Sort Rozovoye oblako enters the State register of selective plants, permitted in the Republic of Crimea in 2015.

In terms of this crop five-years plant gets 100 sm high, 80 sm across diameter, the bush is compact. Leaves are assidenous with opposite position, dark-green color, lancet-lake with biserratevedge, large-sized, 10 sm long and 2,5-3 sm wide. The top side of the leave is smooth, down side is downy a bit. Leave top is pointed. Inflorescence has a shape of spicate

thyrsus, 10-15 sm long, 1,5-2 sm across diameter. Flowers are small-sized, 6-9 mm long and 2,5-3 mm across diameter, corolla has got intensive pink color. (purple) (fig.1).

Fig. 1 Plants of *Elsholtzia stauntonii,* sort Rozovoye oblako, growing on introduction and collection area in Nikita Botanical Gardens

Flower-cup is adeciduous, five-segmental, joined, club-shaped, teeth are folded. The fruit is a nut of light brown color, smooth, rounded, small-sized. Mass of 1000 seeds makes 0,21 g. Under conditions of South Coast of the Crimea vegetation period starts in the end of March – beginning of April. Budding is fixed in July, blooming starts in the 1st decade of September, mass blooming usually opens in the 2nd decade and finishes in the 3rd decade of September. Blooming period keeps on 20-25 days. Seeds ripen in the end of the 1st – beginning of the 2nd decade of October [4]. Crop capacity of raw material makes till 100 center/ha, mass fraction of essential oil is 0,4-0,5% from the raw material, yielding of essential oil is 40-50 kg/ha depending upon weather conditions.

Essential oil of *Elsholtzia stauntonii* is like an orange-colored thin fluid that shares with all plant fragrances. According to organoleptic rate it could be referred to fruit and balsamic type with notes of dry fruits.

As a result of conduced researches in essential oil of *Elsholtzia stauntonii,* sort Rozovoye Oblako, 36 components were revealed, 30 of them were indentified (fig.2, table).

Concerning mass fraction monoterpenic derivatives prevails in the essential oil, being at the same time acidic heterocyclic compound. Dominant components are rosefuran (41,1%) and its oxid – rosefuraneoxid (24,0%); sesquiterpen caryophillen (8,5%) (fig.2); this component composition corresponds to publishing data of this very essential oil and considered as a typical characteristic of the cultivar in total. Derivatives of furan are rarely found in essential oils, these compounds are typical for *Elsholtzia* and *Perilla* genera only [1]. Essential oil of *Elsholtzia stauntonii,* sort Rozovoye Oblako, contains valuable components such as phenolic aroma substences eugenol (1,5%), monoterpenic alcohol linalool (1,1%) as well as sesquiterpens α-humulen (2,7%) and germacrene d (,1%). Mass fraction of each of them doesn`t reach even 1%.

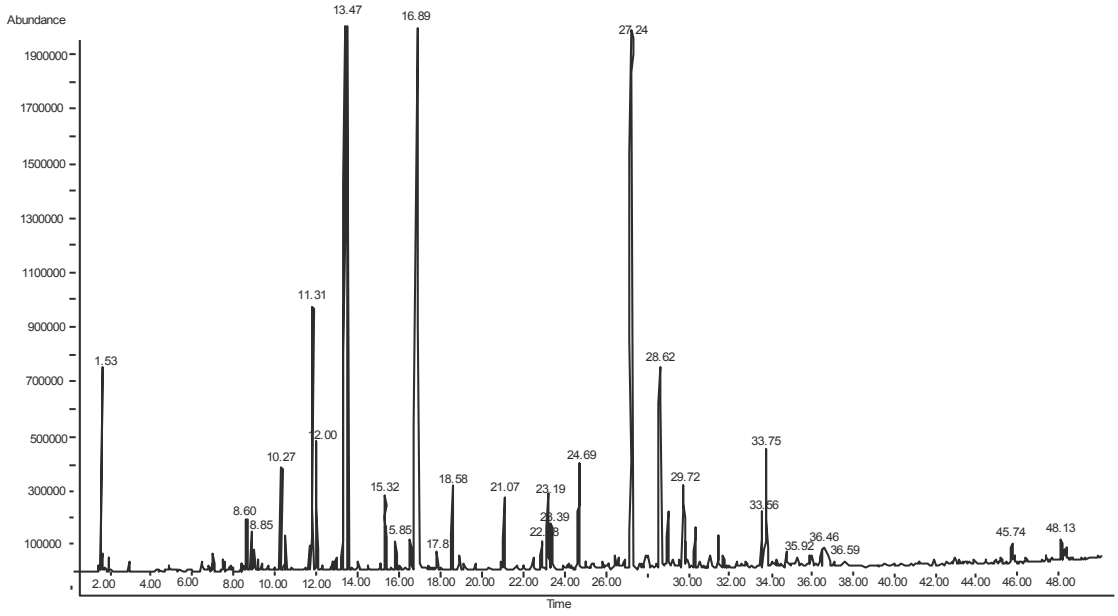


Fig.2 Chromatogram of *Elsholtzia stauntonii* Benth. essential oil, sort Rozovoye Oblako

Besides heterocyclic compounds of pyran, furan and its derivatives *Elsholtzia stauntonii* essential oil also includes sesquiterpenes (15,8%), ketones (6,7%) (artemisia ketone 2,8%, acetophenone 1,4% and etc.), aliphatic alcohols (1,4%), monoterpenic alcohols (1,1%), monoterpenes (0,7%). Mass fraction of all these compounds in the essential oil is insignificant.

Table

Component composition of *Elsholtzia stauntonii* essential oil, sort Rozovoye Oblako

Component	Output time, min	Mass fraction of components, %
Ethanol	1,53	0,90
1-okten-3-ol	8,60	0,40
Oktanon-3	8,85	0,40
Myrcene	9,00	0,20
Para-cymene	10,27	1,00
1,8-cineol	10,55	0,30
γ-terpinene	11,66	0,30
Artemisia ketone	11,82	2,80
Acetophenone	12,00	1,40
Rosefuran	13,47	41,10
Linalool	13,51	1,10
Camphora	15,33	0,90
Rosefuran epoxide	16,89	24,00
Furan	18,58	1,80
Pyran	22,88	1,30
Eugenol	24,69	1,45
Caryophyllene	27,24	8,54
α-humulene	28,60	2,70
Germacrede d	29,72	1,10
α-curcumene	29,84	0,20

Cadinene	31,44	0,40
Spathulenol	33,56	0,80
Caryophyllene oxide	33,75	1,84
Humulene epoxide	34,74	0,20

Conducted investigations corroborate suitability of these sorts cultivation to get valuable essential oils for perfume and cosmetic industry, food production as aromatizers of drinks and as a natural preservative.

Conclusions

Component composition of *Elsholtzia stauntonii* essential oil, sort Rozovoye Oblako, was determined in terms of the research, 30 components were identified. It was found out that principal components are rosefuran (41,1%), rosefuran epoxide (24,0%) and caryophyllene (8,5%). Essential oil is possible to use in perfume and cosmetic industry and food production.

References (3)

1. *Ginsberg A.S.* Uproshchonny sposob opredeleniya efirnogo masla v efironosah // Chimiko-farmatsefticheskaya promyshlennost. – 1932. - № 8-9. – S. 326 – 329.

2. *Dmitriyev L.B., Bakova N.N., Klyuyev N.A., Mashanov V.I., Granberg I.I.* Komponentny sostav efirnogo masla *Elsholtzia stauntonii* Benth // Izv. Timiryazevskoy s.kh. akad. M: Agropromizdat. – Vyp.5. – 1987. – S. 167-170.

3. *Mashanov V.I., Andreyeva N.F., Mashanova N.S., Logvinenko I.Ye.* Noviye efiromaslichniye kultury. – Simferopol: Tavriya, 1988. – S. 145-150.

4. *Rabotyagov V.D., Khlypenko L.A., Bakova N.N., Mashanov V.I.* Annotirovanny katalog efiromaslichnyh, pryano-aromaticheskih i pishchevyh rasteny kollektsii Nikitskogo botanicheskogo sada. – Yalta, 2007. – S. 45.

5. *Jennings W., Shibamoto T*. Qualitative analysis of flavor and fragrance volatites by glass capillary gas chromatography. – NewYork: Academie Press, 1980. – 472 p.

The article was received at editors 18.11.2015.

Khlypenko L.A., Oryol T.I. Component composition of *Elsholtzia stauntonii* essential oil, Rozovoye oblako cultivar. // Bull. of the State Nikit. Botan. Gard. – 2016. – № 118. – P. 21-24.

The article presents data about mass fraction and component composition of *Elsholtzia stauntonii* essential oil, Rozovoye oblako cultivar, and recommendations for use it in perfume and cosmetic industries.

Key words: *Elsholtzia stauntonii; cultivar; essential oil; component composition; rosefuran.*

UDK 581.82:581.135.3

PROCESSES OF ESSENTIAL OIL ACCUMULATION IN PETALS OF *ROSA* (ROSACEAE) AND MYCELIUM *EREMOTHECIUM* (EREMOTHECIACEAE)

Yelena Fyodorovna Semyonovna, Anastasiya Iosifovna Shpichka, Yelena Viktorovna Presnyakova, Nataliya Aleksandrovna Mezhennaya

Penza State University, the city of Penza
440026, the city of Penza, 40, Krasnova str.
sef1957@mail.ru

Introduction

Essential oil-bearing crops unite rather big group of plants, which have such a distinctive feature as capacity to have biosynthesis process and accumulate essential oils. One of these plants that has a high-valuable oil is *Rosa* L. In essential oil industry the following sorts are of great demand: *R. damascena* Mill., *R. gallica* L., *R. alba* L., *R. centifolia* L. as well as hybrids bred on their basis [9,13].

But nowadays due to obstacles, caused by huge effect of ecological factors and labor intensity, plantation cultivation can`t meet such a great demand for natural fragrant substances in the field of food production, perfume and cosmetic industry, chemical and pharmaceutical industry, what drives at development of their alternative sources. It was found out that an amount of synthesized oil in the cell culture of rose is much lower than in petals of intact plant. In this way composition of extracted oils differs from traditional rose oil. In 90s a new method of fragrant product extract was discovered, which is based on stock homotallic ascomycetes of *Eremothecium ashbyi* Guilliermond and *E. gossypii* Kurtzman, close to rose essential oil extracted out of fresh flowers [2, 3, 10, 13].

Nevertheless in the first works, points, related to accumulation of essential oil at the cellular level of study cases, weren`t discussed in comparison aspects and now provoke a great interest [10]. The study purpose was comparison analysis of structural peculiarities of accumulation process in essential oil of *Rose* and *Eremothecium,* composition and quality of synthesized substances on different development stages.

Objects and methods of the research

The study objects were Rosa cultivars: *R. alba* L.*, R. centifolia* L.*, R. gallica* L.*, R. damascena* Mill.*, R. rugosa* Thunb.*, R. canina* L.*, R. cinnamomea* L., *R. odessiana* hort., *R. lutea* Mill., stocks *E. ashbyi* Guilliermond: BKMF-124, BKMF-3009, BKMF-4565D, BKMF-4566D, ВКПМF-36, ВКПМF-340 and *E. gossypii* Kurtzman: BKMF-2627, BKMF-3276, that differ by level of synthesis and monoterpenic accumulation, as well as fragrant alcohols as principal components of rose essential oils.

Researches were carried out with plants, cultivated on collection areas (village Krymskaya Roza – Belogorsk region, the Republic of Crimea), located in north piedmont part of the Crimean peninsula, as well as under conditions of Botanical garden named after I.I. Sprygin (the city of Penza). Plant material was registered on the late phase of onthogenesis (generative period, budding and blooming phase) in acetalcohol (1:3) and 6% in formalin; cross sections were prepared by a razor or applying freezing microtome (agreeable method) [6].

Micromycetes were supported on skew sow and sucrose, potato and glucose, glucose and peptonic with yeasty extract, Sabouraud and Chapeka mediums, wort and maltha agars.

Stock cultivation was conducted in liquid and nutrient mediums under conditions that correspond to those published before [10].

Microscopy of native and colored preparations with methylene blue, iodine, sudan III, black ink was implemented using microscopes MIKMED-1, BIOMED-6 (magnification degree 4, 10, 40, 100). Cameras Nikon Coolpix 2500, Nkon Coolpix 6300, Panasonic DMC-FX100 were applied to make photographs of micro- and macro-objects. Micropreparatons were described according to modern methodical and reference data [6].

Patterns for electronic microscopy (24,36, 48, 56, 64 hours of micromycetes cultivation) were prepared applying 5% solution of glutaraldehyde in phosphate buffer as a fixative (pH 5,8-7,0 depending upon pH of cultural liquid on a certain stage of study stock development). Fixation was keeping on for 24 hours, after that material was washed with phosphate buffer for 10 minute and fixed again for 1,0-1,5 hours with 1% water solution O_sO_4.

Later the patterns were extra stained for 12 h by 5% uranyl acetate prepared in 70% ethanol. The ultrathin sections were made by an ultramicrotome and imbued for 5 min by Reynolds' lead citrate. Then they were investigated via a microscope JEM-100 C (magnification 15000-41000).

For quantification of the essential oil, the method described in the Russian State Pharmacopoeia (XI ed.) [4], or the gravimetry after triple extraction by an organic solvent were used. The solvent was removed by a rotor vacuum evaporator. The oil component composition was tested by gas-liquid chromatography (polar column, flame-ionization detector).

Experimental data was processed statistically in accordance with G.F. Lakin [5], significance level p=0,95.

Results and discussion

The results of the anatomical investigation of rose (dog roses) petals have shown that adaxial epidermal cells are polygonal, adjoin closely to each other, stretch to cone-shaped nipples, and are covered by a highly rugous cuticle. The abaxial cells are elongated, with slightly anfractuose or straight walls, have the cuticle (Fig. 1). The separate groups of the papilate adaxial (in case of *R. alba*, *R. gallica, R. damascena* also abaxial) epidermal cells are able to synthesize and secrete the essential oil which accumulates in small drops under the cuticle and causes its detachment and spot formation. The content of the cells in an epidermis is colored because of pigments dissolved in vacuoles. It adds different roses (*R. damascena*, *R. cinnamomea*, *R. canina*, *R. odessiana*), red (*R. gallica, R. centifolia, R. rugosa*) shades to the petals during the budding and blossoming periods. Small stomata are anomocytic, mostly located on the inferior petal side with low frequency of occurrence.

The parenchymal tissue consists of layers of non-colored rounded or irregular cells with thin walls which form a big intercellular space (*R. canina* has considerably less of it). The number of the parenchymal layers ranges from 4-6 (*R. lutea, R. centifolia, R. cinnamomea*) to 10-12 (*R. gallica, R. alba, R. rugosa*). The strengthening tissue was not found. The vascular tissue is presented by spiral tracheides surrounded by small parenchymal cells adjoined closely to each other. The tracheides are located in groups of 8-10 or 3-4 (*R. gallica, R. rugosa*).

By rose flower tripping the endogenous secretory structures – essential oil lysigenous oval conceptacles are deeply located in the parenchymal tissue and contain drops of the essential oil (Fig. 1, Table 1).

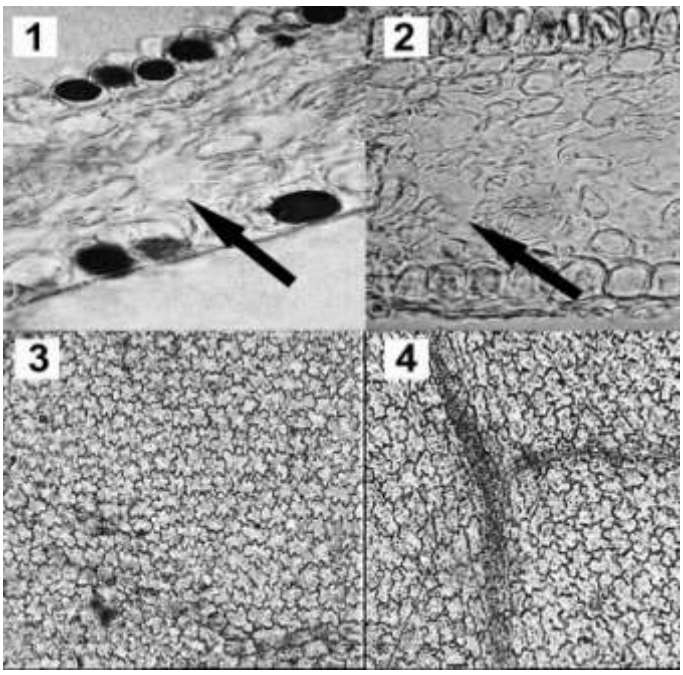


Fig. 1. The anatomical structure of the rose petals: 1 (*R. rugosa*), 2 (*R. gallica*) – transverse sections (lysigenous essential oil conceptacles are pointed by arrows; magnification x40); the surface: 3 – adaxial *R. cinnamomea*; 4 – abaxial *R. canina* epidermal cells (magnification x10)

Table 1

Anatomical-morphological features of secretory structures of different oil-bearing rose species

Cultivar	Glandular epidermal cells		Endogenous structures (secretory conceptacles)			
	availability	localization	localization	frequency, lim pcs/mm^2	sizes, µm	sizes interrelation of lysigenicous conceptacles and parenchymal cells
R. cinnamomea	–	–	close to a surface (2-3 cell layers)	1,29-2,08	89,6±10,2 × 86,1±9,8	0,95
R. canina	+	adaxially	in a depth of parenchyma (4 cell layers)	0,62-1,65	64,3±5,2 × 62,6±4,5	0,63
R. odessiana	+	adaxially	in a depth of parenchyma (3-6 cell layers), uniformly	0,55-1,32	83,0±8,2 × 82,1±8,3	0,88
R. rugosa	–	–	in a depth of parenchyma (4-6 cell layers)	1,58-2,69	88,7±9,2 × 85,4±8,8	0,94
R. alba	+	adaxially and abaxially	close to a surface (3-5 cell layers)	0,52-1,15	106,6±14,2 × 96,5±9,1	0,95
R. lutea	–	–	close to an abaxial epidermis (4 cell	4,98-6,01	84,9±11,1 × 80,7±10,8	1,01

			layers)			
R. gallica	+	adaxially and abaxially	in a depth of parenchyma (4-6 cell layers), uniformly	3,83-4,68	75,5±8,4 × 61,2±7,0	0,83
R. centifolia	+	adaxially	in 2-3 parenchymal layers	2,83-3,68	81,2±9,4 × 66,7±7,6	0,84
R. damascena	+	adaxially and abaxially	close to a surface (2-3 cell layers)	4,83-5,68	87,4±10,2 × 66,7±×6,8	1,13

Note: «+» – structures were found out; «–» – structures were not found out.

Their sizes, localization and frequency ranges. For instance, the conceptacles of *R. canina, R. gallica* are small and evenly distributed in the parenchyma deep. The ones of *R. alba, R. cinnamomea, R. rugosa* are large, rare and located in different way: close to the petals surface (*R. alba*); by groups adjoining to the epidermis (*R. cinnamomea*); in the parenchyma deep (*R. rugosa*).

It should be noticed that cell conversion to the essential oil biosynthesis occurs during the late ontogenetic phases (generative period) and concurs with the budding and blossoming. The interconnection of localization of the endogenous secretory structures and the mass concentration of the volatile fragrant substances per petal raw mass was revealed during the qualitative and quantitative analysis of the essential oil extracted from plant raw materials. It is shown, the maximum levels of the rose oil content is typical for *R. damascena*, *R. lutea* which have the frequent volatile-oil-bearing conceptacles which are closely located to the adaxial and abaxial epidermis and have bigger sizes than the adjoined parenchymal cells (Table 1, 2). Thus, the mass concentration of the essential oil and, conformably, the level of the synthesis and the accumulation of its components in the rose petals are lower when the secretory structures are deeply located in the parenchymal tissue, scarce, and small-sized.

Table 2

Comparative characteristics of the studied rose species

Name	Petals color	Flower type [11]	Essential oil content (EOC), %
R. canina	pale pink	simple	0,0435
R. odessiana	pale with a pink bottom	simple	0,0487
R. alba	white	semidouble	0,0518
R. cinnamomea	pink	simple	0,0702
R. rugosa	red	simple	0,0767
R. gallica	ruddy	double	0,0865
R. centifolia	dark red	thickly double	0,1148
R. lutea	yellow	semidouble	0,1413
R. damascena	pale pink	semidouble	0,1528

As the rose and eremothecium oils have similar qualitative and quantitative component composition, it is supposed that ways of the essential oil synthesis, its intracellar transport, and mechanisms of its excretion in micromycetes could be like in the oil-bearing rose.

Findings are evidence that there are electron light lipid bodies in hyphae of the one-day submerged culture. They are located in the intermembranous space of an agranular endoplasmic reticulum. As a rule these lipid bodies appear in 36 h when the spores germinate and the mycelium starts to form. The quantity of spherosomes changes synchronously in

accordance with the level of the essential oil accumulation in the cultural liquid. In 36-48 h of cultivation the frank vacuolization of the mycelium was noticed. There were the osmiumphilic lipid bodies in the formed vacuoles (Fig. 2, 3). More intensive vacuolization, presented by the numerous small vacuoles, was characteristic for the strains with the high intensity of the essential oil synthesis (for instance, VKPM F-340). Moreover, high level of vacuolization was noticed from the earlier to the later ontogenetic stages.

During all development phases there were the lipid bodies of another type which were electron-dense (osmiumphilic), rounded. The small ones merged to the bigger formation of irregular shape (flows). It was typical for all study strains that in the beginning of the stationary phase, increase of efficiency of the essential oil synthesis was accompanied with the rise of spherosome quantity and sizes. This causes the intensification of affinity for osmium (Table 3).

Table 3

Comparative characteristics of results of essential oil synthesized by *Eremothecium* and *Rosa* species

Strain-producer, species of oil-bearing rose	Ratio			Efficiency of production process within essential oil, mg per g of biomass per h
	2PE/ MTA	geraniol/ citronellol	geraniol/ nerol	
E. ashbyi				
VKMF-4566D	0,08-0,31	0,98-6,90	2,22-10,17	0,825-1,237
VKMF-4565D	0,01-0,37	4,66-12,21	15,64-48,86	1,032-1,682
VKPM F-36 (NRRLY-1363)	0,22-0,39	2,51-7,04	3,65-68,20	0,930-1,358
VKPM F-340	0,11-0,24	37,62-51,94	– **	0,976-1,240
VKM F-3009	0,02-0,12	9,12-15,30	13,6-24,65	0,813-1,298
VKMF-124	0,11-0,28	12,43-16,65	6,96-12,21	0,158-0,239
E. gossypii				
VKMF-3276	0,79-1,29	7,76-13,21	4,79-26,42	0,627-2,198
VKMF-2627	1,12-1,27	13,92-77,33	6,96-34,72	1,514-1,915
R. alba	0,05-0,13	0,26-1,20	1,43-3,20	0,002-0,003
R. gallica	2,33-3,00	2,00-2,40	1,00-4,20	0,004-0,006
R. damascena	0,04-0,08	0,13-1,12	0,55-7,67	0,001-0,005
R. rugosa	0,01-0,14	0,14-0,53	3,07-4,21	0,002-0,006
R. canina	2,02-3,23	– *	– **	0,001-0,003
R. centifolia	0,99-2,14	1,17-35,00	1,05-2,10	0,004-0,006
R. lutea	– ***	– *	– **	0,004-0,007

Note: 2PE – 2-phenylethanol; MTA – monoterpene alcohols; –* – citronellol was not identified; –** – nerol was not identified; –*** – cyclohexanone was identified.

