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PRACTICAL RESULTS OF WORK WITH WILD AND CULTIVATED PLANTS

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The collected articles include material in the field of essential oils effect on different aspects of human higher nervous activity allowing for various compositions: psychoemotional state, mental capacity, neuromotor processes. Characteristics of single and course treatments, aromaprocedures at rest and exercise of medium intensity, essential oil effect of various concentrations and time of treatment are reported here as well.

Essential oil effect on experimental animals and some oil-bearing plants are also described in collected articles.

These works are appropriate for scientists and experts at Psychology, Biology and Medicine.

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NATURAL POPULATIONS OF CULTIVARS FROM *PINUS* L. GENUS IN MOUNTAIN CRIMEA. PROBLEMS OF PROTECTION

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Introduction

Most specimens of the *Pinus L.* genus are valuable forest forming cultivars. The high-level tolerance of the pine to abiotical factors determined its wide spread over different climatic zones [35].

Pine forests of the Crimea peninsula play a very important role in providing the social and environmental needs of society. Their principal habitat is Mountain Crimea. The population of mountain arboreal plants is characterized by large genetic variety that determines their high importance for selection and silvicultural brunch.

In Mountain Crimea three pine cultivars grow naturally: P. *pallasiana* D. Don, P. *pithyusa* Stev. subsp. stankewiczii (sukacz.) N. Rubtz., P. *kochiana* Klotzsch ex C. Koch. [2, 59]. The last one is considered as relative to P. *sosnowskyi* Nakai (P.hamata Sosn.) or as a variety of P. *sylvestris var.hamata* (Stev.) Sosn., which differs by pyramide-shaped swollen and hooked folded corymbs at the bottom of the cone [35].

The most part of pine forests in the Crimea is naturally planted and refers to the protected territory. On the peninsula there is one of the oldest reserves of our country – the Crimean reserve, founded in 1923. As a matter of fact it ensured preservation of the main part of pine woodland. Ever-increasing recreational loads and as a result – fires, determined the necessity of two more reserves in 1973: Yalta mountain forest reserve and " Cape Martyan", which contains plantations of the southern macroslope of the Main Crimean Mountain Range. Since then it has been considered that natural plantations of *P. pallasiana* and *P. kochiana* gained the highest protective level. But stands of trees of *P. pithyusa* have still belonged to forestry enterprises, with the status of preserves (preserve "Cape Aya" – Sevastopol forest enterprise and "Novy Svet" – Sudak enterprise. But the status of preserve doesn`t ensure necessary protection of valuable tree cultivar, *P. pithyusa* is one of them.

Unfortunately, in spite of all entrepreneurial arrangements, at present time reduction of territory and natural population size of Pinus L. in Mountain Crimea has a negative trend. In accordance to records of Yalta mountain forest reserve for period from 1973 till 2013, 1139 fires have happened within its territory, the total afire area has made 2483,53 ha, approximately 22,4% of the reserve forest area. Most of fires took place among *P.pallasiana* plantations.

Based on the current forest condition and its role, at present time the most important task is throughout analysis and assessment of existing approaches efficiency and applied methods of agriculture, protection and renewal of the forest pine biocenosis. It`s necessary on a large scale to implement and use methods, firstly considering social and ecological forest role, what has primary importance for the Crimea Peninsula as a foreground territory of agricultural, resort and recreational development.

Last decades working out scientific and methodical concepts of protection and renewal the disturbed forest cenosis, population and genetic approaches have become widespread. The population analysis, using different methods of Biology and mathematical simulation, is one of the principal and promising directions in learning mechanisms of changeably species, peculiarity of their adaptation in definite growing conditions.

In accordance to modern ideas, population is a group of individuals of one species, that have inhabited definite area for many generations, where the exchange of genetic information is possible. A population is an elemental unit of the evolutional process and form of the species existing. The level of genetic diversity determines evolutional species resistance [8, 24]. Each population has its own evolutional destiny. At the same time level of genetic diversity supplies population resistance and possibility of its stable renewal [1]. The population has complicated biochorological structure according to its density, classification of specimens by age groups, types of growth.

The most important factor in population forming is natural selection. The natural selection is the statistic phenomena, from the point of modern concepts, otherwise the best genotype is more tenacious [8, 56].

The mechanism of the natural selection by means of growing conditions is the basis of approach in assessment of population size of arboreal plants, that is confrontation of species changeability in the region to the landscaping and geographical structure of this region. It`s assumed that in similar landscaping geographical conditions a definite type of forest growth condition is formed, that is the basis of the native forest types, making one population. In this case, vectorized mechanism of the natural selection is taken into consideration, which for a long time on the basis of the similar forest growth conditions has formed stands of trees with common gene pool namely population [33,43].

In a number of studies there is a high level of population adaptation to different growth conditions [44,45,53]. It`s well-known that populations in the similar conditions hardly differ even if to isolate them and on the contrary, population with active gene interchange in the definite selection way are able to change independently. The natural selection reveals its mechanism not only through abiotic environment, but also through organisms interrelations as inside of one species as through interspecific correlations in phytosenosis.

In natural populations there is a genetically determined polymorphism of resistance to effect of various factors. Increasing of changeability is one of the most important adapted responses to stress, as under radically change of environment, population is able to exist only if some specimens of population survive, which will ensure breed forming under new conditions. Selection of variants mostly adapted to new conditions change average of quantitative characters. Thus changeability increasing reflects the rate of adaptational processes inside of population. Inequality of average morphophysiological characteristics is a quantitative measure of changes due to selection. In general adapted potential of the species is determined by ability of quick changing morphologically in accordance to rhythm of variations of the basic essential natural and climatic environmental factors [4, 20].

Conception of plant population stability is associated with critical state which is connected with irreversible changes of their structural and dynamical organization. Objective character of the population critical state is disorder of the usual generations cycle, which makes forming and preservation of viable diasporas impossible. Critical state of population is assessed in accordance to changeability level of its demographic components, which are evaluated on the ground of analysis the level of incomplete age structure of populations in comparison with basic data.

The level of population stability is effected by changes of age composition, individual vitality, time of ontogeny periods, development of reproduction processes [10]. Stable space-time existence of populations is determined by change of demographical elements, their heterogeneity, which is supplied by multiple-aged individuals and polyvariant ontogeny. Polyvariant ontogeny and ability to change vital state are the most important mechanisms of population stability in the range of environmental and phytocenotic conditions [11].

Different factors effect on ratio of demographic elements in pine populations, such as abiotical factors playing the most considerable role. In Mountain Crimea at first stage of

ontogeny moisture regimen has the principal effect on self-seeding process in natural pine populations [13, 51]. Under lack of moisture seed sprouting becomes slower, loss of geminating ability effected by soil micro flora is caused by lack of moisture as well [47].

The moisture regimen of arboreal plants is closely connected with the dynamics of soil moisture. In stand of pine trees soil drying across happens irregularly. In the upper half meter layer the most replete by plant roots, moisture is used faster. On the depth of 20-30 sm moisture is used for transpiration, in the upper 10sm layer it goes for physical evaporation [52]. Growth of young pine plants with root system in 10-15 sm soil layer is limited by herb layer to a large degree. Intensively growing herb plants supplant pine roots out of upper to lower, less fertile soil layers, that reduces growing capacity of seedlings. In seasons with enough moisture herb plants use the same quality of moisture as 19-years dense pine cultures, in dry years herb plants use more moisture than 30-years cultures [25]. According to Shumakov V.S. and Kurayev V.N. (1973), with dense herbage 0,4-0,6 a considerable inhibition of pine is marked [54]. It is one of the reasons of mass death among self-seeding across the area of cutting. In accordance to Gordiyenko M.I. and Gordiyenko N.M. (1988), level of pine self-seeding in pine forests and subors of woodlands is registered before cutting, in a year of cutting and a year after it. Henceforth pine seeds sprout but because of intensive herbage spreading, plantlets don`t meet the competition and majority dies in spring [6].

In this connection phytocenotic mechanisms keeping stability and resistance of pine assemblages play an important role. Golubets M.A. and Tsarick I.V. (1990) consider stability as an integral index of resistance. [5]. They suggest not to indentify notions of biotic systems stability and resistance, but to consider resistance as an ability of a system to reserve its structural and functional properties and renewal them quickly in reply to natural and anthropogenic effects; and stability is considered as an capacity of a system to reserve its basic parameters during the whole existence or during its continuous period of development.