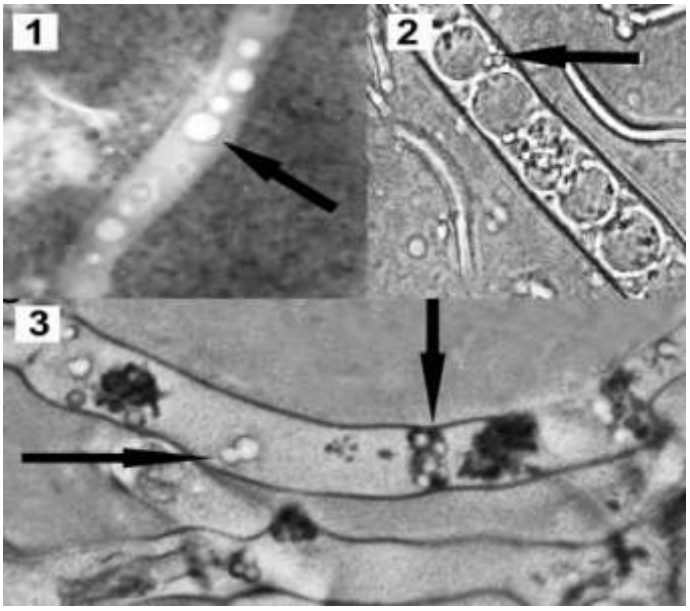


Fig. 2. The anatomical structure of *Eremothecium* oil synthetizing hyphae (lipid bodies are pointed by arrows; magnification x100) stained by: 1 – ink; 2 – iodine; 3 – Sudan III

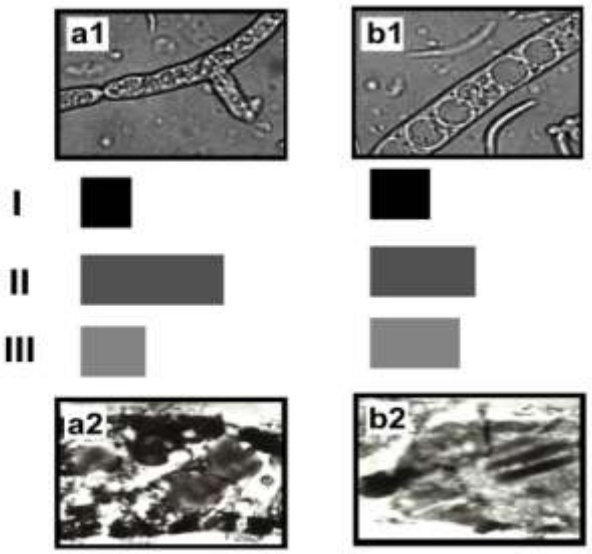


Fig. 3. The cytomorphological features of the *Eremothecium* mycelium and the level changes in the accumulation of the main biologically active substances: I – riboflavin, II – essential oil, III – geraniol; a_1, a_2 – 36 h, b_1, b_2 – 48 h of cultivation in a liquid nutrient medium (magnification: a_1, b_1,– x100; a_2 – x18500; b_2 – x20000)

The interconnection between increase of the total protoplasm affinity to osmium and rise of the total biosynthetical activity of every strain was revealed. At the same time the rounded lipid bodies started to locate near the main cell membranes (plasmalemma, tonoplast). The maximum of the essential oil productivity of the study taxons concurred with the massive flows of the electron-dense substance to the cell membrane. The affinity to osmium for all strains significantly decreased after lipids excretion into the environment. The lipid bodies practically disappeared. The electron-dense content was not found in the vacuoles. Besides the lipid bodies might get into the environment because of the cell lysis as a result of aging.

Analyzing structural organization of the essential oil synthesis and its accumulation among representatives of the *Eremothecium* genus in comparison with *Rosa,* it should be

taken into account, that the study objects have the significant micromorphological differences caused by their taxonomic positions. The fungal cells form hyphae which make mycelium (Fig. 2). And no further tissue differentiation like in the oil-bearing rose occurs. So the described earlier secretory structures are not typical for the *Eremothecium* species. Moreover, the differences, like in the rose cells, in the productional ability of the separate mycromycete cells were not revealed. Concerning the synthesis of other secondary metabolites, that is riboflavin and its forms, it is known that only certain cells of the mycelium (~60%) are able to synthesize and accumulate them, and the rest (~40%) do not have this ability [10]. This assumption is based on the results of the microscope investigation which has shown that vitamin crystalline inclusions were not found in each cell. Moreover, there is no reliable data about permeases, participating in the active transport of the synthesized riboflavin to the environment and genes coding them.

The findings of the histological investigation of the rose species is coordinated with the data described previously for kinds Krymskaya Krasnaya [3] and Anna [1] belonging to *R. gallica* and *R. hybrida,* and *R. rugosa* [14]. Every petal tissue is characterized to synthesize the certain component of the essential oil. For instance, in the petals of the Michurinka kind the synthesis of the main monoterpene alcohols (MTA) took place mostly in the adaxial and abaxial layers of the epidermis, but 2-phenylethanol is mainly produced in the parenchyma. This might be connected with the difference in the influence of endogenous and exogenous factors on the genes participating in the synthesis of the certain substances in the differently located cells. From our point of view, the MTA biosynthesis is associated with the presence (level) of active oxygen, and the synthesis of an aromatic alcohol is intensified in the anaerobic conditions that might be observed in the rose petals tissue.

Due to the fungi organization the plastids, particularly the leucoplasts, are absent in their cells. So the synthesis of the main MTA precursor, isopentenyldiphosphate, from 2-C-methyl-D-erythrol-4-phosphate (Fig. 4, 5) [12] cannot be realized.

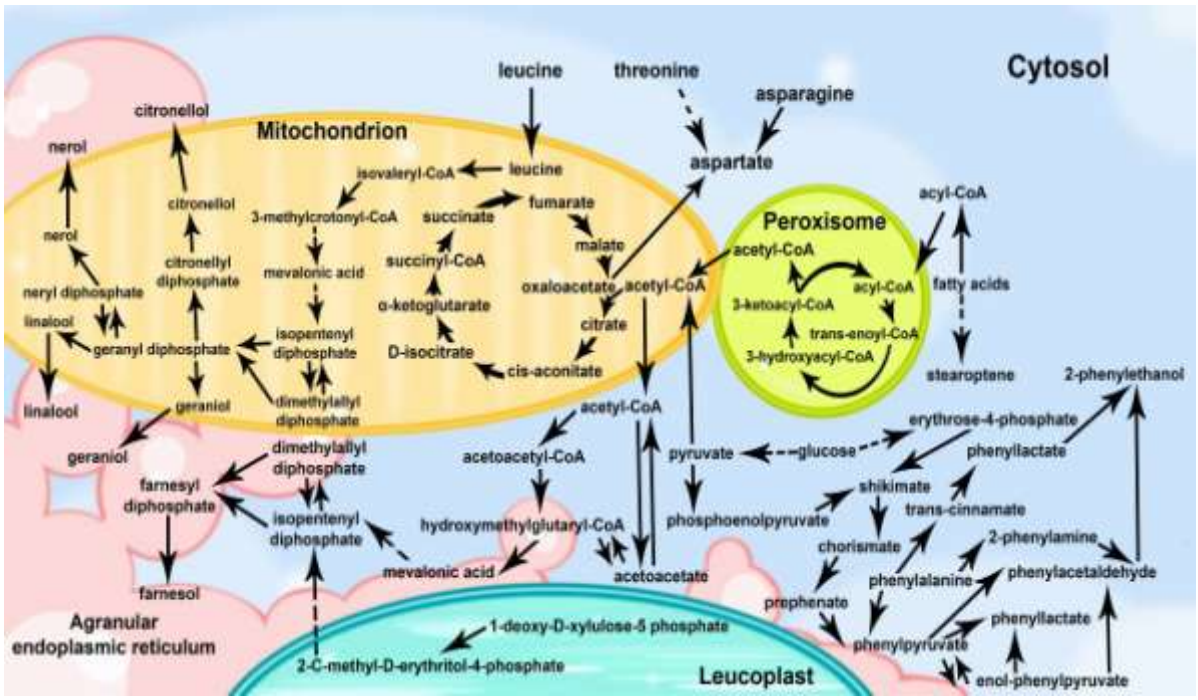


Fig. 4. The hypothetic metabolic model of the biosynthesis of the main essential oil components in the petals of the oil-bearing rose

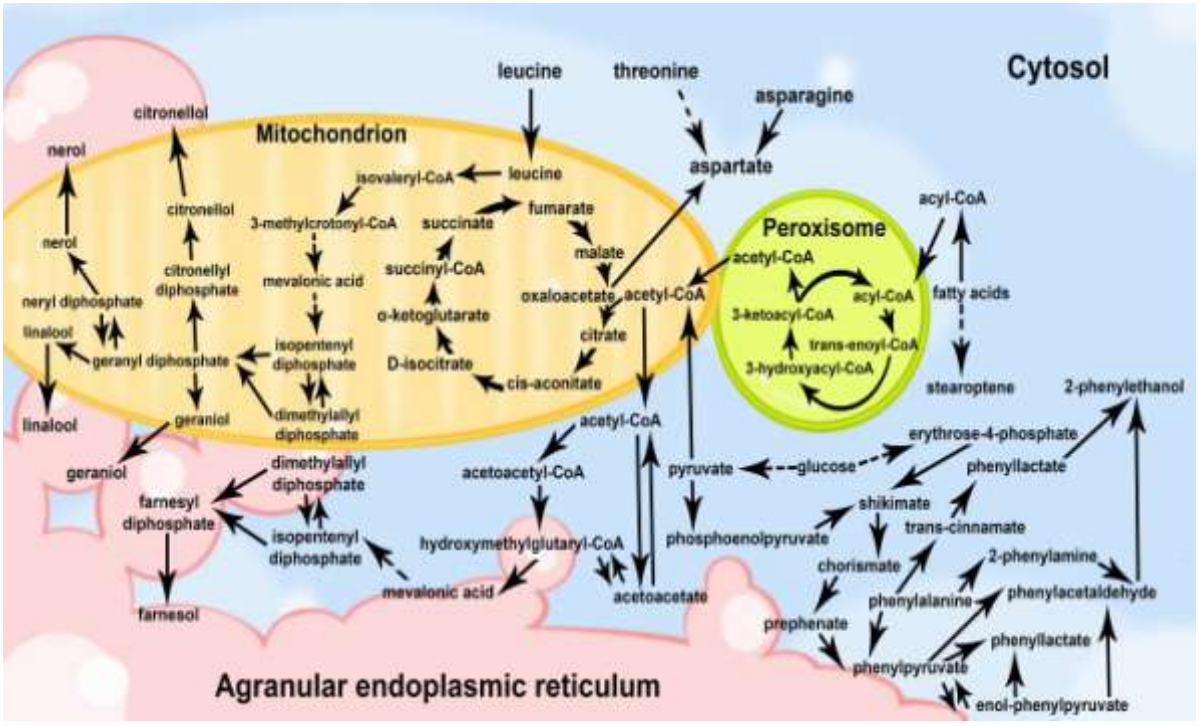


Fig. 5. The hypothetic metabolic model of the biosynthesis of the main essential oil components in the *Eremothecium* mycelium

Perhaps for *E. ashbyi* and *E. gossypii* the synthesis of this precursor can be accomplished only due to mevalonic acid which is produced in a cytosol and mitochondria from hydroxymethylglutaryl-CoA. Isopenthyldiphosphate isomerizes into dimethylallyldiphosphate, and then after condensation of these two compounds farnesyldiphosphate or geranyldiphhosphate might be formed depending on the precursors localization. Such MTA as linalool, nerol, and citronellol come from geranyldiphosphate due to the specific reactions. The formation of the oxidated terpene compounds is connected with the agranular endoplasmic reticulum. Moreover, the production of other lipophilic compounds (stearoptenes, triacylglycerols, fatty acids, etc.) is common for both *Rosa* and *Eremothecium*. It is possible that the structural organization of the plant cells and the hyphae is caused by the intensity of the processes of the secretion synthesis and its transport. The morphological parameters of the vacuoles and the spherosomes (quantity, sizes, localization) characterize the mycelium functional activity like in the rose cells [1]. The increase of the vacuolization is observed from early to late growth stages that correspond to the data related to the oil-bearing rose [15]. Besides it was found that the rounded lipid bodies are located at the same places like in the rose petals: near the plasmalemma and the tonoplast. The maximum of the essential oil productivity of the *Eremothecium* strains concurred with the massive flows of the electron-dense substance to the cell membrane. The same phenomenon was noticed in the rose petal cells [3, 15].

Conclusions

The results of the carried out research permit to make the following conclusions:

1. The rose secretory structures are presented as the glandular epidermis and the endogenous oval conceptacles which are located in the deep of the parenchymal tissue and contain the drops of the essential oil.

2. The small volatile-oil-bearing conceptacles in the parenchyma deep are characteristic for collection samples with the low essential oil content.

3. The over-synthesis of the fragrant substances among species of the *Eremothecium* genus is closely connected with the high cell functional activity which becomes apparent as

the increase of the spherosomes and the filling of the vacuoles by the osmiumphilic substance like in the case of the rose.

4. The fungal secretion of the fragrant substances to the environment is supposed to be one of the regulatory functions of their synthesis ("mechanism of overfill").

The article was received at editors 05.10.2015.

Semyonova Ye.F., Shpichka A.I., Presnyakova Ye.V., Mezhennaya N.A. Processes of essential oil accumulation in petals of *Rosa* (Rosaceae) and Mycelium *Eremothecium* (Eremotheciaceae). // Bull. of the State Nikit. Botan. Gard. – 2016. – № 118. – P. 25-33.

The plantation cultivation of an oil-bearing rose is not able to cover the increasing demand of the industry. Therefore, the interest to fungi strains *Eremothecium ashbyi* Guilliermond and *E. gossypii* Kurtzman, is rising. The features of secretory structures of the *Rosa* and *Eremothecium* species were found out. The investigation of biosynthesis, accumulation, and secretion of essential oils with a rose scent is crucial either for development of new ways to produce them or for rating the biological role of *Rosa* and *Eremothecium* secondary metabolites.

Key words: *essential oil; oil accumulation; spherosomes; secretory structures; Rosa; Eremothecium*

PLANT BIOCHEMISTRY

UDK 582.929.4:577.19

BIOLOGICALLY ACTIVE SUBSTANCES OF NEPETA CATARIA L.

Anfisa Yevgenyevna Paly, Ivan Nikolayevich Paly, Natalya Vladimirovna Marko, Valery Dmitriyevich Rabotyagov

Nikita Botanical Gardens – National Scientific Centre
298648, the Republic of Crimea, the city of Yalta, urb.vil.Nikita
onlabor@yandex.ru

Introduction

Nepeta Cataria L. is a perennial plant that belongs to Lamiaceae family. Overground mass of the cultivar possesses pretty lemon fragrance, pungent taste and takes a great interest in the field of food, perfume and soil boiling industries.

Various preparations that include *Nepeta Cataria* are used in folk medicine as spasmolytic, carminative, tonic and stimulative remedy. Besides, extracts of its overground part can be used to treat gastrointestinal and respiratory diseases, stagnations of gallbladder and bile passages, histerical and depressive attacks [6, 12].

Complex of biologically active substances, such as volatile compounds, phenolic substances and vitamins in plant material of *Nepeta cataria* specifies its medicinal properties [5, 8, 14].

Essential oil (EO) has got a fine herbaceous and citrus fragrance and presents high antimicrobial action. Content and component composition of *Nepeta cataria* EO ranges a lot what depends upon ecological and genetical factors. Essential oil includes geranial, geraniol, camphora, carvacrol, caryophyllene, nepetalactone, nerol, citral, citronellal, citronellol and eugenol [9, 15]. Phenolic compounds of *Nepeta cataria* are presented by hydroxicoric acids – rosemary, caffeic, n-cumarin, ferulic acids; and flavonoids – flavones (apigenin, lutheoline) and flavonols (quercetin, kaempferol, myricetin) [2, 7, 8].

Long-term research in *Nepeta cataria* introduction and selection has been kept on within NBG-NSC, as a result there is a new sort – Peremozhets-3, which is characterized by high crop capacity and EO output [1].

In terms of introduction 2010, new *Nepeta cataria* specimen was involved, it possesses lemon fragrance and can be a source of high crop capacity under conditions of the Steppe Crimea.

Purpose of this work was to investigate qualitative and quantitative composition of biologically active substance in water-ethanol extract of new promising *Nepeta cataria* specimen.

Objects and methods of the research

Overground part of *Nepeta cataria*, yielded during mass blooming became the study research.

Essential oil was extracted out of raw material applying method of hydrodistillation by Ginsberg, with further conversion into dry mass. Distillation time of EO – 1 hour.

Concentration of volatile substances was determined in water-ethanol extract (hereinafter extract), prepared from air-dry plant material. Raw material was dried in the aired room getting constant mass. Extraction was conducted by 50% ethyl alcohol having ratio of raw material and leach – 1:20 (preparation took 10 days at room temperature).

Component composition of volatile substances was determined applying chromatograph Agilent Technology 6890 with mass-spectrum detector 5973. Chromatographic heater – capillary HP-1 (30 m long); inside diameter – 0,25 mm. Thermostat temperature was programmed in a range 50-250 °C with velocity of 4°C/min. Injector temperature - 250°C. Carrier gas is helium, stream velocity – 1,0 sm^3/min. Transfer from gas chromatograph to mass spectrometric detector was heated till 230°C. Source temperature was kept up at 200°C. Electronic ionization was carried out at 70 eV with mass range m/z from 29 till 450. Identification of essential oil components was guided by comparison results obtained in terms of chemical substances mass spectra with publishing data of mass spectra NIST05-WILEY2007 (about 500000 of mass spectra).

Component composition of phenol substances was determined applying chromatograph “Agilent Technologies” (model 1100), completed with running vacuum degasifier G1379A, 4-channeled pump of low pressure gradient G13111A, automotive injector G1313A, thermostat of heaters G13116A, diode-matrix detector G1316A, fluorescent detector G1315B. Chromatograph heater 2,1mm x 150 mm, filled with octadecilsilil sorbent “ZORBAX” SB-C18 and 3,5 mkm graining was applied during analysis. Gradient regime of chromatography that foresees changes of component A (0,1% orthophosphoric acid, 0,3%-tetrahydrofuran, 0,018%- triethylamine) and B (methanol) components in eluting mixture. Feed rate of mobile phase made 0,25 sm^3/min; working pressure of eluent – 240-300 kPa; sample volume – 2 mkl; scanning time 0,5 sec, scale of measurements – 1,0.

Identification of phenol substances was carried out according to time of standard keeping and spectrum characteristics (parameters of spectrum reading - each peak 190-600 nm; wave length – 280, 313, 350, 371 nm.

Results and discussion

As a result of introduction work there is a new specimen of *Nepeta cataria*. Plants were cultivated in the steppe zone of the Republic of Crimea, where they are able to reach 179-190 sm and present a high crop capacity up to 70 center/ha. New specimen was marked out due to organoleptic method, as one with bright lemon fragrance. Mass fraction of essential oil inhere made 0,11% from raw material and 0,51 from the absolutely dry material (2,4% of dry substances).

Investigating component composition and concentration of biologically active substances in *Nepeta cataria* water-ethanol extract it was found out that concentration of volatile substances made 233 per 100 g of dry plant material (table 1, fig.1). Among volatile compounds 15 components were identified. The following volatile compounds are typical for extract of this specimen: monoterpenic alcohols, aldehydes, lactones, as well as sesquiterpenoids. The dominant components of the extract are nepetalactone (34%), citronellol (33%) and geranial (10%). At the same time considerable content of geraniol (7,96%) and caryophyllenoxide (6,19%). Findings coordinate with publishing data [3, 4, 8].

Table 1

Component composition of volatile compounds in *N.cataria* water-ethanol extract

Component	Mass fraction, %
acetic acid	2,70
6-methyl-5-hepten-2-one	0,70
phenylacetaldehyde	1,10
6-methyl-3,5-heptadien-2-one	0,23
phocitral A	0,84
citronellal	1,60
citronellol	33,13
geraniol	7,96
geranial	10,45
geranylformiat	0,56
nepetalactone	33,81
caryophyllene	0,50
caaryophyllenoxide	6,19
myristinic acid	0,35

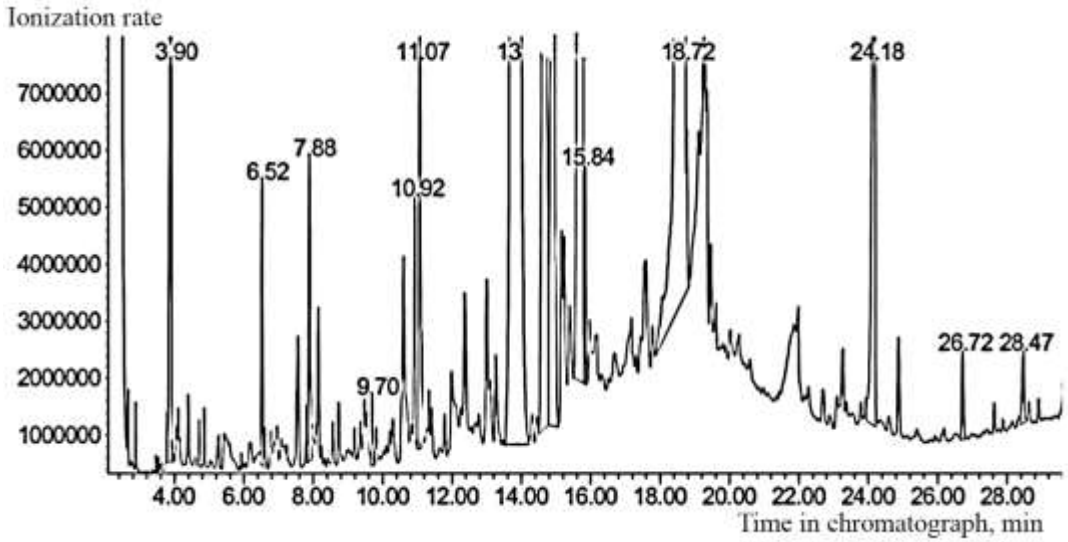


Fig.1 Chromatogram of volatile compounds in *N.cataria* water-ethanol extract

The principal components that specify qualitative parameters of *N.cataria* essential oil are neral, geranial and geraniol. At the same time nepetalactone that also enters this composition causes high antimicrobial, repellent and insecticidal effect of the EO. It`s a well-

known fact, that nepetalactone can act as pheromones for aphides and protect from orthopterans [6, 12]. Typical fragrance of nepetalactone attracts animals of cat family [13].

It was determined that *N.cataria* extract contains high concentration of phenolic compounds, carotenoids and ascorbic acid (table 2).

Table 2

Concentration of biologically active substances in *N.cataria* extract

Biologically active substances	Concentration, mg/100 g
Phenolic compounds	2218,0 ± 64,0
Ascorbic acid	308,0 ±12,0
Carotenoids	2,8 ± 0,07

Phenolic compounds of *N.cataria* are presented by hydroxicoric acids and flavonoids. Concentration of hydroxicoric acids made 1,4 g/100 g of air-dry plant material, flavonoids – 0,8 g/100 g.

Studying component composition of the extract phenolic substances 12 components were revealed, while 8 of them were identified (table 3, fig.2).

Table

Component composition of *N.cataria* phenolic compounds

Component	Output time	concentration, mg/100 g
4- caffeoylquinic acid	19.91	84,94
5- caffeoylquinic acid	20.42	100,58
chlorogenic acid	**26.09**	**1113,00**
lutheoline-7-diglicoside	28.82	133,81
n-cumaroil glycoside	30.62	31,34
n-coumaric acid	31.91	55,00
apigenin-7-diglicoside	**32.79**	**499,88**
lutheoline-7-glycoside	33.67	43,43
apigenin-7-glycoside	34.42	33,08
acacitine-7-glycoside	35.7	65,33
apigenin	37.54	27,92
Unidentified	38.46	29,31

Dominant components are chlorogenic acid and diglicoside of apigenin flavone, their concentration makes 50,0% and 22,5% respectively, from the total content of phenolic substances; our findings coordinate with data of other scientists [9, 10]. Chlorogenic acid possesses strong antioxidant, antivirus, antibacterial antifungal and probiotical properties, presents hypoglycemic, hypocholesteremic, anticancer and hepatoprotective action. Inhibiting oxidation of lipoproteid with low concentration, what causes artery damages, chlorgenic acid makes it possible to prevent cardiovascular diseases [5, 10]. High concentration of chlorogenic acid, apigenin glycosides and lutheoline isomers in *N.cataria* new specimen extract explains its biological value.

Flavones, lutheoline and apigenin reveal a wide range of biological activity as well. They possess anti-inflammatory, spasmolytic, antitumoral, immunoregulatory and antiallergic action, high antioxidant and weak antibacterial activity [3, 11].

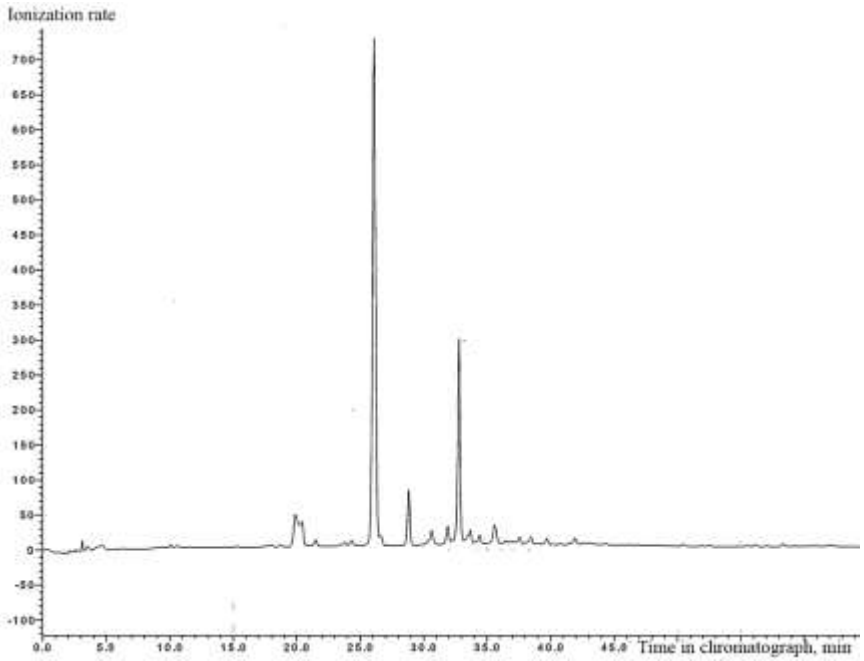


Fig.2 Chromatogram of *N.cataria* phenolic compounds

In this way overground part of study case (*N.cataria* specimen) is of great biological value due to monoterpenic aldehydes (nepetalactone, citronellol and geranial), phenolcarboxylic acids (mainly cholorogenic acid), ascorbic acid and carotenoids and could be used in the field of food, cosmetic and medioprophylactic production, enriched with biologically active substances.