Researching pine biocenosis based on microenvironmental and system approach using quantitative methods, analysis of structure chorologic changes and functions of stand of trees-edificator was carried out. By means of prevailed types of pine forests in Mountain Crimea the principal role of stand of trees-edificator in forming of phytoenvironment, structure and functions of the main components in forest biocenosis was marked out [15, 30, 31].

If to consider biocenosis as a system, mainly created by process of environment forming, tree role in the assemblage is defined by its edificator power [46]. Time when effect of the given individual reflects on other plants state is characterized as a quantum leap. Intensity phytogenetic field depends upon plant size and age. This statement is approved by most researchers, who defined indexes of tree phytogenetic field in their works [21,57]. The most effective places of stand of trees are usually close to large old trees. Though role of a specimen in the assemblage isn`t limited by this function, competitive status of a tree in synusia is quite important. Tolerance and competitive power of species in an over-ground cover differ, edificator power of woody specimens in different environmental conditions is diverse as well.

Future of a young pine generation in forest assemblages is determined by complicated processes of plants interrelations due to competition of adult trees for light, nutrients and soil moisture. Assemblages of diverse types differ markedly from each other in nanorelief, mosaic and area of their microstations and synusias of lower layers. It determines heterogeneity of biocenotic environment regimens, causes variety of growth conditions, significant space differentiation of sprouts, their localization in connection with microstations peculiarity in biocenoses [12]. According to some observations, on the ground of different adverse factors, biochemical interactions play definite role in the root system zone by means of root products of adult trees and other components of phytocenosis [55]. In spite of the principal factor in given conditions, result of the common effect leads to the total or partial elimination of

sprouts and young growth. Survived part of pine young growth under the crown layers of stand of trees differs from open-growing trees of the same age by disordered morphogenesis and changed correlation of physiological processes intensity. Morphological characteristics of inhibition become apparent first of all in changing of general habit and crown form [11,48].

Inhibited young growth being under closed forest crown is characterized by reduction of photosynthesis, breath and transpiration intensity. The pine young growth thrives on that area where negative effect of climax vegetation of lower layers is absent. Conditions of mineral nutrition, moisture, temperature and illumination regimen are more favorable for plants. Such conditions are typical on locations with single and group falls of the oldest trees [28, 37, 58].

On the whole pine self-seeding has ruderal properties, such as: high degree of light-requiring, low shade tolerance of self-seeding and as a result its inability to survive under closed vegetable crown for a long time, drought-, heat- and frost-resistance of sprouts, deep establishment during first years of existence, intensive growth [39,40].

There is an opinion that low-intensive fires create favorable conditions for pine renewal. Most of works indicate that under close conditions of seeding, as a rule number of pine self-seeding is much higher on mineralized or burnt soil than on humic substrate [17].

In general growth and development of pine are close connected with fire effect. For instance, one of the proofs of pyrogenic adaptation of P. *sylvestris* is thermal isolation of the fulcrum bottom by rind with thickness of 1,5sm, it protects phloem and cambium from overheat [38]. Thickness of the rind becomes than bigger than higher above the soil, reaching maximum mark on level of 10-15 sm, then this characteristic goes down slowly in accordance to the vertical temperature profile during ground fires. Among morphophysiological peculiarities of pine trees which favor preservation and survival after fire besides thick rind at the bottom of the fulcrum there are strength of timber and root, strong development of main and "anchor" roots, lateral roots penetration, ability of injured tissue to tar and regenerate fast, high disposition of the crown [35,40].

Fires intensify trend to strong pine population. A small amount of young grow cohort on the areas, which hasn`t been subjected to fire effect for a long time takes place because seeds of dominant trees getting into the sward don`t have an opportunity to sprout [41].

There is a close negative correlation among a number of young pine grow generations and long absence of fires. The whole complicated process of direct and indirect effects of ground fires (destroying of a strong mat layer, increasing of illumination, moisture on the exposed soil surface, enrichment of soil by mineral elements, abolition of phytotoxicant effect, reduction of competition etc.) is a kind of signal for seed sprouting and active development of seedlings [18,19,38,49,50]. Pyrogenic cyclicity of young grow sprouting leads to staged age system of pine forests. Periodicity of renewal and age structure of pine populations are under similar influence of cycles of substrate erosion in mountains and agricultural cuttings, attended by mineralization of soil [32].

Seed renewal of pine on recent burning depends on if there are seeding sources and their allocation, combination of seed years with sufficient precipitation and other factors.