Conclusions

Qualitative and quantitative composition of biologically active substances (phenolic substances, volatile compounds, vitamins) in water-ethanol extract of the *N.cataria* promising specimen was determined in terms of the research.

It was found out that *N.cataria* extract contains the main volatile compounds, typical for essential oil – monoterpenoids: nepetalactone, citronellol, geranial and geraniol and sesquiterpenoid caryophyllenoxide. Dominant components are nepetalactone (34%), citronellol (33%) and geranial (10%).

Phenolic substances of this extract are presented by hydroxicoric acids and flavonoids. The principal components are chlorogenic acid (50%) and dilicoside apigenin (22,5%).

This water-ethanol extract of *N.cataria* possesses high biological value and fine minty-lemon fragrance. This extract can be a ground for food, medioprophylactic and perfume-cosmetic production.

References (6)

1. *Rabotyagov V.D., Khlypenko L.A., Bakova N.N., Mashanov V.I.* Annotirovanny katalog vidov i sortov efiromaslichnyh, pryano-aromaticheskih i pishchevyh rasteny kollektsii Nikitskogo botanicheskogo sada. – Yalta: Nikitsky botanichesky sad, 2007. – 48 s.

2. *Cigremis Y., Ulukanlia Z., Ilcimb A., Akgozc M.* In vitro antioxidant activity and phenolic composition of *Nepeta cataria* L. extracts // International Journal of Agricultural Science and Technology. – 2013. – Vol. 1 №. 4. – P. 74-79.

3. *Dirscherl K., Karlstetter M., Ebert S., Kraus D.* Luteolin triggers global changes in the microglial transcriptome leading to a unique anti-inflammatory and neuroprotective phenotype // Journal of Neuroinflammation. – 2010. – V. 7. – P. 3.

4. *Farah A., Monteiro M., Donangelo C.M.* Chlorogenic acids from green coffee extract are highly bioavailable in humans // J Nutr. – 2008. – V. 138 (12.). – P. 2309–2315.

5. *Giamperi L., Bucchini A., Cara P., Fraternale D., Ricci D., Genovese S., Curini M., Epifano F.* Composition and antioxidant activity of *Nepeta foliosa* essential oil from Sardinia (Italy) // Chemistry of Natural Compounds. – 2009. – 45. – P. 554-556.

6. *Grognet J.* Catnip: Its uses and effects, past and present // Can. Vet. J. – 1990. – 31. – P. 455-456.

7. *Lee S.Y., Lee C.Y., Eom S.H., Kim Y.K., Park N.I., Park S.U.* Rosmarinic acid production from transformed root cultures of *Nepeta cataria* L. // Scientific Research and Essays. – 2010. – № 5. – P. 1122-1126.

8. *Modnicki D., Tokar M., Klimek B.* Flavonoids and phenolic acids of *Nepeta cataria* L. *var. citriodora* (Becker) Balb. (Lamiaceae) // Acta Poloniae Pharmaceutica – Drug Research. – 2007. - Vol. 64. - № 3. - P. 247-252.

9. *Mohammadi Sh., Saharkhiz M.J.* Changes in essential oil content and composition of catnip (*Nepeta cataria* L.) during different developmental stages // Jeobp. – 2011. Vol. 14. – № 4. – P. 396-400.

10. *Olthof M.R., Hollman P.C., Katan M.B.* Chlorogenic acid and caffeic acid are absorbed in humans // J. Nutr. – 2001. – V. 131 (1.) – P. 66-71.

11. *Patel D., Shukla S., Gupta S.* Apigenin and cancer chemoprevention: progress, potential and promise (review) // Int. J. Oncol. – 2007. – V. 30(1). – P. 233-245.

12. *Tucker A.O., Tucker S.S.* Catnip and the catnip response // Econ. Bot. – 1988. – 42. - P. 214-231.

13. *Waller G.R., Price G.H., Mitchell E.D.* Feline attractant, cis, trans-nepetalactone: Metabolism in the domestic cat // Science. – 1969. – V. 164. – P. 1281-1282.

14. *Zomorodian K., Saharkhiz M.J., Shariati S., Pakshi K., Rahimi M.J., Khashei R.* Chemical composition and antimicrobial activities of essential oils from *Nepeta cataria* L. against common causes of oral infections // Journal of Dentistry, Tehran University of Medical Sciences. – 2013. – Vol.10. – № 4. – P. 329-337.

15. *Zomorodian K., Saharkhiz M.J., Shariati S., Pakshir K., Rahimi M.J., Khashei R.* Chemical composition and antimicrobial activities of essential oils from *Nepeta cataria* L. against common causes of food-bornen infections // ISRN Pharmaceutics. – 2012. – DOI:10.5402/2012/591953.

The article was received at editors 10.12.2015

Paliy A.Ye., Paliy I.N., Marko N.V., Rabotyagov V.D. Biologically active substances of *Nepeta cataria* L. // Bull. of the State Nikit. Botan. Gard. – 2016. – № 118. – P. 33-38.

Qualitative and quantitative composition of biologically active substances in aqueous-ethanol extracts of *Nepeta cataria* L. was investigated in terms of the research.

Volatile compound composition in *N. cataria* water-ethanol extract was determined as well.. The main components of extract are nepetalactone, citronellol and geranial.

Content of phenolic compounds was also revealed in aqueous-ethanol extract of *N. cataria*. Apigenin glycosides, luteolin and hydroxycinnamic acids – chlorogenic acid isomers were identified in the extract.

New specimen of *N. cataria* is possible to use as a source of biologically active substances for production of medicine, cosmetics and foods.

The study was funded by the Russian Scientific Foundation (№ 14-5000079).

PLANT BIOCHEMISTRY

UDK 582.998.1:577.19 (477.75)

BIOLOGICALLY ACTIVE SUBSTANCES OF AQUEOUS-ETHANOLIC EXTRACT OF *TAGETES SIGNATA* BARTL. № 13152-8 'VETVISTY' SPECIMEN OF NIKITA BOTANICAL GARDENS COLLECTION

Gury Viktorovich Kornilyev, Anfisa Yevgenyevna Paly, Ivan Nikolayevich Paly, Valery Dmitriyevich Rabotyagov, Sergey Aleksandrovich Feskov

Nikita Botanical Gardens – National Scientific Centre
298648, the Republic of Crimea, the city of
Yalta, urb.vil.Nikita
onlabor@yandex.ru

Introduction

Tagetes L. genus includes more than 30 cultivars growing *in vivo,* mainly in Central America. *Tagetes patula* L. *Tagetes erecta* L., *Tagetes signata* Bartl., syn. *Tagetes tenifolia* Cav. are considered the most popular within crop [5, 18, 27].

Different *Tagetes* cultivars are used to treat liver or kidneys disorder, for pain control – as a diuretic and pathogenic remedy. *Tagetes* L. preparations permit to control nervous tension and improve spirit. The plant is popular in dermatology as well, because it cures blackheads and possesses tonic and regenerative properties, so important for skin [1, 10, 13, 16, 17, 19, 21, 26, 30].

Essential oils (EO) of different *Tagetes* L. cultivars are known as matters with antibacterial, antimicrobial, larvicidal, repellent and fungicidal effect [7, 10, 11, 22-24, 26, 28, 29].

Tagetes flowers are possible to use as a spice "Imeretian Saffron" [30]. Besides some species of *Tagetes* L. are source of carotenoids (for example, in poultry farming – for intensifying egg color) [2, 4, 9], pigments and lutein [3, 20].

Allowing for variety of useful *Tagetes* L. properties, introduction of new cultivars is of great importance now, particularly on South Coast of the Crimea, as well as breeding new sorts with valuable characteriristics.

Nikita Botanical Gardens – National Scientific Centre bred promising (by ornamentality, disease- and pest-resistance, crop capacity, essential oil content) specimenTagetes signata L. № 13152-8 "Vetvisty". It was found out that essential oil composition of this specimen included trans-tagetenon, dihydro-tageton, trans-tageton, cis-tagetenon, limonene, sabinene, trans-ocymen, eugenol and a number of minor components [15].

In this way EO extracting out of raw material is accompanied with oxidation process if to apply method of steam distillation; getting native substances demands their transition into water-ethanol extracts.

It`s a well-known fact, that flower extracts of T.*signata* is a result not only of volatile substances transition, but phenol substances, which present antioxidant properties: hydroxycoric acids (caffeic, rosemary, chlorogenic, p-Coumaric acid), flavonoids and their glycosides (apigenin, hyperoside, isoquercetin, quercetin, rutin) [12, 15]. At the same time there is dependence of chemical composition in raw material from cultivar and sort belonging, soil and climatic conditions of habit.

Allowing for data mentioned above, investigation of chemical composition of T.*signata* specimen water-ethanol extract, № 13152-8 "Vetvisty", cultivated on South Coast of the Crimea, is a vital question.

The study purpose is to research qualitative and quantitative composition of biologically active substances (volatile terpenes, phenol compounds, vitamins) of T.*signata* № 13152-8 "Vetvisty" under conditions of South Coast of the Crimea, to get an opportunity for use it in medioprophylactic production.

Objects and methods of the research

Study research is water and ethanol extract out of specimen T.*signata* №13152-8 "Vetvisty" flowers, yielded during mass blooming.

Specimen was bred in NBG-NSC applying individual selection from seed population. Plant of 50-60 sm high. Stem is straight, branchy from the bottom, well-leafed. Leaves are sectile. Inflorescence is a basket of 19-24 mm across diameter, 5 semiflosculous flowers, corolla is golden-yellow. Blooming period is the whole summer, mass blooming happens from July the 10-20th. Specimen is disease- and pest-resistant. Crop capacity made 84,7 center/ha, essential oil content – 0,46% of the raw material, EO yield made 40,8 kg per hectare.

Concentration of biologically active substances was determined in water-ethanol extract, made of air-dry plant mass, yielded during mass blooming. Extraction was carried out applying 50-% ethanol having ratio of raw material and leach – 1:10 what was prepared for 10 days at room temperature.

Component composition of volatile substances was determined applying chromatograph Agilent Technology 6890N with mass-spectrum detector 5973N. Chromatographic column – capillary HP-1 (30 m long); inside diameter – 0,25 mm. Thermostat temperature was programmed in a range 50-250 °C with velocity of 4°C/min. Injector temperature - 250°C. Carrier gas is helium, stream velocity – 1,0 sm^3/min. Transfer from gas chromatograph to mass spectrometric detector was heated till 230°C. Source temperature was kept up at 200°C. Electronic ionization was carried out at 70 eV with mass range m/z from 29 till 450. Identification of essential oil components was guided by comparison results obtained in terms of chemical substances mass spectra with publishing data of mass spectra NIST05-WILEY2007 (about 500000 of mass spectra).

Component composition of phenol substances was determined applying chromatograph "Agilent Technologies" (model 1100), completed with running vacuum degasifier G1379A, 4-channeled pump of low pressure gradient G 13111A, automotive injector G 1313A, thermostat of heaters G13116A, diode-matrix detector G1316A, fluorescent detector G1315B. Chromatograph heater 2,1mm x 150 mm, filled with octadecilsilil sorbent "ZORBAX" SB-C18 and 3,5 mkm graining was applied during analysis. Gradient regime of chromatography that forsees changes of component A (0,1% orthophosphoric acid, 0,3%-tetrahydrofuran, 0,018%- triethylamine) and B (methanol) components in eluting mixture. Feed rate of mobile phase made 0,25 sm^3/min; working pressure of eluent – 240-300 kPa; sample volume – 2 mkl; scanning time 2 sec, scale of measurements – 1,0. Identification of phenol substances was carried out according to time of standard keeping and spectrum characteristics (wave length – 313 (for phenol acids and their derivatives), 350 (for glycosides of flavonoids), 371 nm (for flavonoids)).

Concentration of carotinoids was determined applying photometric method [14], ascorbic acid – by titration of potassium iodate [6].

Results and discussion

It was found out that concentration of volatile substances in specimen extract (T.*signata* № 3152-8 "Vetvisty") made 32,96 mg/100 g of raw material.
7 components were identified (table 1, fig.1).

Table 1

Volatile substances of water and ethanol extract *Tagetes signata* Bartl. № 13152-8 "Vetvisty"

№	Output time, min	Component	Concentration, %
1	6.38	cyclohexanone	8,49
2	7.25	3,5-dimethylcyclohexe-2-en-1-on	10,2
3	8.27	propylvaleriate	2,28
4	8.36	hexe-1,2,6-triol	2,76
5	14.12	tagetenone	17,3
6	15.11	4-vinylphenyl acetate	8,23
7	16.63	P-vinylguaiacol	50.8

The principal volatile components of this extract are P-vinylguaiacol (50,8%) and typical for T.*signata* № 13152-8 "Vetvisty" tagetenone (17,3%). Dominant group of compounds in the extract is aroma (59%; P-vinylguaiacol and 4-vinylphenyl acetate). The biggest number of components (3,5-dimethylcyclohexe-2-en-1-on, cyclohexanone, hexe-1,2,6-triol, propylvaleriate) belongs to aliphatic compounds, what is 23,7% in amount. Typical for T.signata component – tagetenone is a monoterpenic compound.

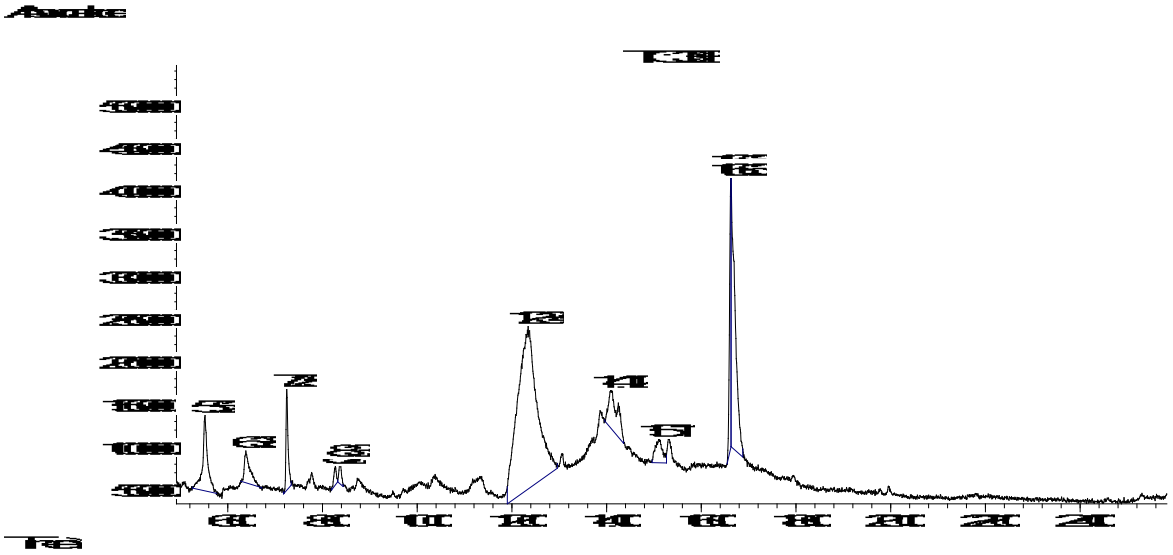


Fig. 1 Chromatogram of volatile substances in water-ethanol extract of *Tagetes signata* Bartl. № 13152-8 "Vetvisty"

It was found out that content of phenolic substances in extract of T.*signata* № 13152-8 "Vetvisty" made 1821 mg/100 g of plant material (table 2, fig.2). 11 components were revealed, 6 compounds – identified.

Table 2

Phenolic substances of water and ethanol extract Tagetes signata Bartl.№ 13152-8 "Vetvisty"

№	Output, min	Component	Concentration mg/100 g
1	15.35	Caffeic acid derivatives	142
2	17.92	Ferulic acid	152
3	18.73	Unidentified flavonoid	132
4	18.96	Unidentified flavonoid	79
5	19.34	Quercetin -3-O-galactoside	301
6	19.92	Rutin	331
7	20.28	Unidentified flavonoid	65
8	20.76	Unidentified flavonoid	332
9	21.40	Quercetin -3-O-glucoside	217
10	23.70	Unidentified flavonoid	38
11	24.39	Lutheoline	32

Among identified substances in study extract the following dominate: glicosides of quercetin – rutin (331), quercetine-3-O-galactoside (301) and quercetine-3-O-glucoside (217 mg/100 g). Hydroxycoric acids are presented by ferulic acid and caffeic acid derivatives, which make 294 mg/100 g in total.

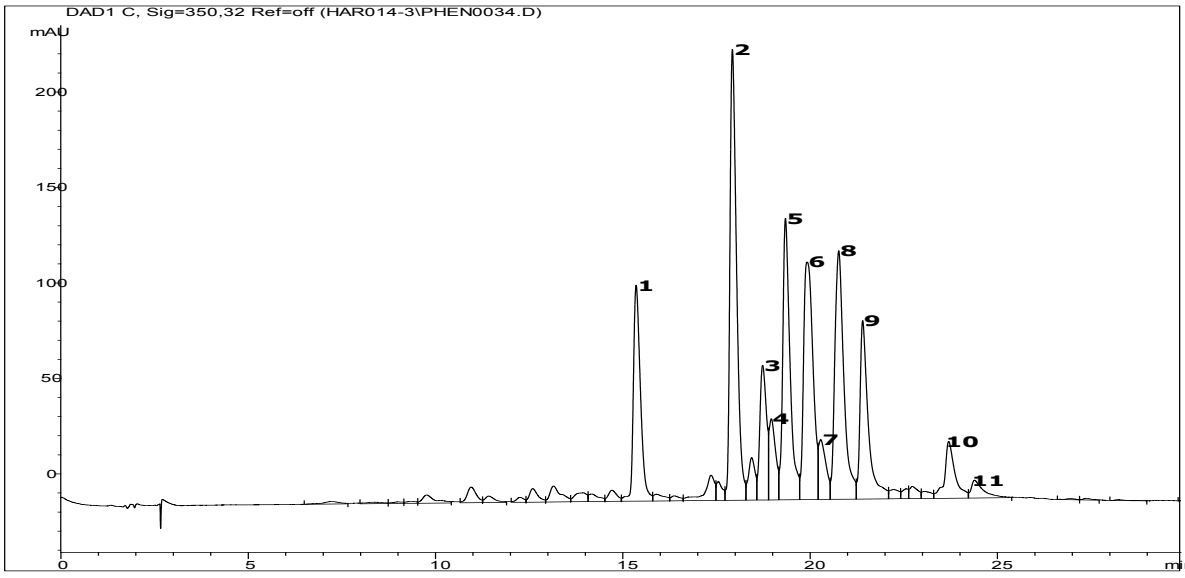


Fig.2 Chromatogram of phenolic substances in water-ethanol extract of *Tagetes signata* Bartl. № 13152-8 "Vetvisty"

Vitamin concentration was determined in the study extract as well. Concentration of ascorbic acid made 14,54 mg/100 g, carotinoids – 5,19 mg/100 g.

In this sway water-ethanol extract of T.signata specimen №13152-8 "Vetvisty" contains not so much of volatile terpenes, but could be considered as a source of P-vinylguaiacol, glicosides of quercetine, ascorbic acid and carotinoids.

Conclusions

In terms of the research qualitative and quantitative composition of biologically active substances (volatile terpenes, phenolic substances and vitamins) in water-ethanol extract of Tagetes signata Bartl. specimen, № 13152-8 "Vetvisty", cultivated on South Coast of the Crimea.

It was found out that among volatile compounds of the specimen, P-vinylguaiacol and tagetenone dominate.

At the same time among phenolic substances glicosides of quercetine (rutin, quercetine-3-O-halactoside, quercetine-3-O-glucoside) and flavonoids prevail. Hydroxycoric acids are presented by ferulic acid and derivatives of coffeic acid.

In extract of T.*signata* № 13152-8 "Vetvisty" we determined vitamin concentration – ascorbic acid and carotinoids.

In general specimen of T.*signata* Bartl. № 13152-8 "Vetvisty" could be considered as a source of biologically active substances, especially of P-vinylguaiacol, glicosides of quercetine, ascorbic acid and carotinoids.

References

1. *Bender K.I., Gomenyuk G.A., Freidman S.L.* Ukazatel po primeneniyu lekarstvennyh rasteny v nauchnoy i narodnoy meditsine. – Saratov: SGU, 1988. – 112 s.

2. *Berdey T.S., Marchishyn S.M.* Doslidzhennya lipofilnoy fraktsii roslyn rodu chornobryvtsi (Tagetes L.) // Farmatsevtychny chasopys. – 2011. - №1. – S. 10-14.

3. Deineka V.I., Sorokopudov V.N., Deineka L.A., Tretyakov M.Yu. Issledovaniye tsvetkov *Tagetes* sp. kak istochnika lyuteina // Khimiko-farmatsevticheskiy zhurnak. – 2007. – T. 41, № 10. – S. 30-32.

4. Deineka V.I., Tretyakov M.Yu., Deineka L.A., Sorokopudov V.N. Nekotoriye osobennosti nakopleniya pigmentov v tsvetkah *Tagetes* sp. // Nauchniye vedomostu Belgorodskogo gosudarstvennogo universiteta. Seriya: Yestestvenniye nauki. – 2007. – T. 5, vyp.5. – S. 123-129.

5. Klassifikator roda *Tagetes* L. (Barkhatsy). – L.: VYR, 1088. – 15 s.

6. Kriventsov V.I. Metodicheskiye rekomendatsii po analizu plodov na biochimichesky sostav. – Yalta, 1982. – 22 s.

7. *Libus O.K., Rabotyagov V.D., Kutko S.L., Khlypenko L.A.* Efirnomaslichniye i pryano-aromaticheskiye rasteniya: Nauchno-populyarnoye izdaniye. – Kherson: Ailant, 2004. – 272 s.

8. Maksimenko N.V., Prokhorov V.N. Otsenka razlichnyh vidov *Tagetes* L. po osnovnym khozyaistvenno-tsennym priznakam // Vestnik Belorusskoy gosudarstvennoy selskokhozyaistvennoy akademii.- 2014. – №4. – S. 112-114.

9. Maksimenko N.V., Prokhorov V.N. Primeneniye razlichnyh genotipov rasteny roda *Tagetes* L. Kak perspektivnyh istochnikov karotinoidov dlya biodobavok v ptitsevodstve // Selskoye khozyaistvo – problemi i perspektivy: sb.nauchn.tr. – 2014. – T. 26. Zootekhniya. – S. 168-174.

10. Malakhov V.A., Zavgorodnyaya A.N., Chernishova T.I. Encyklopediya naturoterapii. – M.: Eksmo, 2008. – S. 75.

11. Malyugina O.O., Mazulin O.V., Smoilovska G.P., Mazulin G.V., Yerenko O.K. Komponentny sklad ta protymikrobna diya efirnoyi olii sutsvit chornobryvtsiv

pryamostoyachyh (*Tagetes erecta* L.) // Farmatsevtychny zhurnal. – 2014. - № 1(14). – S. 86-92.

12. *Marchishyn S.M., Berdey T.S., Kozachok S.S., Demydyak O.L.* Opredeleniye flavonoidov i hydroksikorichnyh kislot v trave *Tagetes erecta* L., *Tagetes patula* L., *Tagetes tenuifolia* Cav. metodom VEZhKh // Meditsina i obrazovaniye v Sibiri: setevoye nauchnoye izdaniye. – 2013. - № 6. – Rezhym dostupa: http://ngmu.ru/cozo/mos/article/text_full.php?id=1259.

13. *Mashkovska S.P., Grigoryuk I.P.* Chornobryvtsi – dzherelo efektyvnyh likiv // Phytoterapiya. Chasopys. – 2003. - № 4. – S. 41-47.

14. Pleshkov B.P. Praktikum po biochimii rasteny. – M.: Kolos, 1969. – 183 s.

15. Rabotyagov V.D., Khlypenko L.A., Bakova N.N., Mashanov V.I. Annotirovanny katalog vidov i sortov efiromaslichnyh, pryano-aromaticheskih, i pishchevyh rasteny kollektsii Nikitskogo botanicheskogo sada. – Yalta: Nikitsky botanichesky sad, 2007. – 48 s.

16. *Oganesyan E.T., Andreyeva O.A., Terekhov A.Yu., Podgornaya Zh.V.* Biologicheski aktivniye veshchestva lipofilnoy fraktsii iz tsveykov *Tagetes patula* i izucheniye protivoozhogovoy aktivnosti na ih osnove // Sovremenniye naukoyomkiye tekhnologii. – 2004. - № 6. – S. 109-110.

17. *Papayani O.I., Dukhanina I.V., Sergeyeva Ye.O.* Izucheniye khimicheskogo sostava i antimikrobnoy aktivnosti sukhogo ekstrakta iz tsvetov barkhatsev rasprostyortyh (*Tagetes patula* L.) // Izvestiya Samarskogo nauchnogo tsentra Rossiyskoy akademii nauk. – 2012. – T. 14, № 5-3. – S. 745-747.

18. *Yurchak L.D.* Kultura chornobryvtsiv v umovah Lisostepu Ukrainy // Introduktsiya Roslyn. – 1999. - № 1. – S. 49-54.

19. *Basher S., Gilani A.H.* Studies on the antioxidant and analgesic activities of Aztec marigold (*Tagetes erecta*) flowers // Phytotherapy research. – 2008. – Vol. 22(12). – P. 1692 – 1694.

20. *Bhattacharyya S., Datta S., Mallick B., Dhar P., Ghosh S.* Lutein content and *in vitro* antioxidant activity of different cultivars of Indian Marigold flower (*Tagetes patula* L.) extracts // J. Agric. Food Chem. – 2010. – Vol. 58(14). – P. 8259 – 8264.

21. *Céspedes C.L., Avila J.G., Martinez A., Serrato B., Calderón-Mugica J.C., Salgado-Garciglia R.* Antifungal and antibacterial activities of Mexican Tarragon (*Tagetes lucida*) // J. Agric. Food Chem. – 2006. – Vol. 54(10). – P. 3521 – 3527.

22. *Chamorro E.R., Ballerini G., Sequeira A.F., Velasco G.A., Zalazar M.F.* Chemical composition of essential oil from *Tagetes minuta* L. leaves and flowers // Journal of the Argentine Chemical Society. – 2008. – Vol. 96(1-2). – P. 80 – 86.

23. *Dharmagadda V.S.S., Naik S.N., Mittal P.K., Vasudevan P.* Larvicidal activity of *Tagetes patula* essential oil against three mosquito species // Bioresource Technology. – 2005. – Vol. 96(11). – P. 1235 – 1240.

24. *Gillij Y.G., Gleiser R.M., Zygadlo J.A.* Mosquito repellent activity of essential oils of aromatic plants growing in Argentina // Bioresource Technology. – 2008. – Vol. 99(7). – P. 2507 – 2515.

25. *Gong Y., Liu X., He W.-H., Xu H.-G., Gao Y.-X.* Investigation into the antioxidant activity and chemical composition of alcoholic extracts from defatted marigold (*Tagetes erecta* L.) residue // Fitoterapia. – 2012. – Vol. 83(3). – P. 481 – 489.

26. *Lima B., Agüero M.B., Zygadlo J., Tapia A., Solis C., Rojas de Arias A., Yaluff G., Zacchino S., Feresin G.E., Schmeda-Hirschmann G.* Antimicrobial activity of extracts, essential oil and metabolites obtained from *Tagetes mendocina* // J. Chil. Chem. Soc. – 2009. – Vol. 54(1). – P. 68 – 72.

27. *Lim T.K. Tagetes tenifolia* // Edible medicinal and non-medicinal plants. – 2014. – P. 469 – 472.

28. *Romagnoli C., Bruni R., Andreotti E., Rai M.K., Vicentini C.B., Mares D.* Chemical characterization and antifungal activity of essential oil of capitula from wild Indian *Tagetes patula* L. // Protoplasma. – 2005. – Vol. 225(1-2). – P. 57 – 65.

29. *Rondon M., Velasco J., Hernández J., Pecheneda M., Rojas J., Morales A., Carmonia J., Diaz T.* Chemical composition and antibacterial activity of the essential oil of *Tagetes patula* L. (Asteraceae) collected from Venezuela Andes // Rev. Latinoamer. Quin. – 2006. – Vol. 34(1-3). – P. 32 – 36.

30. *Vasudevan P., Kashyap S., Sharma S.* Tagetes: A multipurpose plant // Bioresource Technology. – 1997. – Vol. 62(1-2). – P. 29 – 35.

The article was receaived at editors 1.02.2016

Kornilyev G.V., Paliy A.Ye., Rabotyagov V.D., Feskov S.A. Biologically active substances of aqueous-ethanolic extract of *Tagetes signata* Bartl. № 13152-8 'Vetvisty' specimen of Nikita Botanical Gardens collection // Bull. of the State Nikit. Botan. Gard. – 2016. – № 118. – P. 39-45.

The qualitative and quantitative composition of *Tagetes signata* Bartl. № 13152-8 'Vetvisty' specimen was investigated in terms of the research. It was established that among volatile substances there were aromatic (59%; p- vinylguaiacol prevails), aliphatic (23,7 %) and monoterpenoide (tagetenon content is 17,3%) compounds. Phenolic compounds were presented by flavonoids and their glycosides (rutin, quercetin-3-O-galactoside, quercetin-3-O-glycoside), and hydroxycinnamic acids (caffeic, ferulic). The ascorbic acid (14,54) and carotenoids (5,19 mg/100 g) were identified as well. On the whole, the specimen *T. signata* № 13152-8 "Vetvisty" can be considered as a sources of biologically active substances such as p- vinylguaiacol, flavonoid glycosides, ascorbic acid and carotenoids.

Key words: *Tagetes signata* Bartl.*; specimens; aqueous-ethanolic extract; volatile substances; phenolic substances; vitamins.*

PHYTOMONITORING

UDK 635.055:504.753:712.253

LOWER LAYER ORNAMENTAL PLANTS OF ARBORETUM IN NIKITA BOTANICAL GARDENS AND THEIR GROWTH AND STATUS DEPENDING ON ILLUMINATION CONDITIONS

Nikolay Ivanovich Klymenko, Ivan Nikolayevich Paly

Nikita Botanical Gardens – National Scientific Centre
298648, the Republic of Crimea, the city of Yalta, urb.vil.Nikita
runastep@yandex.ru

Introduction

Investigation of shade tolerance of ornamental plants, peculiarities of their light conditions plays a big role for matching of cultivars and crop combinations projecting ladscape compositions. Shade-tolerant plants have wide ecological amplitude; concerning light, good illumination favors their better growth, but at the same time they adapt to weak light easily. As a rule they are divided into more and less shadow-tolerant. Those trees and bushes that grow on open areas and don`t stand continuous shade can be classified as less shadow tolerant (light-requiring) arboreal crops. Photosynthesis gets the highest point if good sun illumination. This group includes: birch, willow, larch, aspen, walnut, locust, pine, ash. More shadow tolerant arboreal plants are presented by trees and bushes standing some shadow, but intensively growing under conditions of good illumination: box tree (*Buxus),* arrowwood *(Viburnum), Euonymus Japonicus,* cherry laurel, Japan laurel (*Aucuba japonica*),

hazel (*Corylus)* and etc. [3, 7]. Lower layer plants should be studied especially, as they grow under conditions of microclimate, created by environment. That`s why expansion of species and form composition of arboreal and shrub plants in landscaping projecting long-term green plantations, resistant to abiotical and biotical factors is an urgent and rather important problem.

In this way the study purpose was to investigate state and growth of ornamental plants from lower layer allowing for illumination to mark out species, promising for landscaping on South coast of the Crimea.

Objects and methods of the research

Researches were carried out in Upper and Lower Arboretum of Nikita Botanical Gardens (NBG). Seven species of ornamental plants with individual characteristics of water regime, drought-resistance and shadow tolerance were chosen as model objects for investigations: *Pittosporum heterophyllum* Franch. (питтоспорум разнолистный), *Buxus sempervirens* L., *Euonymus japonica* Thunb., *Chimonanthus praecox* (L.) Link, *Viburnum tinus* L., *Cornus mas* L., *Laurocerasus officinalis* M. Roem. Principal phenological development phases of study cases are different (table 1). Table 1 revealed that *Euonymus japonica* Thunb., *Chimonanthus praecox* (L.), *Cornus mas* L. started the earliest vegetation (vegetative budding) – the 1st decade of February – the 2nd decade of March.

Table 1

Development phenophases of some ornamental plants in NBG-NSC gardens

Cultivar	Vegetative start (vegetative budding)	Blooming		Growth termination (crown bud setting)
		start	finish	
Eunymus japonica Thunb.	1st d. of February – the 2nd d. of March	3rd d. of June – 1st d. of July	2nd d. of July	2nd d. of June
Chimonanthus praecox (L.) Link	1st d. of February – 2nd d. of March	3rd d. of November – 1st d. of December	1st d. of January	1st d. of June – 1st d. of July
Viburnum tinus L.	1st d. of March – the 1st d. of April	3rd d. of March	3rd d. of April – 1st d. of May	2nd d. of July – 1st d. of August
Cornus mas L.	1st d. of February – the 2nd d. of March	1st d. of March	2nd d. of April	3rd d. of June – 1st d. of July
Laurocerasus officinalis M. Roem.	1st d. of April – the 2nd d. of May	1st d. of April – 2nd d. of May	3rd d. of April – 2nd d. of May	3rd d. of May – 3rd d. of June
Pittosporum heterophyllum Franch.	1st d. of April	1st d. of May	3rd d. of May	1st d. of June – 3rd d. of July
Buxus sempervirens L.	1st d. of March – 1st d. of April	3rd d. of March	2-3rd d. of April	2nd d. of May
Notes: d. - decade				

Laurocerasus officinalis M. Roem. presented the latest vegetation. *Viburnum tinus* L., *Buxus sempervirens* L., *Pittosporum heterophyllum* Franch. took intermediate place. Therefore, study cases covered almost the whole vegetative period, which continues 210 days on South Coast of the Crimea (SCC).

Early growth termination was fixed for *Buxus sempervirens* L. (setting of the crown bud was determine factor) – 2nd decade of May. A bit later *Laurocerasus officinalis* M. Roem. and Eunymus japonica Thunb. finished their growth (the end of May – June). *Chimonanthus praecox* (L.) Link., *Pittosporum heterophyllum* Franch. and *Cornus mas* L.

growth termination happened in June-July. *Viburnum tinus* L. formed its crown bud in the 1st decade of August.

Ornamentality is mainly rated by terms and duration of blossoming if landscape compositions. Among study cases *Cornus mas* L., *Viburnum tinus* L., *Pittosporum heterophyllum* Franch. and *Chimonanthus praecox* (L.) Link revealed the most continuous blooming period (more than a month). Plants of the rest cultivars were blossoming not so long (2-3 weeks). Blooming period of study cases covered rather continuous period since November till December - *Chimonanthus praecox* (L.) Link , up to July - Eunymus japonica Thunb. Majority of cultivars were in blossom in March-May.

Most study cases of the lower layer (besides *Chimonanthus praecox* (L.) Link) grow in Upper park of NBG Arboretum (145-165 m above the sea level), *Chimonanthus praecox* (L.) Link locates in Lower park (115 m above the sea level). Microclimate of study areas is various [5]. Allowing for illumination factor they can be classified into three groups: 1st group of plants grows under shadowed conditions during entire ontogenesis (under coniferous exots), the second – on shadowed land during active vegetation in frost-free season (under foliage trees), and the 3rd group – on rather open areas. The first group included:

- *Pittosporum heterophyllum* Franch. – evergreen ornamental bush with common leathery nitid leaves, grows under *Atlas cedar* crown, 1 m from the stem;
- *Buxus sempervirens* L. – evergreen ornamental bush, locates within shared projective cover of *Atlas cedar*, *Laurus nobilis* and equally spaced with their stems, 2,5m.
- *Chimonanthus praecox* (L.) Link – ornamental bush, blooms in winter, oblong-elliptic leaves fall in winter. It grows under *Cupressus macrocarpa* crown (3,5 m from its stem).

The second group of plants locates under crown of deciduous trees:

- *Eunymus japonica* Thunb. – valuable ornamental evergreen plant, grows under *Laburnum anagyroides* (1 m from the stem);
- *Viburnum tinus* L. – evergreen bush with middle-sized nitid and green leaves and corymbs of white flowers, grows in undercrown space, created by *Circassian walnut* and *Gleditschia triacanthos,* spaced from tree stems 1 and 2,5 m.
- *Laurocerasus officinalis* M. Roem. is a bush with common leathery evergreen leaves, from south, west and north-west surrounded by three trees of *Tilia cordata* spaced from the stems 3,5-4,5 m, while from north it is shadowed by outlying part of *Aesculus glabra* (buck-eye) crown;
- *Cornus* mas L. is a deciduous bush, growing under shadowed conditions [4].

The third group consists of plants belonging to cultivars mentioned above, but not subjected to shadowed conditions of growing.

Studying one-year amount of study cases growth was carried out applying methodic of phenological tree and bush observation [6]. Plant state was determined according technical inventory instruction of green plantations [7]. Light stream was measured by Yu-116 luxmeter allowing for recommendations by V.A. Alekseyev – having total illuminance during midday hours and minimum wind velocity [1].

Results and discussion

Growth investigation of chosen genotype revealed middle length of shoot for plants being under well (enough)-illuminated conditions, that is *Eunymus japonica* Thunb. and *Chimonanthus praecox* (L.) Link (16,5 and 18,8sm respectively), so they are the most intensively growing among study cases (table 2).

Table 2

One-year shoot length as an indicator of shadow tolerance and plant state depending upon growth conditions (2014-2015)

Cultivar	Illumination conditions	Plant state	Middle length of shoot, sm	Shoot being under shadowed conditions, sm	Shadow tolerance indicator
1	2	3	4	5	6
Eunymus japonica Thunb.	illuminated	good	16,5±0,8	10,2	0,38
	shadowed	satisfied	6,3±0,4		
Chimonanthus praecox (L.) Link	illuminated	good	18,8±1,5	13,5	0,28
	shadowed	satisfied	5,3±0,4		
Viburnum tinus L.	illuminated	good	12,6±0,8	5,2	0,59
	Shadowed	good	7,4±0,6		
Cornus mas L.	illuminated	good	13,2±1,1	9,3	0,30
	shadowed	satisfied	3,9 ±0,1		
Laurocerasus officinalis M. Roem.	illuminated	good	10,4±0,5	1,9	0,82
	shadowed	good	8,5±0,4		
Pittosporum heterophyllum Franch.	illuminated	good	11,0±0,2	6,9	0,37
	shadowed	satisfied	4,1±0,4		
Buxus sempervirens L.	illuminated	good	8,2±0,4	3,2	0,61
	shadowed	good	5,0±0,3		

Under conditions of total illuminance *Laurocerasus officinalis* M. Roem. and *Buxus sempervirens* L. presented worse amount of growth – 10,4 and 8,2 sm, so they are considered as weak-growing.

All study cases, growing on shadowed area, length of one-year shoot (on average for 2 years) consideredbly reduced in comparison with plants on well-illuminated areas, but decrease degree ranges (table 2). The most significant reduction of this parameter was fixed for *Chimonanthus praecox* (L.) Link and *Eunymus japonica* Thunb. – 13,5 and 10,2 sm less respectively or 72 and 62% in comparison with other study plants, so these cultivars experienced the most considerable inhibit of growth amount being under shadowed conditions. *Laurocerasus officinalis* M. Roem. and *Buxus sempervirens* L. didn`t reduce amount of growth so much (the lowest parameter). Length of one-year shoot being shadowed is 1,9 and 3,2 sm less (18 – 39% respectively), what indicates rather insignificant shade negative effect on shoot growth. The rest plants took intermediate place.

Correlation analysis of shoot growth amount and reduction degree of study cases being shaded revealed direct relation between average length of one-year shoots under conditions of illumination and degree of its reduction being shaded (r-0,91, n-7). It proves observations conducted before: plants with more intensive growth reduces amount of growth to the large degree being shaded and can be considered not so shade tolerance.

A new parameter of shade tolerance was introduced to determine degree of one-year shoot length being shaded. It can be calculated by ratio of one-year shoot length in shade to the same parameter but under conditions of illumination. Than more shade tolerance than less differences in amount of growth under both conditions (shade and total illumination), than more shade tolerant a plant is.

On average during two-years observations the highest point of shade tolerance was fixed for *Laurocerasus officinalis* M. Roem. (0,82), lower - *Viburnum tinus* L. and *Buxus sempervirens* L. (about 0,59-0,61). Therefore these cultivars can be considered raher shade

tolerant growing in certain conditions, in comparison with other study cases. *Chimonanthus praecox* (L.) Link and *Cornus mas* L. revealed the lowest shade tolerance which ranges 0,28-0,30, and so they are considered weak shade tolerant. *Eunymus japonica* Thunb. *Pittosporum heterophyllum* Franch. took in this line intermediate place, as they had almost similar parameters of shade-tolerance (0,37-0,38); they are medium-shade tolerant.

According to shade-tolerance study plants were arranged in descend order as follows: *Chimonanthus praecox* (L.) Link → *Cornus mas* L. → *Eunymus japonica* Thunb. → *Pittosporum heterophyllum* Franch. → *Viburnum tinus* L. → *Buxus sempervirens* L. → *Laurocerasus officinalis* M. Roem. This line corresponds to shade tolerance parameters, presented in the article [4].

These results are preliminary which characterize plants under certain conditions. These cultivars rate should be kept on for 2-3 years if to get more correct data. Shade tolerance can be used for other cultivars as well.

Rating of study plant common state revealed that high illumination favored better state according to chosen scale (table 2). They were strong, well-developed with dense foliage evenly spread across branches, leaves have got common size and color, no signs of disease or persts damages, no stem of skeletal branches damages as well. Being shaded majority of plants had satisfactory condition. Growth slowdown, uneven crown development, decrease of leaves, insignificant mechanical damages and hollows on trunks pointed that fact. But *Viburnum tinus* L., *Laurocerasus officinalis* M. Roem. and *Buxus sempervirens* L. didn`t get any signs of inhibition being shaded and developed rather well. It proves their relative shade-tolerance under certain ecological conditions.

Dynamics of illumination regime is possible to follow at figures 1, 2, 3, 4 and 5. The highest parameters of illumination were fixed for cultivars, located on open areas. The lowest – for plants being shaded by high trees. Maximum illumination of 32000 Lx reduces till 947 Lx for plants on shaded areas. Low illumination plays role of limiting factor in study cases development (table2).