The first stage of post-fire renewal dynamics is characterized by maximum evenness of the space distribution of young pine grow, which being in the assemblages with 30% of injured stand of tree is regular or casual, but being in the assemblages with 70% of injured stand of tree this value changes as fire remoteness increases from group fire till accidental [7].

In subclimaxes the reason of changes of young grow density in definite periods can be irregularity of apolexis of even-aged and stage multiple-aged stands of tree.

The most widespread interpretation of the fire effects is change in phytocenosis state, which is possible to observe immediately after fire or next 5-7 years after it. These consequences can be considered as "short-term". They include burnt trees and accretion changes of post-fired stands of trees, burn injuries and damage of tree crowns, lost young grow and undergrowth, changes of soil environment, living and dead ground cover [9, 26].

Nevertheless result of fires is not only qualitative and quantitative changes of stands of trees. Sequence of interdependent and correlated post-fired phenomena has effects as follows: change of environmental regimens in growing conditions, emergence of derivative assemblages on burnt areas, replacement of species and age generations. In general post-fired phenomena determine specific and direction of post-fired forest formation [14, 16, 18]. Above-mentioned consequences are possible to reveal and value if to assess allocation of vegetational succession stages in post-fired period within this or that region and their silvicultural and biological peculiarities [49]. At the same time special environmental background is formed, which determines peculiarities of post-fired vegetational successions. Post pyrogenic environmental background combined with burnt seeding areas predetermines ecological and dynamic lines of vegetation forming within elementary natural complexes. Each line is divided into morphologically different periods of regenerative aged post-fired dynamics, which may be considered as genetically correlated types of biocenosis [50].

Nowadays one of the most important questions is a dynamics of abiotical factors in pine stands of trees, damaged by fire. This problem takes on special significance due to increasing of anthropogenic fires and necessity to improve efficiency of reafforestation work within burnt timbers, what is highly actually for ecosystems of Mountain Crimea. In general, to reveal dynamics` regularities of ecological factors and their effect on development of forest phytocenosis is the key condition in forming of high-productive plantations [14, 15, 30, 34].

At present anthropogenic effect becomes a crucial factor in forming of forest ecosystems. It concerns compulsory monitoring of long-term purposeful monitoring systems, providing effective control and prognostication of mainly anthropogenic changes in the natural environment. Long-term monitoring process of the natural populations of the cultivars from *Pinus* L. genus in Mountain Crimea is essential to control pine forests state, assess their structure, productivity, level of recreational exploitation, prognostication of probable changes, destroying stability of the forest biocenosis. Thereupon factor of time takes on primary importance, the sooner tentative areas are formed and monitoring is actuated, the initial characteristics of researching objects more correspond to the performance of their natural virgin state. More long-term chronological lines of observed parameters allow improve informative capability of performances, characterizing tendencies in the state dynamics of researching objects.

Protection of different plant cultivars, solution of problems, preservation of biodiversity demand constant improvement in assessment system of the vital state, which is necessary for objective analysis of changes in the natural populations development, prospect to preserve their ecological potential. The most important problem of present is to form database of bioecological characteristics of natural populations of forest forming tree breeds of Mountain Crimea. The database should rely on performances of vital state, peculiarities of specimens response to environmental changes. One of the most perspective ways to solute mentioned-above problems is to apply biophysical methods for assessment of the plants vital state in the field conditions.

Analyzing plant state, biochemical methods gain a large importance, as that allows detect negative effects of different factors before their damage influence becomes apparent. That`s why, applying monitoring system for forest ecosystems demands wide use of biochemical indicators of arboreal plants state.

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Koba V.P., Plugatar Yu.V. Problem of natural populations of types of *Pinus* L. genus protection in Mountain Crimea // Works of the State Nikit. Botan. Gard. – 2014. – V. 139 – P. 3 – 10.

The article presents modern conceptions concerning population-genetic methods to analyze mechanisms of cultivars changeability, specific features of their adaptation in terms of dynamics of growing conditions. It is shown that presently anthropogenic impact gains an importance of the determining factor in forming and development of forest phytocenosis. One of the main tasks to provide objective control and prognosticate changes of natural populations state including cultivars of *Pinus* L. genus in Mountain Crimea is monitoring system and forming database of their bioenvironmental characteristics.

Key words: ecology, adaptation, phytocenosis, protection, natural populations, *Pinus* L.

UDK 504.064.3:574

A DYNAMIC MODEL OF THE WATER RELATIONSHIPS OF SOME BUSH CULTIVARS GROWING IN LOWER LAYER UNDER CONDITIONS OF PARK PHYTOCLIMATE ON SOUTH COAST OF THE CRIMEA

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Introduction

In the course of landscape architecture, aesthetic features of trees and bushes have a great significance for choosing of necessary plant species. They are characterized by changing size, form, colour, foliage capacity, that depends on their growth, development, seasonal cycles of their vital functions [1, 2, 10]. Each plant especially detached one is unique with its own characteristics of height, habitus and crown form. At the same time in group plantations trees and bushes are capable to adapt to each other, creating not just a set of separate plants, but a single correlated group capable of self-regulating and forming balanced plant mass.

While forming size-space park composition the prior significance goes to plant habitus, their height, macrobiosis and dynamics of their interrelations development.

Sharp fluctuations of meteorological factors, changing every year, often become a reason of stress situation for plants. One of such stress effect, emerging on different ontogenesis stages of any plant organism, is water stress. Considerable part of the Crimean territory is subhumid with irregularity of precipitation and frequent dry periods. Furthermore plants often have a lack of moisture deficit due to high temperature in summer, sharp fluctuations of weather conditions, environmental pollution, irrational utilization of water resources and incorrect agrotechnical measures [8]. Plant resistance to agroecological factors is one of the difficult questions in introduction of plants, especially concerning ornamental cultivars. Therefore response of lower layer plants to changes of environmental factors, assessment of these factors impact on moisture regimen are of great interest researching plant drought- and shadow-resistance, quite necessary parameters for further development of recommendations at growing under specific conditions [3,6].

In accordance to stated above, the research objective was defined: to investigate parameters variations of plant water exchange applying phytomonitoring methods and develop general biological assessment criterion of genotypic drought-resistance of an introduced cultivars that allows define plant tolerance degree to stress susceptibility [3, 7, 9].

Objects and methods of the research

This investigation was carried out using methodology and phytomonitoring instrument base. Following rapid methods were used:

- Determination of xylem (timber) moisture deficit;
- Measurement of xylem stream linear velocity in trunks of arboreal plants.

To define xylem moisture deficit, method of heat pulses was used. It is a matter of xylem, xylem stream makes 98-99% of the total stream (xylem and phloem stream), heat point moves up by xylem stream. In this technical decision results of heat-pulse measurements gained the principal significance to define xylem moisture level. It is generally known, this method is applied to determine linear velocity of xylem stream, though only this

pulse time component was used. The amplitude component isn`t of great importance for scientists. A new method of this parameter determination was developed and patented [5].

Sensor for measurement of this parameter was installed at altitude of 0,6 – 1,1 m above ground.

This sensor determined the linear velocity of xylem stream as well [12, 14, 15]. This parameter lets determine correlation coefficient of water stress and drought-resistance of investigated plant cultivars. Water stress coefficient is calculated by formula:

$$C\ w.s. = Vm./Va., sep.unit$$

Where: Vm. – xylem stream linear velocity in the morning;

Va. - xylem stream linear velocity in the afternoon.

Applied rapid-methods having simultaneous measurement of environmental parameters under microclimate conditions (illumination, air temperature, air humidity, temperature and moisture level of soil, air humidity deficit) allowed study some ecophysiological characteristics of investigated bush cultivars in the lower layer. Environmental parameters were measured by standard methods, applied in meteorology [11].

As pattern objects 8 plant species have been selected, various in their water relationships, drought- and shadow-tolerance level (table 1): *Pittosporum heterophyllum* Franch., *Buxus sempervirens* L., *Euonymus japonica* Thunb., *Chimonanthus praecox* (L.) Link, *Viburnum tinus* L., *Cornus mas* L., *Laurocerasus officinalis* M. Roem., *Aucuba japonica* Thunb.