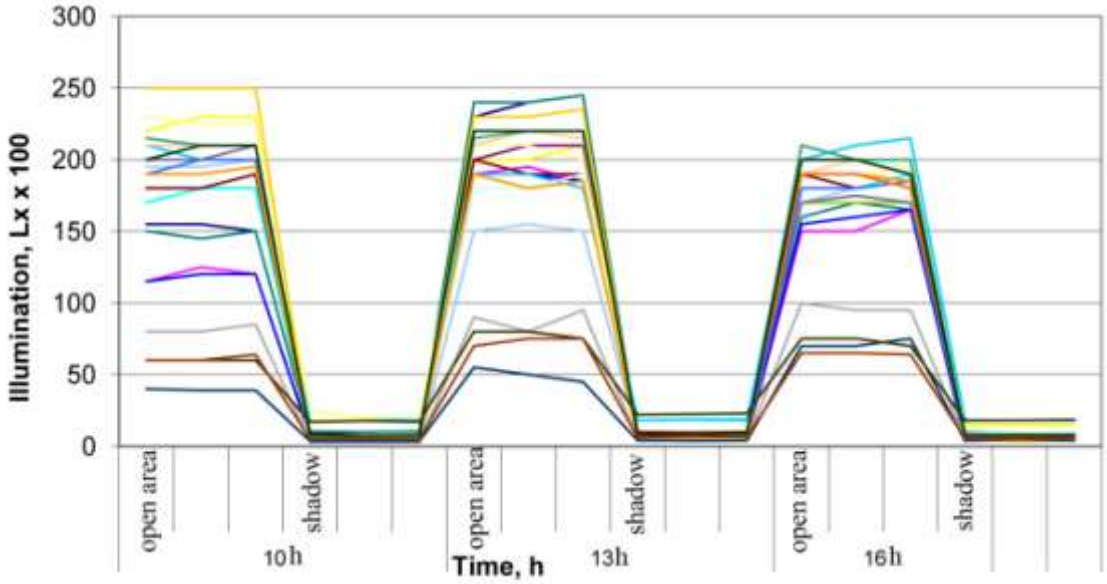


Fig.1 *Eunymus japonica* Thunb. Illumination 18.06.15-07.08.15

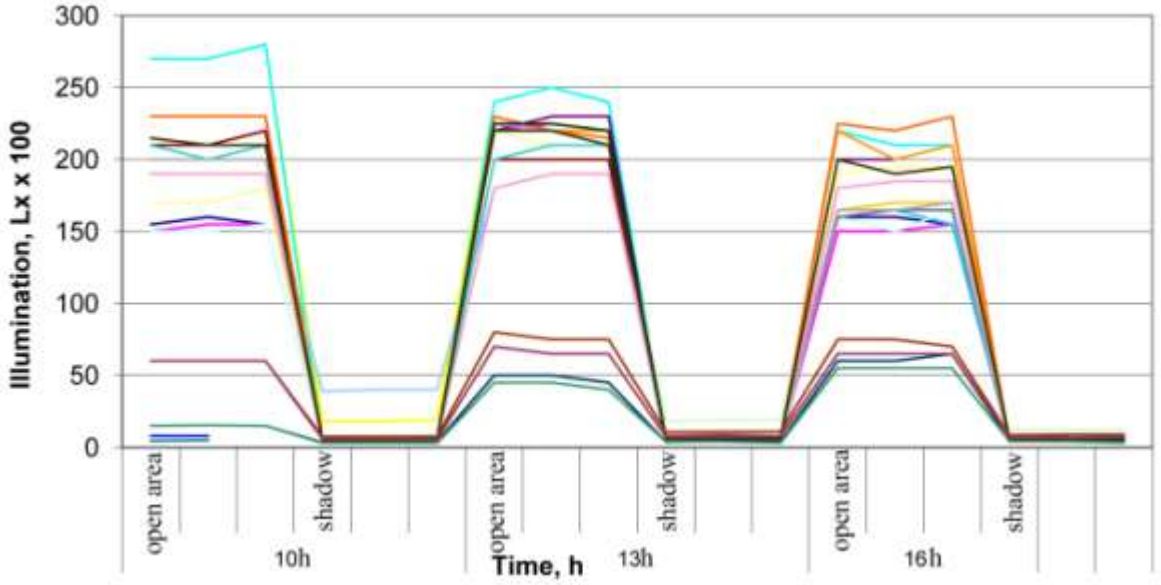


Fig.2 *Chimonanthus praecox* (L.) Link illumination during 18.06.15-07.08.15

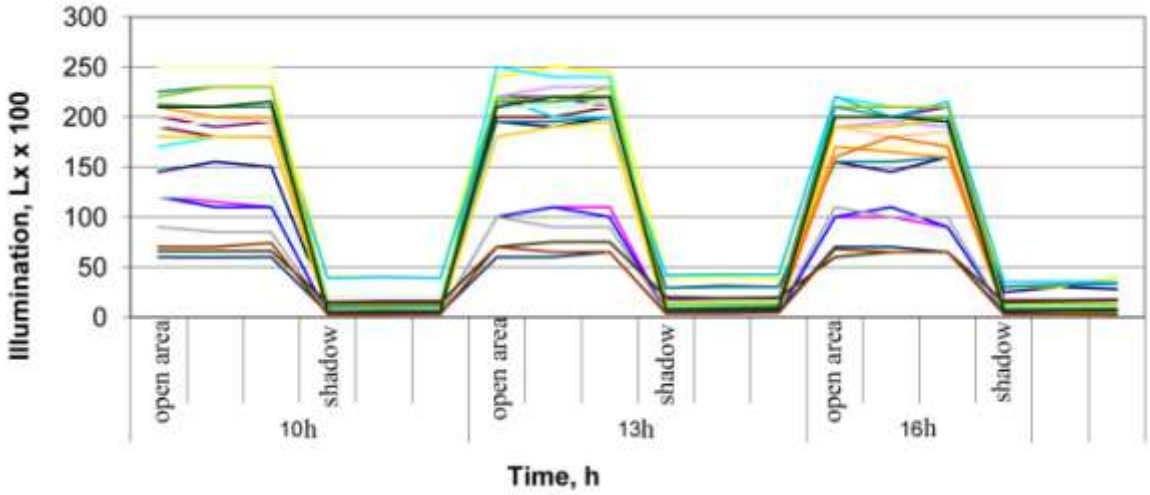


Fig.3 *Viburnum tinus* L. illumination during 18.06.15-07.08.15

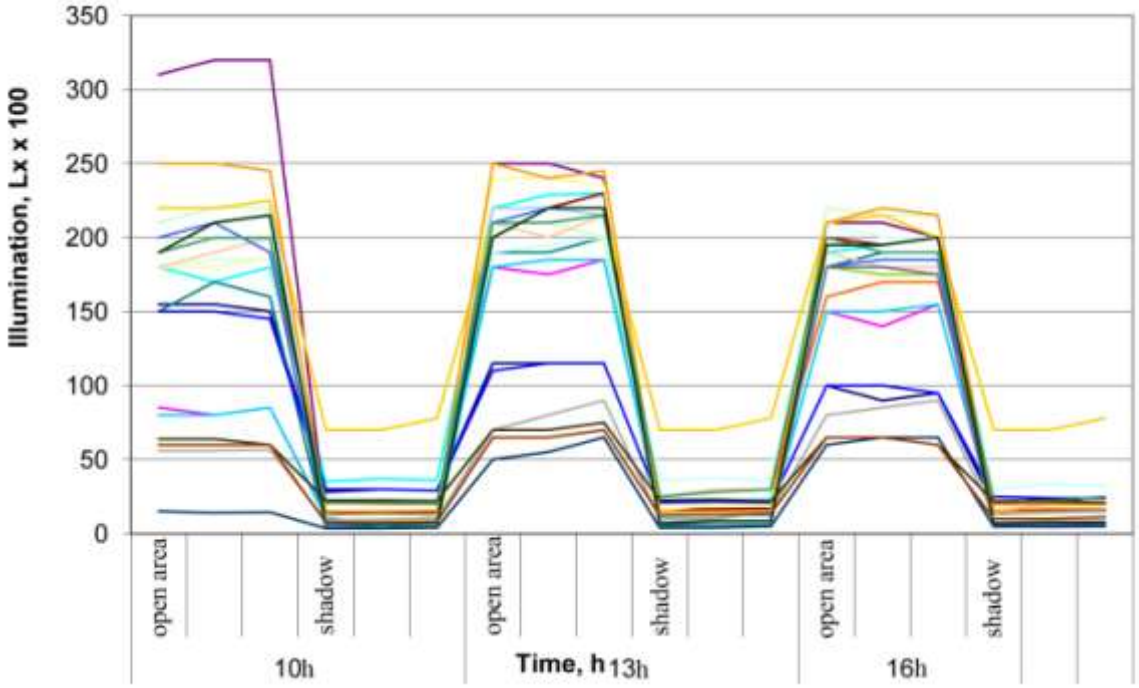


Fig.4 *Cornus mas* L. illumination during 18.06.15-07.08.15

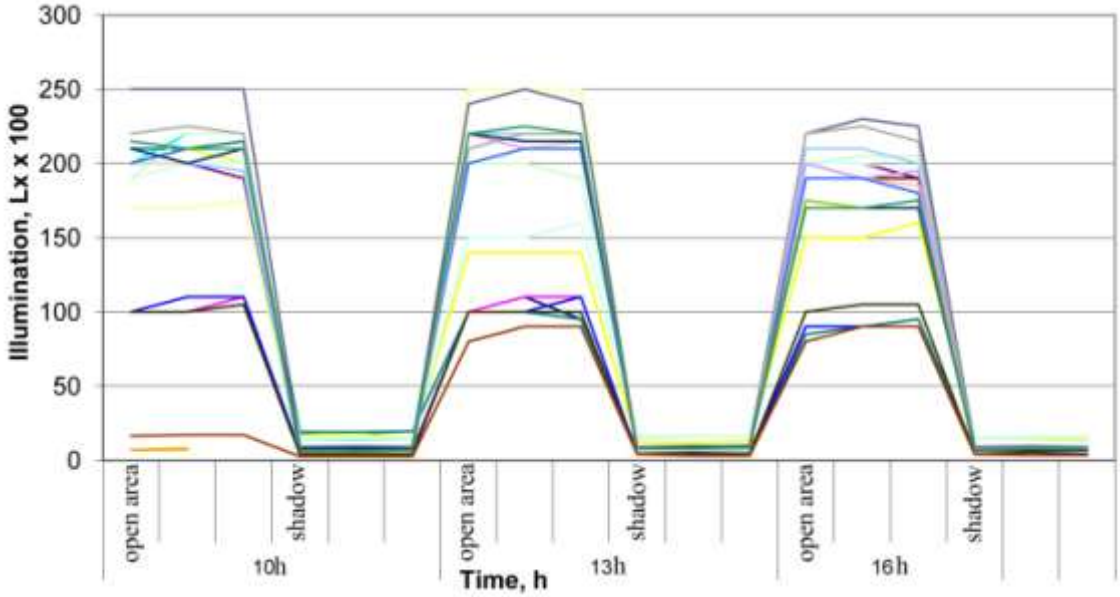


Fig.5 *Laurocerasus officinalis* M. Roem. illumination during 18.06.15-07.08.15

Illumination parameter is characterized with cyclicity for all study cultivars. In the midday parameters take higher points comparing with morning and evening, while morning factors are lower than evening. Such a dynamics is typical for both, plants growing in the shade or on open garden areas.

Conclusions

1. It was determined that shoot length of bush plants growing on sunlit areas, was significantly higher in comparison with bush plants growing in the shade.
2. Parameter of shade tolerance was suggested in terms of this research. Study cultivars were arranged in increasing order according to this factor: *Chimonanthus praecox*

(L.) Link→ *Cornus mas* L. → *Euonymus japonica* Thunb. → *Pittosporum heterophyllum* Franch. → *Viburnum tinus* L. → *Buxus sempervirens* L. → *Laurocerasus officinalis* M. Roem.

3. On open areas plants of all study cases are possible to use in landscaping on SCC. For shaded places *Viburnum tinus* L., *Buxus sempervirens* L. *Laurocerasus officinalis* M. Roem. are more preferable as the most shade-tolerant cultivars.

4. Illumination parameter is characterized with cyclicity. Its dynamics is typical for both, plants growing in the shade or on open garden areas. In the midday parameters take higher points comparing with morning and evening, while morning factors are lower than evening.

References

1. *Alekseyev V.A.* Svetovoy rezhym lesa. – L.:Nauka, 975. – 225 s.

2. Instruktsii po tekhnicheskoy inventarizatsii zelyonyh nasazhdeny v gorodah i posyolkah gorodskogo tipa Ukrainy / Utverzhdeno prikazom Gos.komiteta stroitelstva, arkhitektury i zhylishchnoy politiki Ukrainy ot 24 dekabrya 2001g. №226.

3. *Kazimirova R.N., Antyufeyeva V.V., Yevtushenko A.P.* Printsipy i metody agroekologicheskoy otsenki territorii dlya zelyonogo stroitelstva na Yuge Ukrainy. – K.: Agrarna nauka. – 2006. – 118 s.

4. *Kovalyov M.S., Plugatar Yu.V., Ilnitsky O.A., Korsakova S.P.* Dinamicheskaya model vodnogo rezhyma nekotoryh vidov kustarnikov nizhnego yarusa v usloviyah phytoklimata parkov YuBK // trudy Nikit.bot.sada. – 2014. – Tom 139. – S. 15-31.

5. *Kulikov G.V.* Vechnozelyoniye listvenniye derevya i kustarniki // Trudy Nikit.bot.sada. – 1971. – T.50. – S. 49-86.

6. Metodicheskiye ukazaniya po phenologicheskim nablyudeniyam nad derevyami i kustarnikami pri ih introduktsii na yuge SSSR / Sostaviteli: I.V. Golubyeva, R.V. Galushko, A.M. Kormilitsyn. Yalta: GNBS, 1977. 25 s.

7. *Sokolova T.A.* Dekorativnoye rasteniyevodstvo. Drevovodstvo: uchebnik dlya stud.vyssh.ucheb.zavedeny / T.A. Sokolova. – M.: Izdatelsky tsentr “Akademiya”, 2004. – 352 s.

The article was received at editors 18.12.2015.

Klymenko N.I., Paliy I.N. Lower layer ornamental plants of Arboretum in Nikita Botanical Gardens and their growth and status depending on illumination conditions. // Bull. of the State Nikit. Botan. Gard. – 2016. – № 118. – P. 45-52.

The article covers analysis results of annual shoot growth and status of ornamental plants belonging to seven cultivars growing in Arboretum lower layer of Nikita Botanical Gardens. Correlation of annual shoot growth and illumination, that was revealed in terms of the research, makes it possible to differentiate plant cultivars according to shade tolerance; this data permits to recommend them for cultivation in a certain region on South Coast of the Crimea allowing for its microclimatic characteristics.

Key words: *ornamental plants; shoot growth; shade tolerance; phytomonitoring.*

FLORA AND VEGETATION

UDK 582.542.11:502.753 (477.75)

RHYTHMOLOGICAL DIFFERENCES IN DEVELOPMENT OF *LAGOSERIS CALLICEPHALA* AND *LAGOSERIS PURPUREA (ASTERACEAE)*.

Aleksandr Rostislavovich Nikiforov[1], Aleksandra Aleksandrovna Nikiforova[2]

Nikita Botanical Gardens – National Scientific Centre
298648, the Republic of Crimea, the city of Yalta, urb.vil.Nikita
nikiforov.a.r.01@mail.ru

[2]Tavrichesky Federal University,
295007, the Republic of Crimea, the city of Cimferopol, 4, academician Vernadsky av.
nik.a.815@mail.ru

Introduction

Investigating Crimean representatives of *Lagoseris* Bieb genus (*L. purpurea* L., *L callicephala* (Willd.) Boiss. and *L. robusta* Czer. (last cultivar is commonly treated as a hybrid *L. callicephala* and *L. purpurea*)), perennial herbaceous plants, their morphological peculiarities and geographical isolation of their small population were revealed [8-10]. Nevertheless, recently systematical originality of well-known cultivars is disputed: they are united into one taxon - *L. purpurea* or *Crepis purpurea* (Willd.) M. Bieb. [3, 11]. Actually, according to present descriptions of overground organs and rhizomes, morphostructures of Crimean cultivars of *Lagoseris* genus are virtually identical. Plants characterize organs, similar by structure: stem and root system, underground rhizome, thickened and short caudex, overground rosellate shoots with downy blue-gray deeply separated into lacinias leaves, prolonged flower-bearing stems with bracteosus pink and purple inflorescences-baskets, cypsela-fruits. [8-10].

Since 2005 we observe seasonal development of *L. callicephala* plants under different conditions *in situ* (Upper layer of the Mountain Crimea).: north-east edge of Nikita yaila (1200 m above the sea level), scree at the Shagan-Kaya rock (Gurzuf yaila, 1430 m above the sea level) and scree at the Eklizi-Burun rock (ChatyrDag, 1527 m above the sea level). Besides, since 2011 plants of this cultivar were cultivated *ex situ* 300 m above the sea level in the climate of South Crimea seaside zone.

In 2012 we obtained seeds of *L. purpurea,* its populations are located within inside mountain range and Piedmont Crimea (collections by S.Svirin on the scree slopes of the Belbek river canyon). After its plants introduction in 2013, we had annual registration of their principal phases start and finish in seasonal development: vegetation, blooming, fruit-bearing and dissemination, as well as peculiarities of shoot and flower-bearing stems morphogenesis.

Study object is season development of *L. callicephala* and *L. purpurea,* characteristics of vegetative shoots and flower-bearing stems development.

Study tasks:

- To reveal influence of thermal conditions on shoot morphogenesis process;
- To determine main rhythmological parameters, typical for seasonal development of *callicephala* and *L. purpurea*;
- To find out phenological dates, cycles and conditions of vegetative and generative shoots development of study cases.

Study purpose is to compare cyclicity in development of vegetative and generative sphere of *L. callicephala* and *L. purpurea* plant shoots.

Phenological observations were conducted according to V.N.Golubyev method [1]. Annually growing conditions were recorded, that were typical for seasonal periods of growth, resting and dying of shoots and leaves, at the same time order of generative germs setting and conditions of flower-bearing stems development were specified.

Results and discussion

Until recently phases of seasonal development of *L. callicephala* and *L. purpurea* were recorded *in situ* only. In this way blooming terms of *L. callicephala* were determined since June till July, *L. purpurea* – since May till June [2, 8, 10], while for common taxon *L. purpurea* blooming period was fixed from June till July [11].

During long-term observations of *L. callicephala* seasonal development *in situ* it was found out that seed germination and phase of revegetation concerns the period of stable growth of daily average air temperature from +6°C till +8°C and higher (in April). Shoots that renew spring growth, have been developed since last spring till the beginning of frost period (end of November). At the same time in late summer plants have autumn generation of shoots with undeveloped leave plates. As soon as frost period begins all green leaves die. Apical and accessory bud at died leaves, buds of caudex and root winter having deep dormant season. Next season shoots of three generations start vegetation: two survived after winter – late-spring-autumn and late-summer-autumn, as well as not-wintered spring generation of shoots of buds, bursted during current spring. Shoots of wintered late-spring-autumn generation continue vegetative development up to the end of May. Increasing daily average air temperature till +11 - 12°C we have setting of generative germs. Then, if daily average air temperature increases (middle of June, +14°C), plants prolong flower-bearing stems. Blooming and fruit-bearing are connected with thermal optimum of yaila climate – the end of July, August (table 1). After short-term dessimination (using anemochory) in August, flower-bearing stems die.

Table 1

Phenological dates and duration of the principal phases in ***L. callicephala*** **development** ***in situ***

Observation year	Vegetation beginning and finish	Blooming beginning and finish	Fruit-bearing beginning and finish
2012	15.04 – 16.11	14.06 – 1.08	22.07 – 27.08
2013	16.04 – 15.11	15.06 – 30.07	24.07 – 25.08
2014	15.04 – 15.11	15.06 – 1.08	24.07 – 25.08
2015	18.04 – 14.11	15.06 – 1.08	25.07 – 25.08
Avearge phenodate	17.04 – 15. 11	15.06 – 1.08	24. 07 – 25. 08

Ex situ L. callicephala plants develop in another way. Seed germination starts since winter thaws (January-Feebruary), though germination maximum occurs in the end of March – beginning of April (+7-8 °C). During the first year of biocycle main shoot and first rossete laterals develop. Simultaneously two generations develop: main shoot form spring-summer-autumn growth, while laterals – summer-autumn growth. By autumn one more generation of shoots and leaves has been formed. During cool season three generations of shoots keep leaves green, though majority of spring-summer leaves dies gradually. New leaves develop if daily average air temperature increases up to +5 °C and higher (the end of February – beginning of March): this time survived buds burst, and form shoots of spring generation. In crown bud of two-years main shoot having daily average air temperature +11 °C generative germs happen: plant begins the phase of generative development. Flower-bearing stem becomes longer if daily average air temperature gets 14 °C: in this point flower buds develop, flower baskets open (the end of April, beginning of May). Blooming period continues till the middle of June. Fruit-bearing occurs in June-July (table2). After dissemination flowerbearing

stem dies. Vegetative rossete laterals (spring-summer-autumn, autumn-spring-summer-autumn) enter generative phase of development having certain thermal conditions next year of biocycle.

Table 2

Phenological dates and duration of main phase in *L. callicephala ex situ*

Observation year	Beginning and finish of growth	Beginning and finish of blooming phase	Beginning and finish of fruit-bearing phase
2012	2.03 – 26.11	24.04 – 15.06	20.06 – 17.07
2013	1.03 – 25.11	25.04 – 17.06	22.06 – 15.07
2014	26.02 – 25.11	25.04 – 17.06	22.06 – 15.07
2015	1.03 – 24.11	25.04 – 15.06	22.06 – 15.07
Average phenodate	1.03 – 25. 11	25.04 – 16.06	21.06 – 16. 07

In this way *in situ L. callicephala* develops as a summer-green plant, while *ex situ* – summer-winter green. Difference in duration of development cycle of monaxonic monopodial shoot and beginning date of generative phase *in situ* and *ex situ* is grounded by considerable difference in vegetative sphere development term of expected generative shoot: *ex situ* vegetative sphere occupies almost all year round, but *in situ* – during frost-free season (from April to October) and revegetates next April. General regularity of *L. callicephala* shoot development under different conditions is transition into generative phase happens after wintering only and after shoot vegetative sphere is developed that takes two years or more. That's why juvenile plants of *L. callicephala* never enter generative phase of development during the first year of their biocycle.

According to vegetative rhythm *L. purpurea* is characterized as a summer-winter green plant [2]. It was found out that seed germination *ex situ* connects with daily average air temperature +7-11 °C (March, April). Plants develop synchronously as daily average air temperature degree increases, passing virginal phases of onthogenesis. In the beginning of June having temperature +17+18 °C main shoot begins generative development – generative germs are set. In case of daily average air temperature +19+20°C and higher (June) the plant launches blooming. Synchronically with flower-bearing stem, system of rossete laterals, sourced from accessory bud, begins to develop. Under thermal conditions mentioned above, laterals produce flower-bearing stems. Blooming continues up to mid-autumn due to constantly developing laterals with set generative germs. But if daily average air temperature decreases +15 °C below (middle of October) generative organs don`t set anymore: next laterals have only vegetative sphere that develops. As a result the most powerful plants form up to 30 or more flower-bearing stems during season.

Air temperature decrease in November till +10 °C or below slows down generative development of ready flower-bearing stems. Before wintering shoots with developed vegetative sphere function, flower-bearing stems keeps flower buds and inflorescences, though fruits aren`t able to ripen. Vegetative rosette shoots winter with green leaves of late summer-autumn generation and keep on their development next spring (the end of February, the beginning of March). Setting of generative germs depends upon thermal conditions of the beginning of April, while blooming starts in May (air temperature +12-14 °C).

Therefore, in comparison with *L. callicephala*, plants of *L. purpurea* have different way of flower-bearing stems development and occupies larger range of thermal conditions. As a result blooming phase of *L. purpurea* is much more continuous. It is casued by difference in rate of main and laterals vegetative sphere development, necessary for their transition into generative state. Vegetative development of *L. callicephala* shoot makes no less than two seasons, but blooming always depends upon daily average air temperature

increase after wintering. Cycle of *L. purpurea* shoot vegetative development also depends upon temperature regime, but having themal maximum durig late-spring-summer-mid-autumn period it is rather shortened. That's why if *L. callicephala* laterals, developed at axial flower-bearing stem, bear function of assimilation in spite of environmental conditions, *L. purpurea* laterals cycle completely during season mentioned above. At the same time a number of flower-bearing stems of *L. callicephala,* during season, depends upon conditions of vegetative shoots development, formed last season, for *L. purpurea* only thermal factor is important, that is mid-autumn decrease of air temperature.

It`s a well-know fact that plant rhythms are endogenous and possible to be controlled by microclimate, and association to its conditions is worked out during cultivar adaptation to environmental conditions [4-7]. So, rhythmological peculiarities of seasonal development become apparent as far as certain climatic conditions supply (and other external factors), which affect on realization of plant morphostructural potential. Shoot capacity of *L. purpurea* of complete cycling during frost-free season is a key character of this cultivar. Shoot morphogenesis of *L. callicephala* implements being effected by different rhythmological parameters.

Conclusions

1. Development of shoot generative sphere of *L. callicephala* and *L. purpurea* coinsides with period of daily average air temperature increase after wintering, but blooming – with seasonal thermal maximum.
2. In spite of thermal regime vegetative sphere development of *L. callicephala* occupies several seasons.
3. Shoot development of *L. purpurea* during summer transient: they cycle completely without break.
4. Rhythmological characteristics of *L. callicephala* and *L. purpurea* are specified genetically, that is determined by various bioecological endogenous characters of study cultivars.

The article was received at editors 05.11.2015

Nikiforov A.R., Nikiforova A.A. Rhythmological differences in development of *Lagoseris Callicephala* and *Lagoseris Purpurea (Asteraceae)*. // Bull. of the State Nikit. Botan. Gard. – 2016. – № 118. – P. 58-63.

For the first time *Lagoseris callicephala* and *Lagoseris purpurea* were investigated from the point of view of seasonal differences. It concerns either blooming and fruiting terms or development peculiarities of vegetative sphere of shoots, development of flower-bearing stems and correlation of generative phase with different thermal conditions.

Key words: *Mountain Crimea, Lagoseris callicephala; Lagoseris purpurea, seasonal development.*

UDK 581.144.1

LEAF STRUCTURE OF HERBACEOUS PLANTS, CULTIVARS OF APIOIDEAE DRUDE SUBFAMILY (APIACEAE LINDL.) AS STUDY CASES.

Sergey Yuryevich Naumov

Lugansk National Agricultural University,
91008, the city of Lugansk, LNAU, campus
mr_sun@ramler.ru

Introduction

Leaves are classified into simple and compound. If a leaf contains ony one plate, it is considered simple one. But another situation if one footstalk with common bottom has got several separate plates, sometimes even with own footstalk, this leaf is compound. Development of compound leaf resembles branching in one surface, that can get the second-third order; in this way leaves generate twice and thrice, many times and etc. (4, 11, 21, 28 and etc.). A. Takhtadzhyan notes that in comparison with compound leaf a simple is never divided into separate sharply marked segments, so-called leaflets [3]. Leaflets of typical compound leaves are supplied with joints, that is why such leaves don`t fall whole [16]. This characteristic of compound leaves is typical for arboreal and shruby plants, concerning herbaceous – only some families, among them *Fabaceae* Lindl, possess it [13, 16, 29]. But in some families of herbaceous plants there are leaves that difficult to classify for sure – simple or compound as they are not characterized with separate leaflet fall. Leaves of majority cultivars from *Apioideae* family are in this categoty, as even after total dying they hold on the stem. Publishing materials don`t express common opinion concerning their structure. Most authors tend to consider leaves of this subfamily specimens as simple with various degree of segmentation [14-17, 22], others don`t have any clear point of view and that` why moreoften contradict oneself [1], but there are specialists that consider them compound [10, 23, 26]. In this way purpose of our study was to identify type of leaves based on findings concerning some specimens of Apioideae subfamily.

Objects and methods of the research

Study objects were cultivars of *Apioideae Drude* subfamily. Seeds of *Levisticum officinale* Koch. From All-Russian Institute of plant growing named after N.I. Vavilov, seeds of some cultivated crops belonging to *Apium graveolens* L., *Daucus sativus* (Hoffm.) Roehl., *Foeniculum vulgare* Mill., *Petroselinum crispum* (Mill.) A.W. Hill. were bought. Wild-growing cultivars of *Bupleurum rotundifolium* L., *Conium maculatum* L., *Daucus carota* L. were yielded during botanical expeditions on south-east of Ukraine. On the territory of Nikita Botanica Gardens (NBG), the Crimea, *Bupleurum asperuloides* Heldr., *Bupleurum fruticosum* L. were yielded and investigated. Plants of *Bupleurum woronowii* Manden. were collected during expedition along Chatyr-Dag mountain.

Plant cultivation was conducted either in laboratory of photoculture, or under field conditions. All these plants were regularly observed during their biocycle. Morphological leaf peculiarities of cultivars from *Apiaceae* family and some other herbaceous plants were studied using green and herbarium material. Photography and computer technology were applied to get leaf pictures. But to illustrate this research the author used original photos, processed with Adobe Photoshope, Corel Draw.

Anatomical organization of footstalks was investigated using cross sections. Rosette leaves of virginal specimens were in use. Cross sections of 5-8 multiple repeatability were made in the middle of footstalk of developed leaves, as this very part has got the most constant structure [15]. Material was fixed in Karnua mixture. Serial microtome cross section 10-12 mkm were made applying agreeable method. Section color was created by Erich hematoxylin with tincturing of gentian violet or methylene blue [27]. Footstalk and petiolule structure was investigated applying microscope MBI-3, microphotos were successfully made by camera Olympus SP350 [9].

Results and discussions

Study and classification of any organs or organisms must be implemented if developed completely. It concerns leaves either.

Applying of morphological methods only can`t get definite answers in the field of leaf development study, classification into simple or compound, as on early development stages of simple divided and compound leaves their ontogenesis has similar characteristics due to cell proliferation of costal and intercostal tissue [11, 13, 23, 25, 26]. Recent years intensive investigations of model plants favored to identify certain number of genes, that control growth and development of plants. As a result we found out that compound leaves appear out of developing simple, first-class induction KNOTTED-like genes (KNOXI, as well as genes ARP, FLO, PHAN) [20, 21, 24]. These genes were revealed in *Solanum lycopersicum* L., and they are so active that cause higher division of compound leaves [24]. They were found within specimens of *Apioideae* either and in particular *Pimpinella anisum* L. and *Daucus carota* L. At the same time leaves of *Pimpinella anisum* are classified as simple, developed out of compound primordial; that fact points that other cultivars of *Pimpinella* genus like majority of *Apiaceae* specimens, have got highly divided leaves. That is authors suppose that simple leaves of *Pimpinella anisum* have got repeated origin in spite of compound primordial [19]. Perhaps, they didn`t allow for fact of heterophilia what is typical for anisum and other subspecies of *Apioideae* family. Before we determined that during ontogenesis of *Pimpinella anisum* simple leaves appear first that turn into odd-pinnate compound leaves with 9 leaflets [12].

Different methods (physiognomic, structural, rhythmological, anatomic) in analysis of leaf structure typical for representatives of *Apiaceae* family revealed that study cultivars, belonging to Apioideae subfamily have got majority of leaves, developed in terms of ontogenesis, possess all morphological characteristics of compound leaves. *Bupleurum* genus is exclusion, as leaf ontogenesis is an open case study because this taxon of species has got leaves with both venation netted and parallel. Leaves of most *Apioideae* cultivars bear more than two leaf plates, fixed to rhachis by pronounced petiolule that helps leaflets locate in different surfaces towards light. Rhachises as petiolutes of lateral leaves keep typical structure inherent in footstalk of different cultivars – some of them are rounded others – chanelled. But none of them has got stripes of leaf plates, that`s why we cannot call lateral leaflets as segments of simple leaf. Besides leaf footstalks, petiolules and rhachises of study cases from *Apioideae* family are composed of three types of tissue: integumentary, main and connective; connecting bundles are separated from each other by layers of the main parenchyma (like initial stem structure of some herbaceous plants [18]); notable for radial symmetry, they can be considered as axial structures.

Majority of study cases, as mentioned before, compound leaves development resembles branching, beginning with separation in footstalk and rhachis of connecting bundles and approaching the second-forth order; in this way morphological structure of branching nodes is similar to branching nodes of leaves, obviously compound, branching nodes of tricompound leaves *Melilotus albus* Medik.

In general petioputes have the same structure as footstalks. In particular, *Conium maculatum* has got large petiolules of the first and second orders with the central space what is non-typical for either leaf plate or its central vein, but characterizes petioputes. Space in rhachis and peoputes becomes smaller and turns into white friable parenchymal tissue as reaching the leaf top. Such characteristics of node, footstalks, rhachises and petiolules structures were marked almost for all study case from *Apioideae* subfamily [5, 7, 8].

Representatives of Apioideae subfamily quite often have such a phenomena as dying of leaflets on compound leaves without any damage signs. Leaflets didn`t fall, held on till the very leaf dying. It was fixed for *Apium graveolens, Conium maculatum, Daucus carota, Peucedanum ruthenicum* and others.

Non-allowing for data mentioned before we can suppose if necessary characteristic of compound leaves is their division into leaflets with petiolules, the problem could be solved due to comparison of petioputes and footstalks structure. That is, if a petiolules is cylindrical and its structure resembles footstalk structure, we deal with a compound leaf. Anatomical researches revealed leaflet structure of compound leaves is similar to the first simple leaves in terms of the same cultivar. In particular, petiolules of leaves keep the structure, typical for footstalks, but some cultivars, such as *Apium graveolens* have got even more complicated structure in comparison with footstalk of the first true leaf [fig.1). Comparing with footstalk of the first true leaf (fig.1D) petiopte of lateral leaflet includes five close collateral bundles, three of them are large and two located on ledge of the channel – small (fig.1E). Besides in petiolule opposite connecting bundles there are bands of angular collenchyme, what is non-typical for footstalk. Petiolule isn`t a part of a segment or a leaf plate.

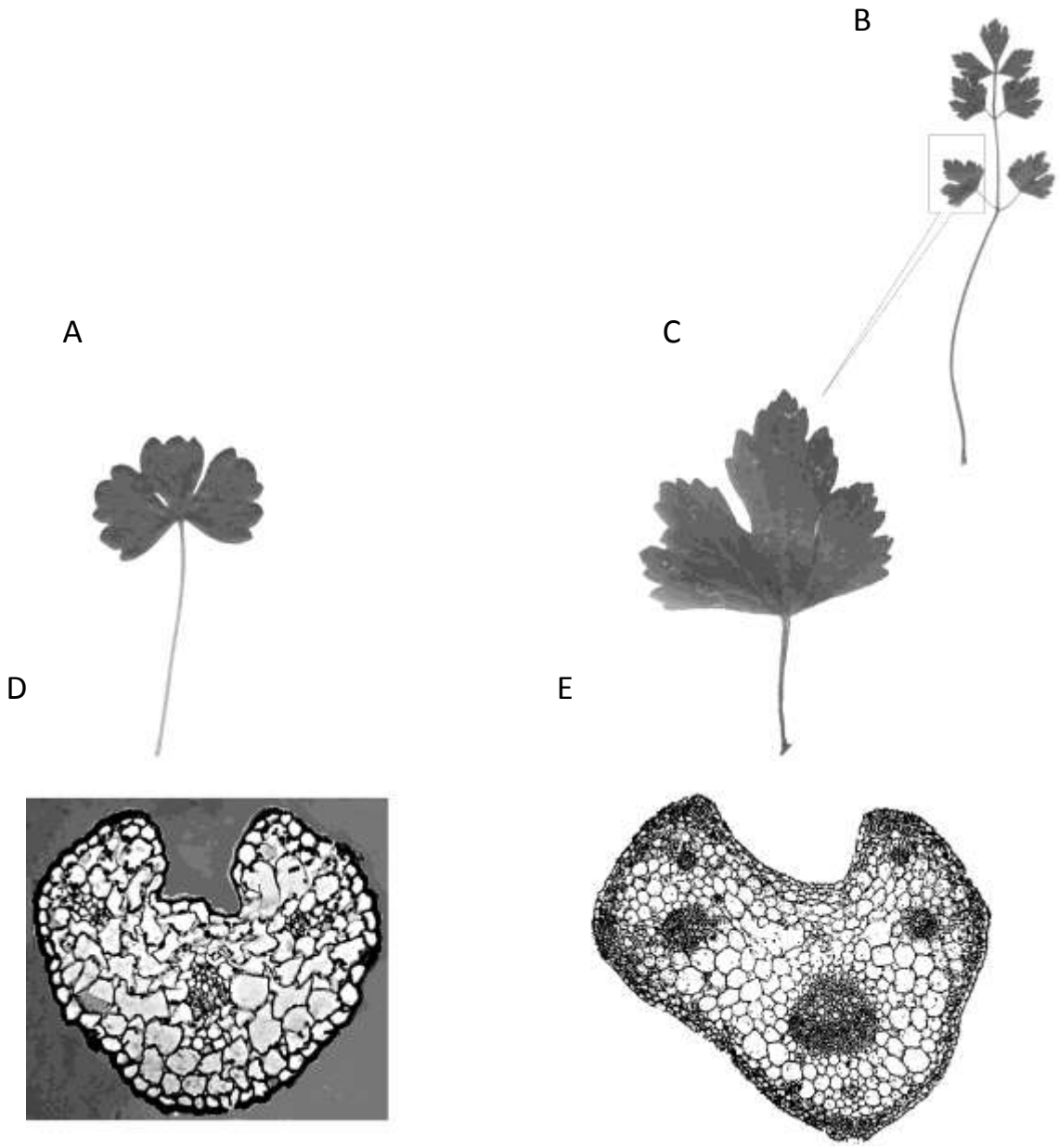


Fig.1 The first true leaf (A), odd-pinnate compound leaf (B) and cut off lateral leaflet (C) *Apium graveolens:* D – footstalk section of the first true leaf (hematoxylin, 20x7); E – petiopute section of lateral leaflet (hematoxylin, 10x10)

The first true leaf of *Petroselinum crispum* is simple, divided into three segments (Fig.2A). The footstalk is long, channeled almost triangular in cross section, covered with one layer of epidermis and two layers of chlorenchyma (fig.2D). Majority of footstalk is filled with oval cells of the main parenchyma which contains three connecting bundles. Tricompound leaf develops after that (*P. Crispum) (*Fig.2 B). Petiolele of the lower pair of lateral leaflets has similarities with structure of the first true leaf footstalk. Difference is that in petiolule opposite connecting bundles under epidermis cells and in ledges of channel there are bands of collenchyme, what is non-typical for footstalks of the first leaves (Fig. 2 E). Besides linear size of footstalks is more than petiolule`s. Such insignificant differences in structure of simple leaves footstalks and compound leaves petiolules in terms of the same cultivars from *Apioideae* subfamily are typical for other study cases as well: *Daucus sativus, D. carota, Foeniculum vulgare* and etc. [5-8].

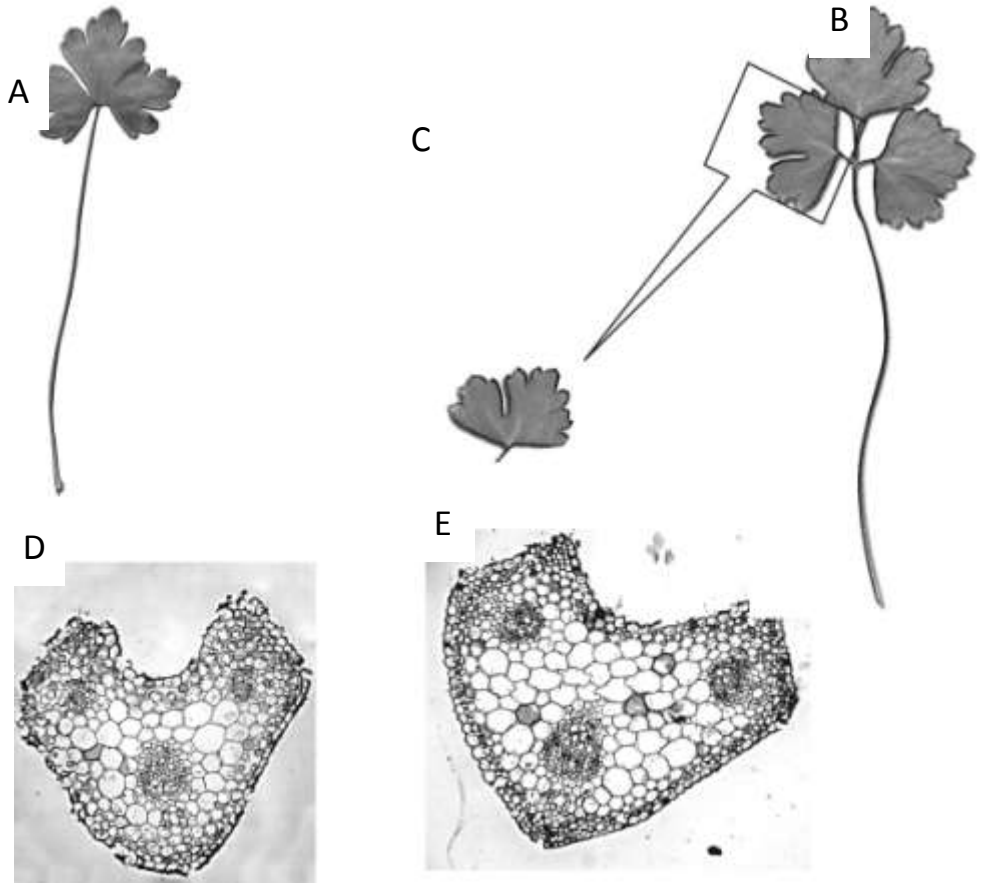


Fig.2 The first true leaf (A), tricompound leaf (B) and cut-off lateral leaflet (C) *Petroselinum crispum:* D – section of the first true leaf footstalk (hematoxylin, 8x7); E – section of lateral leaflet petiopute (hematoxylin, 8x7)

There are two hypotheses, capable to explain homology of simple and compound leaves. The first one was suggested by Sattler R. and Rutishauser R., it equalizes separate leaflets of the compound leaf with simple leaves. The second hypothesis, suggested by Kaplan D., supposes the compound leaf as a simple leaf equivalent [20]. Study findings prove the first hypothesis, as according to anatomic structure leaflets of compound leaf are similar with simple leaves (the same plant), but in mostly they have more complicated structure [5-9].

Conclusions

1. Study leaves of *Apioideae* subfamily are rather compound due to all characteristics revealed in terms of the research. Typical joint in nodes, that allow leaflets of compound leaf to fall, weren`t found. We consider term "joint" for compound leaves isn`t correct, as during setting and development of compound leaves, processes of part joint don`t happen. It`s a well-know fact, that compound leaf develops as a single unit [11, 13, 21, 23, 25, 26 and etc], as it was mentioned before branching of connecting bundles with accompanying elements of strengthening tissue happens in nodes.

2. Based on study results, we suppose that leaves of *Apioideae* specimens consisting of some separate leaf plates should be believed as compound, in spite of type of their falling: either separate falling happens or a leaf dies entirely. In this way, Linney K. [4] classified simple and compound leaves using degree of their disjoint as it is.

Gratitudes

The author is thankful to senior scientist of Botanical Institute named after V.Komarov (St.-Petersburg, Russia), Doctor Oskolsky A.A. for useful consultations and advice discussing the article.

References

1. Botanichesky atlas / Pod obshch.red. B.K. Shyshkina. – M.-L.: Selkhozizdat, 1963. – 504 s.

2. *Gendels T.V.* Osobennosti morphogeneza lista dvudolnyh // botan.zhurnal. – 1988. – T.73, № 11. – S. 1554-1559.

3. Zhyzn rasteny v 6-ti tt. / Pod obshchey red.akad. a.L. Takhtadzhyana. – M.: Prosveshcheniye, 1980. – T. 5. Ch.1. – 430 s.

4. *Linney K.* Philosophiya botaniki. M.: Nauka, 1989. – 456 s.

5. *Naumov S.Yu.* Anatomichni doslidzhnnya lystkiv *Petroselinum crispum* (Mill.) A.W.Hill. // Ukr.botan.zhurn. – 2007. – T. 64, № 6. – S. 901-904.

6. *Naumov S.Yu.* Formoobrazovaniye listyev *Daucus carota* L. v techeniye ontogeneza // Zbirnyk naukovyh prats LNAU. – Lugansk: "Elton-2", 2007a. - № 75 (98). – S. 87-93.

7. *Naumov S.Yu., Bril O.A.* Anatomicheskiye issledovaniya vaskulyarnoy sistemy listyev *Daucus carota* L. // Zbirnyk naukovyh prats Luganskogo NAU. – 2007. - №79 (102). – S. 32-40.

8. *Naumov S.Yu., Zlobina A.A.* Izmeneniye vaskulyarnoy sistemy listyev prikornevoy rozetki *Foeniculum vulgare* Mill. // Zbirnyk naukovyh prats Luganskogo NAU. – 2008. - № 83. – S. 73-80.

9. *Naumov S.Yu., Sokolov I.D.* Dosvid vykorystannya tsyfrovyh prystroyiv u biologii // Naukovy vistnyk Volynskogo derzhavnogo universytetu im.L.Ukrayinky. – 2004. - №4. – S. 76-79.

10. *Satsyperova I.F.* Borshcheviki flory SSSR – noviye kormoviye rasteniya. – L.: Nauka, 1984. – 223 s.

11. *Serebryakov I.G.* Morphologiya vegetativnyh organov vysshyh rasteny. – M.: Gos. Izd-vo "Sov.nauka", 1952. – 391 s.

12. *Sokolov I.D., Naumov S.Yu., Minyayeva I.V., Shelikhov P.V.* Formoobrazovaniye listyev v ontogeneze anisa (*Anisum vulgare* Gaertn.) // Visnyk Donetskogo universitetu. Seriya A. Prirodnychi nauky, 2001. – № 2. – S. 294-297.

13. *Ten A.G.* Rost lista bobovyh rasteny v ontogeneze // Voprosy morphologii, rosta i razvitiya rasteny: Trudy Tselinogradskogo SKhI. – 1973. – T.9, vyp.3. – S. 25-49.

14. *Tikhomirov V.N.* Proiskhozhdeniye, evolutsiya i sistema semeistva zontichnyh (Umbelliferae Juss. – Apiaceae Lindl.): Avtoref.dis.dokt.biol.nauk / MGU im. M. Lomonosova. – M., 1977. – 39 s.

15. *Tikhomirov V.N., Yanitskaya T.O., Pronkina G.A.* Zontichniye Sredney Rossii. Opredelitel po vegetativnym priznakam. – M.: Argus, 1996. – 88 s.

16. *Fyodorov Al.A., Kirpichnikov M.E., Artyushenko Z.T.* Atlas po opisatelnoy morphologii vysshyh rasteny: List. – M.-L.: Izd-vo AN SSSR, 1956. – 302 s.

17. Flora SSSR / red. B.K. Shyshkina. – M.-L.: izd-vo AN SSSR, 1950. – T. XVI. – 648 s.

18. *Ezau K.* Anatomiya semennyh rasteny. – M.: "Myr", 1980. – 558 s.

19. *Bharathan G., Goliber T.E., More C., Kessler Sh., Pham T., Sinha N.R.* Homologies in leaf form inferred from *KNOX1* gene expression during development // Science, 2002. – Vol. 296, N 5574. – P. 1858-1860.

20. *Champagne C., Sinha N.* Compound leaves: equal to the sum of their parts? // Development, 2004. – Vol.131. – P. 4401-4412.

21. *Cronk Q.* The Molecular Organography of Plants.-Oxford: University Press, 2009.-259 p.

22. *De Candolle Aug. P.* Prodromus systematis naturalis regni vegetabilis. – Paris, 1830. – V. 4. – 683 p.

23. *Eberwein R.K.* Bau und ontogenese unkonventioneller Blätter des typs 'unifaziale Phyllome' und deren beitrag zur theorie des spermatophytenblattes: Dis. Doktors der Natur. / Rheinisch-Westfälischen Technischen Hochschule Aachen. Österreich, 1995. – 77 p.

24. *Eckardt* N.A. Evolution of compound leaf development in Legumes: Evidence for overlapping roles of *KNOX1* and *FLO/LFY* genes // Plant cell. – 2007. – Vol.19. – P. 3315-3316.

25. *Gerrath J.M.,* Lacroix, C.R. Heteroblastic sequence and leaf development in *Leea guineensis* // Int. J. Plant Sci., 1997. – Vol. 158 (6). – P. 747-756.

26. *Guedes M.* Leaf morphology in the Umbelliferae: Rachis, unifaciality, stipels and pinna insertion // Phyton. 1984. – Vol. 24. – P. 257-272.

27. *Lillie R.D.* Histopatologic technic and practical histochemistry. New York: 1965. 645 p.

28. *Meyen S.V.* Plant morphology in its nomothetical aspects // Bot. Rev. 1973. – Vol. 39, № 3. – P. 205-260.

29. *Takhtajan A.L.* Diversity and Classification of Flowering Plants. New York: Columbia University Press, 1997. – 643 p.

The article was received at editors 23.12.2015

Naumov S.Yu. Leaf structure of herbaceous plants, cultivars of Apioideae Drude subfamily (Apiaceae Lindl.) as study cases. // Bull. of the State Nikit. Botan. Gard. – 2016. – № 118. – P. 57-63.

In the course of comparative morphological and anatomical researches of leaf ontogeny mechanisms within a number of cultivars belonging to Apioideae subfamily, it was revealed their leaves are truly compound.

Key words: *Apioideae; simple leaf; compound leaf; petiole; petiolule.*

UDK 581.522.68

INTRODUCTION OF *EREMURUS ROBUSTUS* (REGEL) REGEL IN LUGANSK REGION

Sergey Yuryevich Naumov[1], Lyudmila Ivanovna Sigidinenko[2]

Lugansk National Agricultural University
91008, the city of Lugansk, Agricultural University
sergey.naumov@mail.ru

Introduction

Eremurus robustus is a contractive endemic in Tian Shan and Pamiro Altay with breaking-up area [6, 8]. In this way as far as anthropogenic changes of natural flora intensify preservation of endemic cultivars becomes the urgent problem that should involve all possible methods for solution. At the same time high demand for ornamental plants, *Eremurus robustus*, requires not only increasing of planting material but significant assortment gain of exotic and rare plants in that region. *Eremurus robustus* was cultivated in 5 botanical gardens of USSR, Donetsk Botanical Garden (DBG) possessed a huge *Eremurus robustus* collection in 80s of the last century [4]. But today only one cultivar of *Eremurus robustus* is cultivated in DBG, its population was found out in Lugansk region either [3]. That`s why renewal of *Eremurus robustus* collection in Donetsk region is of great importance for its further use in landscape of Donbass cities.

The study purpose was to identify peculiarities of plant development under conditions of Lugansk city and obtaining of viable seeds.

Objects and methods of the research

3 rhizomes of study object (*Eremurus robustus)* were presented by Nikita Botanical Gardens (NBG) in 2012. In autumn 2012 they were planted in different districts of Lugansk city and its outskirts on private areas of scientists from Biology Department in Lugansk Agricultural University. Unfortunately, only two specimens wre observed, as the third one grew in combat zone.

Phenological observations were conducted according to agreeable research methods in botanical gardens [2]. At the same time they allowed for main phenological phases of plant development: draws after winter dormant season, leaves of root system, development of the floral shoot, flower budding, beginning and finishing of blooming, beginning of fruit ripening, complete fruit ripening, dying of overground vegetative and generative organs. All development phases were fixed applying camera Nikon D40. These photos are posted on-line “Plantarium” (Electronic plant determinant on-line) [10]. Measurements were made by devices tested metrically.

Results and discussions

In the middle of March 2015, vegetation beginning was fixed with hibernating bud of renewal on the soil surface (fig.1a). It should be noted that leaves presented intensive growth and by the end of April root rosette was developed completely and made 25 thick and juicy leaves, which almost got their maximum length. Nearly at ones in spite of enough amount of moisture, leaves tops started to dry, but their growth at the bottom didn`t stop. Developed leaves are wide-linear with carina. External leaves at the bottom reached 6-8 sm wide and 50-60 sm long, soft and smooth (fig.1b).

a  b 

Fig .1 Renewal bud (a) and root rosette of leaves (b) of *E. robustus*

In the beginning of the second decade of April germ of inflorescence occurred between leaves, and by May the 1st it developed to root rosette above leaves. Flower-bearing stem of study cases isn`t branching, bare, cilindric 2-2,5 sm across diameter in the middle, green-colored. It is characterized by intensive development, its acropetal growth keeps on even during blooming and possible to reach 1 m 62 sm (the first study specimen) and 2 m 15 sm (the second one).

On the opening stage of development inflorescence seems downy due to its well-developed bracts - awl-shaped leaf-lets 2 sm long. Bracts membranous with one vein are widened at the bottom and densely covered with trichomes. As far as inflorescence develops flower buds advance bracts and they become not so visible.

Inflorescence is a coma cluster, develops acropetally (fig. 2a). Budding stage begins in the end of the first May decade. The first flower opening was fixed on May the 13th (fig. 2b). Flower buds are characterized by more intensive pink color, while flowers are white with hardly notable pink touch. Floral envelope has six segments and two circles. There are 6 stamens, baculum and stigma are filamentous. Pedicle is long and exceeds even flower size, during blooming it locates a flower almost at the angle of 90° towards inflorescence axis. In general blooming period takes no more than two weeks. Well-developed inflorescence of much stronger specimen gets 100 sm long, the second specimen – abut 60 sm. On the first plant there were 615 flowers, the second – 235. In this way inflorescence density of the first case makes 6 flowers/sm, while the second one – about 4 flowers/sm.

Fig.2 Developing inflorescence on the budding stage (a), flower and flower bud (b) in the bottom of inflorescence of *E. robustus*

During blooming in the bottom of inflorescence fruits were set in acropetal order, making global-shaped densed box with three channels being green at first and as fas as ripening reached light-brown color (fig.3a). First fruits were fixed on May the 23rd. Well-developed fruits got 2 sm across diameter. In the end of blooming a number of withered fruits were calculated. Stronger specimen of *Eremurus robustus* had 89 fruitcases, the second one – 59. Setting rate for the first case made 14,5%, the second one – 25,1%.

By the third decade of June vegetative organs of plant started to die and by the beginning of July dried entirely. At the same time fruitcases started bursting and sowing ready seeds. On average each fruitcase had 7 seeds. Seeds were three-edged with a small scarious wing (fig 3b).

Fig 3. Ripenng fruitcases (a) and ripe seed (b) of *E. robustus*

In conclusion it should be noted that in natural and climatic conditions of Lugansk region *E. Robustus* is a typical ephemeroid that gets over all phases of life cycle. At the same time being *a* flowering plant characterized by high ornamental qualities permits to

recommend it for landscaping of Donbass cities. Investigation of biological peculiarities and capacity to propagate by seed will be continued.

Conclusions

1. Advance study of *Eremurus robustus* growth and development revealed that the plant successfully goes over all stages of life cycle forming viable seeds.
2. *Eremurus robustus* is a typical ephemeroid under weather conditions of Lugansk region.
3. *Eremurus robustus* is characterized by high ornamental qualities and can be recommended for cultivation within Donbass region.

References

1. *Yena A.V.* Prirodnaya flora Krymskogo poluostrova: monographiya. – Simferopol: N.Orianda, 2012. – 232 s.
2. Metodika phenologicheskih nablyudeny v botanicheskih sadah SSSR. – M.: Gl.botan.sad AN SSSR, 1975. – 27 s.
3. Pryrodno-zapovidny fond Luganskoyi oblasti // O.A. Arapov, T.V. Sova, O.A. Savenko, V.V. Ferents ta inshi. Dovidnyk. – 30ye vyd., dop. i pererob. – Lugansk: Tov. "Virtualna realnist", 2013. – 224 s.
4. Redkiye i ischezayushchiye vidy prirodnoy flory SSSR, kultiviruyemiye v botanicheskih sadah i drugih introduktsionnyh tsentrah strany. – M.: Izd-vo "Nauka", 1983-304 s.
5. Skvortsov A.K. Flora Nizhnego Povolzhya. – M.: T-vo nauchnyh izdany KMK, 2006. – T.1. – 435 s.
6. Flora SSSR. V 30 t. / Gl.red. i red. toma V.L. Komarov. – M.-L.: Izd-vo AN SSSR, 1935. – T.4. – S. 37-52.
7. Khokhryakov A.P. Eremurusy i ih kultura. – M.: Izd-vo "Nauka", 1965. – 128 s.
8. 8. *Eremurus robustus* Regel – http://redbooksam/rastenija/33-eremurus-robustus-regel.html. - Searched on 22 july 2015.
9. 9. *Mosyakin, S.L.; Fedoronchuk, M.M.* Vascular plants of Ukraine. A Nomenclatural checklist. – Kiev: M.G. Kholodny Institute of Botany, 1999. – 346 p.
10. 10. Plantarium (определитель растений on-line) – http://www.plantarium.ru/page/view/item/14934.html. - Searched on 22 july 2015.

The article was received at editors 22.12.2015

Naumov S.Yu., Sigidinenko L.I. Introduction *of Eremurus robustus* (Regel) Regel in Lugansk. // Bull. Nikit. Botan. Gard. – 2016. – № 118. – P. 64-67.

Introduction of *Eremurus robustuS* (Regel) Regel plants was successfully carried out. Planting stock was provided by employees of Nikita Botanical Gardens. Three years later under conditions of Lugansk city plants started blooming, formed fruits and viable seeds.

Key words: *Eremurus robustus, introduction, growth and development rhythms.*

PLANT PROTECTION

UDK 633.812:632:631.234

PHYTOSANITARY STATE RATING OF LAVANDIN INDOORS

**Yelena Borisovna Balykina, Olga Vladimirovna Ivanova,
Valery Dmitriyevich Rabotyagov, Yury Sergeyevich Khokhlov**

Nikita Botanical Gardens – National Scientific Centre, the city of Yalta
298648, the Republic of Crimea, the city of Yalta, urb.village Nikita
vip.polskaya@mail.ru

Intrpduction

The main regions of lavender cultivation are England, France, Italy, Spain, Bulgary, the Crimea and Krasnodarsky krai. Essential oil (EO) contained in its new inflorescences (1,2-2,3%) makes lavender especially valuable (main components: linalool and linalilacetate). Principal EO components are widely used in perfume and food industry, medicine [1,3]. Lavender is an evergreen plant, light- and warm-requiring, draught-resistant; mature plant can resist even hard frosts (-25°C). Poor soils of light and middle granulometric composition are quite favorable for lavender, while heavy soils with groundwater occurance near the surface aren`t quite proper [13].

Propagation method of lavender is cutting, plantings are setting in autumn. Lavender is being operating for 20 years starting with the second year of vegetation. Each 6-7 years plantations are renewed: in spring bushes are cut 3-5 sm high. Yielding happens during mass blooming [12].

Lavender is originated from Mediterranean [10]. And over all cultivation regions, *Lavandula angustifolia* Mill. is mainly cultivated. *Lavandula latifolia* Medic. is not so widespread and can be found only in collection plantings of botanical gardens and other research establishments. Both cultivars are parent forms of lavandin, though have sharp differences in morphological and biochemical peculiarities [10]. *Lavandula angustifolia* Mill. contains high-quality essential oil with 40-50% of esters and 40% of unsaturated terpenic alcohols [7, 8]. *Lavandula latifolia* Medic. is more drought-resistant and longevous in comparison with *Lavandula angustifolia* Mill., but not so frost-resistant (stands frosts till -16°C).

Scientists of “Aroma and medicinal plants” laboratory in Nikita Botanical Gardens successfully bred sorts of *Lavandula angustifolia* Mill. “Record” and “Prima”, intensively used for lavandin production. They are characterized by high winter-resistance, efficiency and high-oil concentration. Its oil rating in perfume industry reaches 4,5 points [10].

Lavandin is an interspecific hybrid of lavender, well-known even since XVIII century [7, 9]. It is one of perspective oil-bearing plants, as best lavandin clones exceed lavender 1,5-2 times in crop capacity and essential oil content, while EO yielding per hectare – 4 time [12]. Essential oil, the main cause of lavandin cultivation, is appreciated on the world market, its demand increases year by year [8, 10]. Lavandin EO possesses fresh herbaceous and tarry touch, lower but intensive flavor in comparison with lavender fragrance. It is commonly used in perfume industry and medicine [3, 9].

As a result of experimental researches scientists of “Aromatic and medicine plants” laboratory bred interspecific hybrids of lavender with various number of initial cultivars genomes and in different combinations [10, 11]. Study research was to identify biocomplex

of diseases and pests effecting this crop and comparison of damage level on lavender and lavandin study specimens, cultivated indoors.

Objects and methods of the research

Researches were carried out in "Enthomology and phytopathology" laboratory of NBG-NSC during spring 2015. Study objects were sorts of *Lavandula angustifolia* Mill. (Record, Prima, Belyanka) and lavandin specimens with different number of genomes in initial cultivars ("Aromatic and medicine plants" laboratory) with a range of damages, planted in the greenhouse.

Analysis of damage level was based on "Linear rate" of observing the cut specimens in the greenhouse what is the determine factor for protective steps [5].

Extraction of phytopathogens isolates was conducted out of root zone, segment of root collar, stem shoots and leaves. Method of "Accretion" was applied to analyse infected inside tissue: material pieces of study crops, 2 mm, were being sterilized in concentrated solution $KMnO_4$ or in alcohol solution for 10-15 sec. After washing in sterile distilled water isolates were put on the surface of Ka and Czapek's mediums in Petri cups. Bacterium colonies or myceliums developed around objects were sifted out onto uninfected plants for further investigation [2].

Shtemerding method was applied to reveal active stage of endoparasitic nematodes, created galls on lavandin roots. It makes possible to intensify nematode output getting macerative tissue out of infected plant part. This suspension is carefully filtered, sediment is shifted into funnal or Oostenbrink`s cup [5]. Output of active nematodes is over in 2-3 days.

Results and discussion

Study analysis revealed that recessive form of *Lavandula angustifolia* Mill., sort "Belyanka" is the least infective disease- and pest-resistant (damage level – 75%). Sort "Record" presented not so high damage level – 40%, allohaploid (lavandin Temp) – 23%, quite resistant was allotriploid with two genomes of *Lavandula angustifolia* Mill. and one genome of *Lavandula latifolia* Medic. – 11%. Amphidiploid was found the most damage-resistant; it includes two genomes of *Lavandula angustifolia* Mill. and two genomes of *Lavandula latifolia* Medic. (damage level – 5%) (table 1).

Table 1

Disease damage leel of lavandin specimens indoors

№	*Specimen*	*Form*	*Cut*		*Dying out, %*
			Total amount	Damaged	
1	Belyanka	*sort*	115	86	75
2	Record	*sort*	107	43	40
3	Lavandin Temp	*allohaploid*	93	22	23,7
4	Lavandin	*allotriploid*	65	7	11
5	Lavandin	*amphidiploid*	84	4	**5**

Disease signs were found on all tissue types of dying out plants. Stem was drying up, pale brown with gray coating. Down leaves started intensive fading. Small zone of green viable shoots was fixed on the top of the bush what concerns ascending development of disease (fig.1).

Fig.1 Lavandin Temp bush with damage signs

Root collar had some symptoms of wet rot. Applying section of root zone revealed larvas of Phytometra-gumma (fig.2).

Fig.2 Place where larva of Phytometra gumma was found

In the center of root on its fibrils there were a huge number of nodes – galls (fig.4). High concentration of gall nematode (*Meloidogyne spp.)* was revealed in terms of laboratorial analysis.

Fig.4 Lavandin root with numerous galls, generated by nematodes

Colonies of phytopathogenic bacterium (*Erwinia sp*. genus) which cause wet rot of plants were sampled from the zone of root collar [2]. It`s a well-known fact that nematodes often come as bacterium carriers which can provoke tissue maceration. At the same time phytopathogenic fangus - *Cephalosporium sclerotigenum* F.et R.Moreau – was found in stem (fig.5),that is a stem fading agent of industrial crops [6]. This agent causes damages of xylem waterworks with further dying of plants.

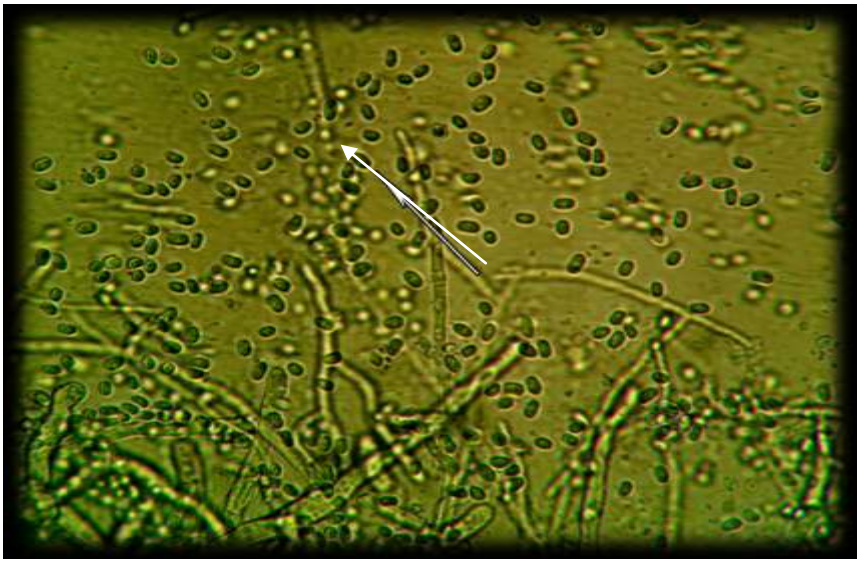


Fig.5 *Cephalosporium sclerotigenum* – fading stem agent. The arrow points fungus spores (conidia)

Fungi of *Alternaria* sp. genus were sampled from fading leaves and sowed. They cause botch and fading on many plants [4] and as a result considerably intensify infection load of damaged crops (Fig.6).

Fig. 6 *Alternaria* sp. – agent of blotch and leaf fading on lavandin. The arrow points fungi spores (conidia)

Conclusions

Interspecific lavender hybrids – lavandins, besides a number of advantages in comparison with parent forms are more resistant to disease caused by pests and phytopathogenic agents. The best results of immune level obviously belong to forms with bigger number of chromosomes [10]. Amphidiploid of lavandin having (2n=4x=AALL=96 chromosomes) 5% of damaged plant organs it is characterized with fertility. Allotriploid (2n=3x =AAL=72 chromosomes) – 11% of damaged plant organs, hybrids are sterile. Allohaploid (2n=2x=AL=48 chromosomes) – 23,7% of damaged plant organs, hybrids are sterile as well. Such hybrids could favor sanitation of planting and seed material.

Taking into consideration that sources of all infections, fixed on lavandin plants are well conserved in damaged plant residue, soil and seeds, carried onto other areas by means of equipment, the following plant protection measurements are necessary for lavandin sanitation being cultivated indoors: thermal disinfection of soil (steaming with 100°C for 3 hours), crop rotation with resistant crops, addition of high concentrated potassium fertilizers with microelements against gall nematodes, crops treating with fungicidal preparations to overcome bacterial infection indoors.

The study was granted by the Russian Scientific Foundation (№ 14-50-00079)

References

1. *Akimov Yu.A., Ostapchuk I.F.* Deistviye efirnyh masel na patogennuyu mikrofloru organov dyhaniya // Osnovniye napravleniya nauchnyh issledovany po intensivnosti efirnomaslichnogo proizvodstva: Tez. Dokl. 4 simpoziuma po efirnomaslichnym rasteniyam i maslam. – Simferopol, 1985. – Ch.2. – S. 42

2. Bakterialniye bolezni rasteny / pod redaktsiyey Gorlenko M.V. – M.: kolos, 1977, S. 146 – 154.

3. *Voitkevich S.A.* Efirniye masla dlya parfumerii i aromaterapii. – M.: Pishchevaya promyshlennost, 1999. – 284 s.

4. Zhyzn rasteny. Griby // pod redaktsiyey Gorlenko M.V. – M.: Prosveshcheniye, 1976. – T. 2. – S. 406-411.

5. Metody opredeleniya bolezney i vrediteley s.-kh. rasteny // Per. s nem. Popkovoy K.V., Shmygli V.A. – M.: Agropromizdat, 1987. – 224 s.

6. Mikroorganizmy – vozbuditeli bolezney rasteny // Spravochnik, pod redaktsiyey Bilay V.I. – Kiyev, Naukova dumka, 1988. – S. 119-124.

7. *Mukhortova T.G.* Morpho-biologicheskiye i khozyaistvenniye osobennosti lavandina v usloviyah Kryma: Avtoref. Diss.kand. s/kh nauk. – Donetsk, 1974. – 20 s.

8. *Nevstruyeva R.I.* Lavandiny na yuzhnom beregu Kryma // Agrobiologiya, 1960. - № 1. – S. 147.

9. Noviye efirnomaslichniye kultury // Mashanov V.N. i dr. – Simferopol: Tavriya, 1988. – 160 s.

10. *Rabotyagov V.D., Svidenko L.V., Derevyanko V.N., Boiko M.F.* Efiromaslichniye i lekarstvenniye rasteniya introdutsirovanniye v Khersonskoy oblasti. – Kherson, Izd-vo "Ailant", 2003. – S. 82-90.

11. *Rabotyagov V.D.* Sinteticheskoye sozdaniye alloploidnyh form v rode, i ih morphologicheskiye osobennosti // Genetika, 1986. – T. 27, № 12. – S. 2091-2012.

12. Rastitelniye resursy SSSR. – Leningrad, Nauka, 1988. – 357 s.

13. Selskokhozyaistvenny entsyklopedichesky slovar // pod redaktsiyey Golyshyn N.M., Grebtsova V.G., Kashtanov A.N., Korbut L.A., Kuznetsov L.N. i dr. – M.: Sovetskaya entsyklopediya, 1989. – S. 267-269.

The article was received at editors 14.12.2015

Balykina Ye.B., Ivanova O.V., Rabotyagov V.D., Khokhlov Yu.S. // Bull. Nikit. Botan. Gard. – 2016. – № 118. – P. 68-73.

Biocomplex of diseases and pests, typical for interspecific hybrids of Lavandula with various number of genomes was investigated in course of the research. Susceptibility of Lavandula and Lavandin specimens, cultivated in greenhouse were compared as well. Principal measurements of plant protection, necessary for improvement of Lavandin indoors were also determined.

Key words: *oil-bearing crops; plant protection; signs of susceptibility; phytopatogens; Lavandin; nematode.*

UDK 582.572.225:58.073

POLLINATION FEATURES OF *ALLIUM SICULUM* SUBSP. *DIOSCORIDIS* WITHIN CRIMEA NATURAL PRESERVE

Yelena Vasilyevna Burziyeva, Marina Ivanovna Rudenko

FSBE "Complex "Crimea" Crimean natural preserve,
298515, the Republic of Crimea, the city of Alushta
lju.zamurueva@mail.ru

Introduction

As traditional nature conservation measurements, aimed at preserve and renewal of biodiversity, study of pollination and seed formation (especially rare cultivars) with the help of insects-pollinators in nature preserves are of great importance. Especially protected natural territories is a special study model of exist historically developed, balanced concervative ties

in system palt-animal. One of the most rare and poorly studied cultivars of East Europe flora is *Allium siculum* Lindl. subsp. *dioscoridis* (Sm.) K. Richt.[1] (*Amaryllidaceae*).

A. siculum subsp. *dioscoridis (Nectaroscordum bulgaricum* Janka) is a relict cultivar growing on north-east border of Crimean- Balkan-Asia Minor habitat [3, 4]. Only the Crimea presents localities of its growing in Russia (about 10 localities). It enters the Red Data Book of Moldova (category – Endangered) [6], the Red Data Book of Ukraine (category – rare) [5], the Red Data Book of the Republic of Crimea (category – rare). It is highly ornamental, in some countries of Balkan Peninsula seeds of this plant are commonly used as seasoning.

Objects and methods of the research

Study object is a coenopopulation of *A. siculum* subsp. *dioscoridis (Nectaroscordum bulgaricum* Janka) growing on territory of the Crimean natural preserve (mountain range Konyok massif Babugan of Main range of the Crimean Mountains).

Long-term dynamics of blooming and fruit-bearing is presented according to data "Nature records of the Crimean natural preserve" for 1986-2014 [3] and field authors` observation. Pollination efficiency was determined applying the following method: a part of inflorescence was isolated from insect effect by caproic caps to identify self-pollination capacity, in the end of blooming allowing for parameters of fruiting and seed formation of the same specimen in the coenopopulation we analyzed efficiency of open and isolated inflorescences.

To investigate species composition of visitors on *A. siculum* subsp. *Dioscoridis* flowers, we captured insects during blooming, mainly from 9:30-12:00 in spite of weather conditions. They were put into cupping glass with fixator. Totally there were 100 insect specimens. Capture was sample as some of them belong to dominants and possible to identify without it.

Results and discussion

Study coenopopulation locates on north macroslope of the main range of the Crimean Mountains in mixed forests on the pass Kebit-Bogaz 525 m above the sea level on 25° slope within coenosis *Fraxinus excelsior* subsp. *excelsior* + *Acer hyrcanum* subsp. *stevenii* – *Allium siculum* subsp. *dioscoridis*. At the same time 34 cultivars of 17 families can be found in here. Soils are brown of mountain-forest type. The first layer is 10 m high, the second – 5, maximum herbaceous layer height is 102 m above the sea level and minimum – 12 sm. The total projective surface makes 40%.

A. siculum subsp. *Dioscoridis* belongs to polycyclic monocarpic plants with perennial life cycle [4]. Average vegetation density of coenopopulation makes 19,1 specimens per m^2. Age range is sinistral, absolute maximum is fixed for virginal individuals. Population of generative specimens (g) ranges from some specimens to 2000 [4, 5]. In terms of coenopopulation area of 800 m^2 is fenced off for long-term *A. siculum* subsp. *Dioscoridis* observation (constant sample plot – CSP). Investigating population dynamics of generative specimens of *A. siculum* subsp. *Dioscoridis* and its fruiting on CSP "Kebit-Bogaz" the principal peaks of its generative specimens population were registered (maximum population) in 1992, 1995, 2000, 2005, 2011; recession (minimum population) – 1991, 1994, 1997, 2001, 2004, 2008, 2014. The main fruiting peaks happened in 1994, 1999, 2002, 2011 and 2014, recession – 1995, 2000, 2013, 2015 (fig.1).

[1] Taxon name corresponds to annotate checque-list of the Crimean vascular plants: Yena A.V. Natural flora of the Crimean peninsula: monograph – Simferopol: N.Oreanda, 2012. – 232 s. [1].

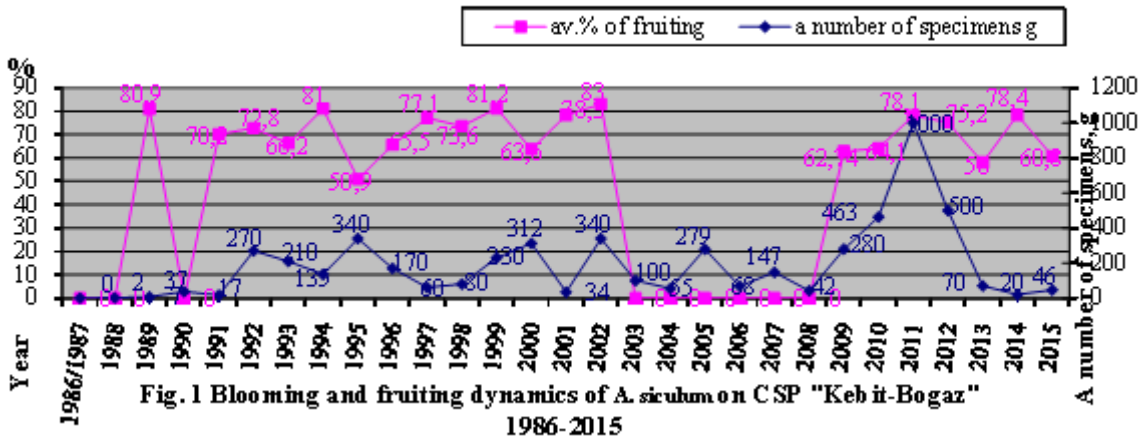


Fig. 1 Blooming and fruiting dynamics of A. siculum on CSP "Kebit-Bogaz" 1986-2015

Fruiting data for period 2003-2007 is absent. In this way peaks/recessions of generative specimens population and fruiting don`t indicate exact periodicity and range from 3 to 7 years. At the same time there is no dependence between average percent of fruiting and increase the number of generative specimens on the site (fig.1). Maximum number of generative specimens on the area was fixed in 2011 and made 1000 specimens, minimum – the third year after fencing (1988) and made only 2 specimens. Fruiting rate within coenopopulation ranges from 50,9 to 83% (fig.1) what is rather high. In this way according to our long-term observations some individuals in the coenopopulation have fruiting rate about 40% and no more than 88% in case of open pollination.

Blooming of *A. siculum* subsp. *Dioscoridis* coincides with period of +15° average air temperature, it lasted 22-35 days depending upon weather [4, 5]. Inflorescence is a multiflowered friable umble, bundled and drooping, that has got 1045 greenish and white large (12-15 mm) flowers on long pedicles (up to 60 mm), widening like a disk to the point of flower attachment. Floral envelope consists of pulpy pearl petals with 3-7 petals, colored with white greenish and pink touch. Color, intensive fragrance and a lot of nectar attract insects. Anthers are not covered, pollen is easy to move away and insects landing on flowers contact it in this or that way. Open pollination is typical for *Allium*, where pollen agents could be different insect species, mainly hymenopterous like bumblebees, bees; but if pollen gets stigma out of inside stamen anthers self-pollination is possible [2].

We could investigated *A. siculum* subsp. *Dioscoridis* level, efficiency and types of self-pollination applying inflorescence isolation from pollen agents in natural conditions. In case of isolated flower-bearing stems regular inflorescences with flowers could form, their fruiting parameter ranged from 34,0 til 66,7%, and on average it made 52%. Therefore autogamy level is rather high what provides regeneration of this species even under unfavorable conditions. Besides, geitonogamy is an often phenomena when representatives of Diptera – *Empis livida* L. are quite active pollen agents from one flower onto stigma of another one (fig.2).

Fig.2 *Empis livida* on inflorescence of *A. siculum* subsp. *dioscoridis* (photo by rudenko M.)

In general, Diptera that better prefers more humid and shadowed locations mainly *E.livida* can be found on inflorescences whether it`s cloudy or sunny, and not-moving if to disturb. Males and females have equal rate on flowers. On average one representative of *Empididae* approximately 5 flowers per one flight, creeping the whole inflorescence. Specimens of this species make mass landing on *Smirnium perfoliatum* L. (Apiacea) flowers and some other cultivars growing withing and out of *A. siculum* subsp. *Dioscoridis* coenopopulation. At the same time geitonogamy involves representatives of Hymenoptera, that is genus *Bombus, Andrena, Halictus, Dolichovespula, Paravespula. Bombus haematurus* Kriech with single visit of about 4 flowers in the inflorescence, *Dolichovespula sylvestris* Scop. and *Paravespula vulgaris* L. – 1-3 flowers, *Andrena nitida* Mull. and *Halictus* sp. – 2–3 flowers.

Based on these findings we can conclude that insects-pollinators are necessary for better fruiting and higher level of pollination. Otherwise we have got decrease of species seed productivity with difference of average parameters of fruiting in isolated and opened inflorescences makes: av. .$\%_{min}$=16,9; av.$\%_{max}$=16,3 (table 1).

Table 1

Average fruiting parameters of isolated and open inflorescences of *A. siculum* subsp. *Dioscoridis*

Parameter, %	Autogamy	Open pollination	Parameters difference
Average minimum (cp.$\%_{min}$)	34,0	50,9	16,9
Average maximum (cp.$\%_{max}$)	66,7	83,0	16,3
Average perennial	52,0	71,4	19,4

Insects taken on flowers of *A. siculum* subsp. *Dioscoridis* during field period of 2010-2015 belong to 16 species 10 genera 6 families and 2 orders - Diptera: Empididae and Hymenoptera: Vespidae, Andrenidae, Halictidae, Anthophorida, Apidae. Species composition of *A. siculum* subsp. *Dioscoridis* pollinators and their food chains are presented in table 2.

Table 2

Species composition of *A. Siculum* visitors on Konek (2010-2015)

№	Species	Frequency rate (visual)	Food ties
1	2	3	4
1.	*Dolichovespula sylvestris* Scop.	often specimens g > 400	Predator (*Andrena*, *Halictus, Empis* amd etc.), nectare consumer, pollinator
2.	*D. media* F.	single	Predator (*Andrena, Halictus, Empis* and etc.), nectare consumer, pollinator
3.	*Paravespula vulgaris* L.	often specimens g > 400	Predator (*Andrena, Halictus, Empis* and etc.), nectare consumer, pollinator
4.	*P. germanica* F.	single	Predator (*Apis, Andrena, Halictus, Empis* and etc.), nectare consumer, pollinator
5.	*Vespa crabro* L.	single	Predator (*Andrena, Halictus, Empis* and etc.) nectare consumer, pollinator
6.	*Andrena haemorrhoa* F.	single	Nectare consumer, pollinator
7.	*A. nitida* Mull.	Mass landing	Nectare consumer, pollinator
8.	*Nomada goodeniana* Kirby	single	Nectare consumer, kleptoparasite *Andrena nitida*, accidental pollinator.
9.	*N. ruficornis* L.	single	Nectare consumer, kleptoparasite *Andrena haemorrhoa*, accidental pollinator
10.	*Anthophora plumipes* Pall.	often specimens g > 400	Nectare consumer, pollinator
11.	*Apis mellifera* L.	mass specimens g > 400	Nectare consumer, pollinator
12.	*Bombus haematurus* Kriech.	mass	Nectare consumer, pollinator
13.	*B. terrestris* L.	single	Nectare consumer, pollinator
14.	*B. hortorum* L.	single	Nectare consumer, pollinator
15.	*Halictus* sp.	mass specimens g > 400	Nectare consumer, pollinator
16.	*Empis livida L.*	mass	Nectare consumer, pollinator

According to conducted observations we found out that species composition and dynamics of insects visiting within coenopopulation range depending upon number of *A. siculum* subsp. *Dioscoridis* generative specimens – more flowers-more insects – more insect variety.

Since 2010 till 2012 a number of generative specimens made more than 400 (g specimens .> 400) on the experimental area all species pointed in the table 1 were found out. Visually analyzing the following species were fixed on flowers: *Apis mellifera*, *Bombus haematurus, Andrena nitida, Paravespula vulgaris*, *Dolichovespula sylvestris, Empis livida.*

Dynamics of insect visiting the inflorescences per 1 hour:

Hymenoptera: Apidae, the highest rate of visiting – 54%,

Vespidae – 17%,

Andrenidae – 12%,

Halictidae – 3%,

Anthophorida – 1%;

Diptera: Empididae – 13% (fig.3, A).

Fig. 3 Dynamics of insects from different families visiting the *A. siculum* subsp. *Dioscoridis* per 1 hour observation, specimens g > 400 (A) and g specimens < 100 (B)

Insect visiting of one inflorescence is much more intensive – up to 74 landings per hour (visit frequency wasn`t considered). Workers of *Apis mellifera*, *Bombus haematurus* and *Empis livida* were found as the most mass species-pollinators.

Having decreased a number of inflorescences less than 100 specimens in 2013-2015 caused abrupt reduction of inflorescence visitors – no more than 3-4 visits per hour. Predators Vespidae, kleoparasites and pollinators Anthophorida weren`t fixed on experimental area at all. The most mass pollinator *Apis mellifera* wasn`t found as well. *Bombus haematurus, Andrena nitida, Halictus* sp. and *Empis livida L.,* while *Andrena haemorrhoa* had single landing, and *Bombus terrestris* was fixed on *Smirnium perfoliatum* growing next experimental area (fig.3B).

Conclusions

As a study result *A. siculum* subsp. *dioscoridis* belongs to antecological complex of the forest entomophilous plants with a wide range of pollinators and possible way of self-pollination (autogamy and geitonogamy). In case of autogamy fruiting rate can reach 67%, but open pollination – 83%, what proves high efficiency of pollination. Insects as pollen agents in case of geitonogamy and open pollination play rather important role. The most effective pollinators are *Bombus haematurus, Andrena nitida, Halictus* sp., *Apis mellifera u Empis livida.*

Findings could be useful for development of preservation and renewal strategies concerning *A. siculum* subsp. *Dioscoridis* as a component of natural biotope. Recommendations in introduction of this rare and ornamental plant are also based on study results.

References

1. *Yena A.V.* Prirodnaya flora Krymskogo poluostrova: monographiya. – Simferopol: N.Orianda, 2012. – 232 s.

2. Zhyzn rasteny: V 6 t. / Gl.red. A.L. Tahtadzhyan.T. VI. Tsvetkoviye rasteniya. – M.: Prosveshcheniye, 1982. – 543 s.

3. Letopis prirody Krymskogo prirodnogo zapovednika: Rukopis / Krymsky prirodny zapovednik. – Alushta, 1986 -2014.

4. *Rudenko M.I.* Ekologo-biologicheskiye osobennosti *Allium siculum* (Ucria) Lindl. subsp. *dioscoridis* (Sm.) K. Richt. v Krymskom eksklave // Bulleten Glavnogo Botanicheskogo sada. – 2013. – Vyp.199. – S. 35-40.

5. *Rudenko M.I., Burziyeva Ye.V.* Nekotoriye osobennosti opyleniya raritetnogo vida *Allium siculum* subsp. *dioscoridis* / M.I. Rudenko, Ye.V. Burziyeva // Zapovedniki Kryma. Bioraznoobraziye i okhrana prirody v Azovo-Chernomorskom regione. Materialy VII Mezhdunarodnoy nauchno-prakticheskoy konferentsii (Simferopol, 24-26 oktyabrya 2013). – Simferopol, 2013. – S. 243-247.

6. Chervona kniga Ukrainy. Roslynny svit / Red. Ya.P. Didukh. K.: Globalkonsalting, 2009. – S. 61.

7. The Red Book of the Republic of Moldova. Chisinau: Stinta, 2002. – P.65.

The article was received at editors 14.12.2015.

Burziyeva Ye.V., Rudenko M.I.. Pollination features of *Allium siculum* subsp. *dioscoridis* within Crimea natural preserve. // Bull. Nikit. Botan. Gard. – 2016. – № 118. – P. 73-79.

The article covers analysis of long-term blooming and fruiting dynamics, and correlation between these processes of a rare protected *Allium siculum* cultivar, subspecies Dioscorida. Type of pollination and its efficiency in case of mutual work with insects and without were discussed within research as well. This work presents long-term dynamics of insect attendance at various families of *Allium siculum* inflorescence allowing for maximum and minimum number of generative specimens. For the first time a list of insects which pollinate just this very cultivar and their food chains were annotated in here.

Key words: *insects-pollinators; Allium siculum subsp. Dioscoridis; Crimea Nature Preserve.*

INFORMATION FOR AUTHORS

"GNBS Bulletin" is published by Nikitsky Botanical Gardens – National Sientific Centre (NBG-NSC).

RULES OF ARTICLE EXECUTION AND PRESENTATION

1. Articles should be in Russian or English, not published or registrated in other journals, collections of works before (exept thesis reports and material of conferences, symposiums, meetings and etc.).

2. Articles must contain brief and clear summery about current state of the subject, description of research methods, issue and discussion of findings. Article title should correspond its contain. An article must be divided into structural parts, cited in the pattern below. Part "Introduction" should include research currency (problem statement and its connection with important scientific and/or practical tasks), analysis of references, used while solving the problem, and research objective.

3. Article is typed in MS Word for Windows (*.doc or *.docx). The following page setup should be used: format – A4, page orientation is book, border size is 2,5sm, print – Times New Roman 12 pt (except summeries, key words, figures and tables, which are typed in 10pt – see patterns below), indent – 1,25 sm, line spacing of the main text is 1 (single spacing), text is typed without word division, justified alignment, text is not paged. During execution and formatting of the text or its structural elements, please follow patterns carefully!

4. Publication size shouldn`t be more than 8 pages. Relative volume of illustrations mustn`t surpass 1/3 of total. The list of references shouldn`t contain more than 30 sources in survey articles and only 15 sources in articles with author`s own research results. Initials are not spaced, but initials and surname are separated by spacing. It`s not allowed to carry over surname to another line (I.I. Ivanov, Ivanov I.I.).

5. Summeries are presented in two languages (Russian and English). Summery and key words are placed before "Introduction" in language the article was written (type – 10pt, **"Key words"** are bolded, key words are italicized). Key words or word combinations are separated by semicolon. After references summery and key words in English are placed. Summery size is 500 characters; a number of key words is 5-7. Execution and formatting setup of these elements should correspond to the pattern (see below).

6. Printed version of manuscript (1 copy) is enclosed by electronic version of files in formats *.doc or *.docx (can be sent to e-mail of editorial office).

7. The manuscript is signed by all authors. One page is taken for authors` data: place of work, position, academic degree, address of establishment, contact details for feedback (telephone number and e-mail of all authors). An article is attached by assignment from establishment where the work was carried out. Articles of postgraduates and candidates are attached by review of research supervisor.

8. All articles are anonymously reviewed.

9. Editors of journal have a rule to abridge texts of manuscripts as agreed upon with authors.

If editors return an article as requiring improvements authors have one month for it.

10. Article heading should contain: surname, name, middle name of all authors in full (in Russian); full organization name – place of work of each author in nominative case, country, city (n Russian). If all authors of an article work at the same establishment, place of work can be specified ones; e-mail for each author; correspondent postal address and telephone number for contact with article authors (one for all authors is allowed).

Manuscripts of articles are sent by address:

Redaction of academic publications
Of Nikitsky Botanical Gardens
Urban village Nikita, Yalta, Republic of the Crimea, 298648
Tel.: (0654) 33-56-16
E-mail: redaknbg@yandex.ru

PATTERN OF ARTICLE EXECUTION

UDC 635.055:504.753:712.253(477.75)

ANCIENT TREES OF ARBORETUM OF NIKITSKY BOTANICAL GARDENS

Lyudmila Ivanovna Uleiskaya[1], Anatolij Ivanovich Kushnir[2], Yekaterina Stepanovna Krainyuk[1], Vladimir Nikolayevich Gerasimchuk[1]

[1]Nikitsky Botanical Gardens – National Scientific Centre, Yalta 298648, Republic of the Crimea, Yalta, urban village Nikita
E-mail: mymail@mail.ru
National University of Bioresources and nature management, Kiev
Postal code, Kiev, 5, Sadovaya str.
E-mail: mymail@mail.ru

For the first time analysis of vital state and ecologic and ornamental characteristics … (summery)…
Key words: *key words, key words, key words, key words, key words, key words, key words, key words*

Introduction

Text text text text text text text text text text text text text text text text text text text text text text text text text text text text text text text

Objects and research methods

Text.

Results and discussion

Text.

Conclusions

Text.

Gratitudes (optional)

Text

References

1. Hydrochemistry… Literary source…
2. *Ivanov I.I.* Literary source source source source source source source source source source source source source source source source source
3. Determiner of higher…Literary source…
4. *Petrov P.P.* Literary source…

5. *Sidorov S.S.* Literary source…

Uleiskaya L.I., Kushnir A.I., Krainyuk E.S., Gerasimchuk V.N., Kharchenko A.L. Centuries-old trees of Arboretum in Nikitsky Botanical Gardens // Works of the State Nikit. Botan. Gard. – 2012. – Vol. 134. – P. 168 – 174.

The analysis of vital state, ecological and ornamental characteristics of….

Key words: *key word; key words; key words; key words; key words; key words; key words; key words; key words.*

END OF PATTERN

Typing and correction of the text should be carried out in accordance with the following rules:

1. MS Word is used to create tables.
2. Words shouldn`t be hand-carried.
3. **Don`t put full stop after:** UDK, title of article, authors` surnames, organization name, heading, figure captions, names of tables, notes and references to tables, dimensions (h-hour, s-second, g-gram, min-minute, day, C - degree centigrade, m-meter) and in subscripts as well. Full stop should be put after contractions (mes. – mesyats (month), ned. – nedelya (week), g. – god (year), mln. – million).
4. Names of species and genera of plants and animals are presented in Latin in Italics according to actual international codes of biological nomenclature, together with author`s name and (if necessary) year of description (author and year of description are typed in usual print), for instance: *Quercus pubescens* Willd. But further mention of the same taxon, its generic name is contracted, author`s name isn`t typed (*Q. pubescens*). If the article contains taxonomic list with full names (including taxons` authors) it`s possible not to indicate author`s name. Authors` names are typed either fully or in standard contractions (Recommended!) in accordance to Authors of plant names (2001). References to authorities of either nomenclature combinations are necessary. Latin names of taxons which belong to higher class than genus are not italicized. Names of plant cultivars are single quoted (**‘**…**’**), if there is no the word “cultivar” before them; all words in cultivar name begin with capital letters (for instance, peach ‘Zolotoj Yubilej‘, but cultivar Zolotoj Yubilej).
5. **Requirements for quotation:**

- ellipsis in quotation is enclosed in curly brackets <…>. If there was a punctuation mark before or after omitted text, it is dropped;
- if an author uses a quotation and emphasizes some words in it, after explanatory text of these words there must be full stop, then dash and article author`s initials (first letters of his/her name and surname), warning text is taken in parentheses. For example: (italics added. – A.S.), (underlined symbols added. – A.S.), (spacing added. – A.S.).

6. Decimal fractions are separated by commas: 0,1 or 0,5.
7. Dash isn`t put in the beginning of line.
8. Double or multiple space isn`t allowed.
9. Such contractions as “i t.d., i t.p.”, indices, subscripts and mathematical signs are not spaced.
10. Numerals and signs %, ° are not spaced.
11. Before measures and signs №, §, © there is a space.
12. Hiphen is used only in compound words such as „vsyo-taki” (anyway), „khimiko-pharmatsevticheskij” (chemical and pharmaceutical), which are not spaced. Dash is used in all other cases and two-sided spaced (18 – 30, 1999 – 2014).
13. Tables and illustrations are put into the text after their first mention. Optimal table size is one page.

14. Before figure, after it and its caption (before text) there are indents of 1 line. Figure caption is centering and typed in lower-case bold letters 10pt, single-space (**Fig. 1** – there is no full stop after numeral). Figures and captions should be placed into a table of 1 column and 2 lines, option "Remove table borders" should be activated to avoid their display when printing (see pattern below).

15. There is an indent of 1 line before table and after it. Word **"Table"** with its number is placed on the right side, table caption is centering below; lower-case bold letters of 10pt, single-space (**Table 1 –** there is no full stop after numeral). Table text is typed in lower-case non-emphasized letters of 10pt with single-space. The first words of table column headings are started with the capital letters, subheadings are started with lower-case letters if they are combined in one sentence with heading, with capital letters if subheadings are independent. Measures are pointed after commas. Execution and formatting parameters must correspond to the pattern – see below.

Repeated text in a table column may be replaced by quotation marks (« - »). Quotation marks instead of numerals, notes, signs, mathematical and chemical symbols are not recommended.

If a table size exceeds 1 page all its columns are numbered by Arabic figures, its continuation on the next pages is typed on the right side in 10pt print (for example, "Continuation of table 1").

FIGURE PATTERN

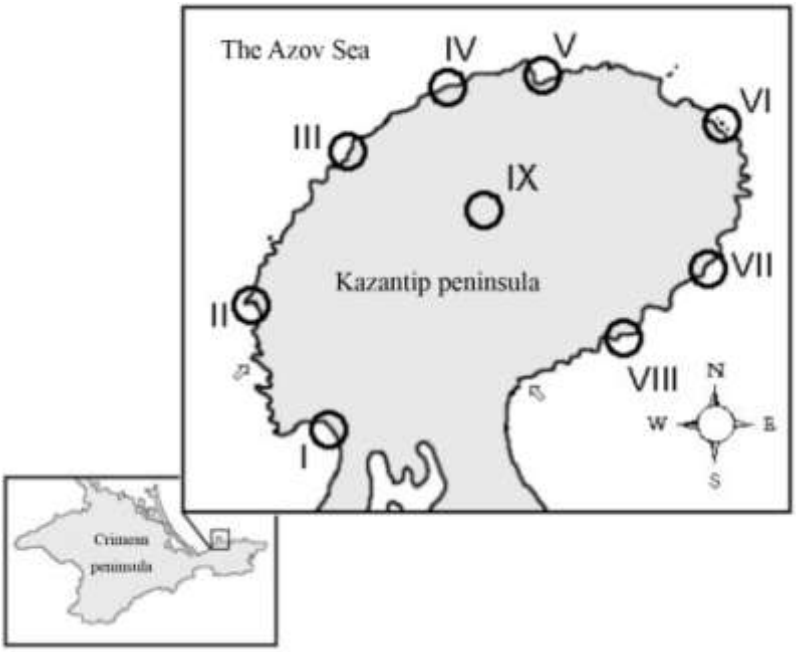


Fig. 1 Sketch map of inspected region (stations I-VIII)

TABLE PATTERN

Table 1

Cultivar composition and biomass of macrophytobenthos within offshore zone of Blessed Trinity Cape

Cultivar	Biomass, g/m^2 (stations I-IV)					
	PLR (±0,25 m)		SLR(–0,5-5 m)			
	I	II	III	IV	V	VI
Ulothrix flacca (Dillwyn) Thur.	F		F			
Chaetomorpha aërea (Dillwyn) Kütz.	F	F	15,00 ±3,92	1,67±0,72		F

Notes:
Hereinafter: PLR – pseudolittoral, SLT – sublittoral. F – few (less than 0,01 g in a sample).
Empty table cells mean absence of cultivar in samples.

16. Bibliographical references in article text are taken in square brackets, several sources are separated by commas in the order of number increasing.

List of references is formed according to State Standard R 7.0.5-2008. Bibliographical reference. General requirements and rules of formation (reference to State Standard http://protect.gost.ru/document.aspx?control=7&id=173511

List of references is made alphabetically, firstly works typed in Cyrillic symbols are enumerated and after it works in Latin. Bibliographical descriptions of works, published in other languages (for example Arabic, Chinese and etc.) should be presented in English and point origin language (in brackets after page number).

17. In the list of references names of cultivars and genera are italicized; numbers of volumes (issue, № or no) are typed by Arabic figures.

18. Line drawings, maps, graphics and photos are enumerated by Arabic figures as they are mentioned in the text. References to drawings and tables in a text are taken in parentheses and pointed in shorthand form with a small letter (tab. 1, fig. 1), if they are mentioned again, add the word “see” (see tab. 1, see fig. 1)

Examples of bibliographical descriptions in references:

Books:

1. *Novosad V.V.* Flora Kerchensko-Tamanskogo regiona. – K.: Naukova dumka, 1992. – 275 s.
2. *Ostapkp V.M., Boiko A.V., Mosyakin S.L.* Sosudistiye rasteniya yugo-vostoka Ukraini. – Donetsk: Noulidzh, 2010. – 247 s.
3. Ekologichesky atlas Azovskogo morya / Gl.red.akad. G.G. Matyshov. – Rostov-na-Donu: Izd-vo UNC RAN, 2011. – 328 s.
4. Authors of plant names: A list of authors of scientific names of plants, with recommended standard forms of their names, including abbreviations / Eds. R.K. Brummitt and C.E. Powell. – Kew: Royal Botanical Gardens, 1992, reprinted 2001. – 732 p.

Periodicals and serials:

5. *Bagrikova N.A.* Analiz adventivnoj fraktsii flori prirodnih zapovednikov Kerchenskogo poluostrova (Krym) // Ekosystemi, ih optimizatsiya i ohrana. – 2011. – Vyp. 4(23). – S. 3 – 9.
6. *Nikiforov A.R.* Elementarnij pobeg i sezonnoye razvitiye rastenij *Silene jailensis* N.I.Rubtzov (Caryophyllaceae) – reliktovogo endemika Gornogo Kryma // Ukr.botan.journ. – 2011. – T. 68, № 4. – S. 552 – 559.
7. *Sadogurskij S.E.* Makrophitobentos vodoyomov ostrova Tuzla i prilegayushchih morskih akvatorij (Kerchenskij proliv) // Algologiya. – 2006. – T. 16, № 3. – S. 337 – 354.

8. *Hayden H.S., Blomster J., Maggs C.A., Silva P.C., Stanhope M.J., Waaland J.R.* Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera // European Journal of Phycology. – 2003. – Vol. 38. – P. 277 – 294.

Abstract of a thesis:

9. *Belich T.V.* Raspredeleniye makrophitov psevdolitoralnogo poyasa na Yuzhnom beregu Kryma: Avtoref. Diss…kand. biol. nauk: 03.00.05 / Gosudarstvennij Nikitskij Botanicheskij Sad. – Yalta, 1993. – 22 s.

10. *Yena An.V.* Phenomen Florystychnogo endemizmu ta yogo proyavi u Krymu: Avtoref.dys. … d-ra boil. nauk: 03.00.05 / Instytut botaniki im. M.G. Kholodnogo NANU. – K., 2009. – 32 s.

Abstract of a paper:

11. *Sadogurskaya S.A., Belich T.V.* Algoflora pribrezhnoj akvatorii u mysa Troitsi (Chornoye morye) // Aktualniye problemi sovremennoj algologii: materialy IV mezhdunarodnoj konferentsii (Kiev, 20-23 aprelya 2012 g.). – K., 2012. – S. 258-259.

12. *Bagrikova N.A.* Syntaxonomical checklist of weed communities of the Ukraine: class Stellarietea mediae // 19-th International Workshop of European Vegetation Survey Flora, vegetation, environment and land-use at large scale (Pécs, 19.04–2.05, 2010): Abstr. – Pécs, 2010. – P. 51.

Section in a collective monograph:

13. *Bagrikova N.A., Kolomijchuk V.P. Astragalus reduncus* Pall. // Krasnaya kniga Priazovskogo regiona. Sosudistiye rasteniya / Pod red. d.b.n., prof. V.M. Ostapko, k.b.n., dots. V.P. Kolomijchuka. – K.: ALterpres, 2012. – S. 198 – 199.

14. *Korzhenevskij V.V., Rudenko M.I., Sadogurskij S.Yu.* PZ Krymskij // Phytoriznomanittya zapovidnykiv i natsionalnyh pryrodnyh parkiv Ukrainy. Ch.1. Biospherni zapovidnyky. Pryrodni zapovidnyky / Pid red. V.A. Onyshchenko i T.L. Andriyenko. – K.: Phytosotsiotsentr, 2012. – S. 198 – 220.

Multivolume editions:

15. Hydrometeorologiya i hydrokhimiya morej SSSR, T. IV. Chornoye morye. Vyp.1.Hydrometeorologicheskiye usloviya / Pod red. A.I. Simonova, E.N. Altmana. – SPb: Hydrometeoizdat, 1991. – 426 s.

16. Algae of Ukraine: Diversity, Nomenclature, Taxonomy, Ecology and Geography. Vol. 1. Cyanoprocaryota – Rhodophyta / Eds. Petro M. Tsarenko, Solomon P. Wasser, Eviator Nevo. – Ruggell: A.R.A.Gantner Verlag K.G., 2006. – 713 p.

Internet resources:

17. *Guiry M.D., Guiry G.M.* 2013. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. – http://www.algaebase.org. – Searched on 05 August 2013.

If a literary source has four or more authors, **all names should be specified.**

As VAK requires electronic copies of published articles are placed into database of Scientific electronic library elibrary.ru (for numbering of Russian index of scientific citation). Therefore author`s consent for article publication extends to placement of its electronic copy at electronic library.

Bulletin SNBG is published according to resolution of Academic Board
Of Nikitsky Botanical Gardens –
National Scientific Centre
Dated 29.09.2015, record № 11

Number 118

Responsible for issue
Shyshkin V.A.
Desktop publishing
.Golovnyova Ye.Yu
Translation from Russian into English
Golovnyova Ye.Yu.

http://bult.nbgnsc.ru

Certificate of registry PI № FS77-61874 dated 25.05.2015
Signed for printing 09.07.2015. Format 210 x 297. Offset paper – 80 g/m^2
Rizograph printing, accounting printing sheets 10,0. Number of copies 500. Order № 9A/10.

Edition of scientific publications
Nikitsky Botanical Gardens –
National Scientific Centre
urb.vil.Nikita, Yalta, Republic of the Crimea, RF, 298648
Tel: (0654) 33-56-16
E-mail: redaknbg@yandex.ru

Original layout was used for printing at «ARIAL»press, Co Ltd.
295034, Republic of the Crimea, Simferopol, 31-a/2, Sevastopolskaya str.,
Tel. (0652) 70-63-31, +7 978 717 29 01.
E-mail: it.arial@yandex.ru