Table 1

Bioecological characteristic of some bush cultivars growing in the lower layer of parks on South Coast of the Crimea

Cultivar	Frequency of occurrence	Shade tolerance	Drought-resistance	Blossoming, fruitage	Ornametality	Function
Pittosporum heterophyllum	S	++	++	Fr.	1	2
Buxus sempervirens	M	+++	++	Fr.	1	1, 2
Euonymus japonica	O	++	++	Fr.	1	2
Chimonanthus praecox	S	++	++	Fr.	3	2
Viburnum tinus	S	+++	++	Bl.	1	2
Cornus mas	O	++	+++	Fr.	3	2
Laurocerasus officinalis	O	++	++	Fr.	1	1, 2
Aucuba japonica	S	+++	+	Bl.	1	2

Frequency of occurrence: S –single instance (from 1-5 specimens); O – often (from 20-100 specimens.); M – mass (over 100 specimens.)

Shade tolerance: +++ – very shade enduring, ++ – less shade enduring

Drought-resistance +++ endure drought conditions without discernible damage, in summer period capable to grow using just natural moisture; ++ are in need of artificial irrigation in dry season (these cultivars are resistant to air drought but require soil moisture); + necessary regular watering during the whole summer season; - plants suffering of air drought and having soil moisture deficit even being regularly watered

Ornamentality degree: 1 – a plant is ornamental all year round, 3 – ornamental only in blossoming and fruiting season

Function: 1 – medicinal and preventive properties, 2 – aesthetic and ornamental effect, 7 – vertical gardening

Results and discussion

These plant cultivars of the lower layer, besides *Chimonanthus praecox* (the lower park, altitude above the sea level – 115m), grow in the upper park of Arboretum in Nikitsky Botanical Gardens (altitude above the sea level ranges from 145 to 165m) in various microclimate conditions [10]. They can be divided into three main groups: 1. Growing under conditions of shade during the whole ontogenesis; 2. Being in the shade during the active vegetation period in frost-free season; 3. Growing on the comparatively open ar *Viburnum tinus* ea.

1. Growing under conditions of shade during the whole ontogenesis:
   - *Pittosporum heterophyllous* – evergreen ornamental bush with simple leathery nitid leaves, grows under cedarwood atlas crown, distantly 1 m from the tree stem.
   - *Buxus sempervirens* - evergreen ornamental bush, grows in area of combine projective cover of cedarwood atlas and laurel, equidistant from their stems - 2,5m.
   - *Chimonanthus praecox* - ornamental bush, blossoming in winter with deciduous oblong and elliptic leaves for this period. It is located under Monterey cypress crown (3,5m from the tree stem).
   - *Aucuba japonica* - ornamental evergreen bush, grows under crowns of evergreen trees: holm oak, wellingtonia and thuja orientalis (4m, 5m and 2,5m from the tree stems).
2. Plants, being in the shade during the active vegetation period in frost-free season, grow under deciduous tree crown:
   - *Euonymus japonica* – valuable ornamental evergreen plant, grows under golden shower crown (1m from the tree stem);
   - *Viburnum tinus* – evergreen bush with small nitid leaves and corymbs of white flowers, grows under English walnut (Juglans regia) and black locust (Gleditschia triacanthos) crown, 1m and 2,5m from the tree stem
   - *Laurocerasus officinalis* is a bush with simple leathery evergreen leaves; in South, West and north-west this plant is surrounded by three tillet (Tilia cordata) trees, 3,5 – 4,5 m from tree stem; in the North *Laurocerasus officinalis* is shaded by peripheral part of horse chestnut (Aesculus hippocastanum) crown.
3. *Cornus mas* is a deciduous bush growing on the comparatively open area.

Table 2 presents a brief biometrical characteristic of studied bush cultivars growing in the lower layer and trees-edificators which make under-crown space for these brushes, their location relative to edificators is given as well.

Table 2

Biometrical characteristics of plants growing in the lower layer and their location relative to the stem in the under-crown space

Cultivar	Biometrical characteristic of plants				Plant location relative to the stem in the under-crown space		Biometrical characteristics of trees growing in the upper layer			
	Age, years	Height, m	Crown structure	Crown shape	Distance from the stem, m	Direction	Cultivar	Age, years	Height, m	Crown radius. m
Pittosporum heterophyllum	30	4	friable	inversely egg-shaped	1	N	Cedrus atlantica	80	20	4
Buxus sempervirens	60	4	dense	inversely egg-shaped	2,5	E	Cedrus atlantica	150	27	5
					2,5	N	Laurus nobilis	60	11	3
Euonymus japonica	30	3,5	friable	inversely egg-shapede	1	N	Laburnum anagyroides	40	12	3
Chimonanthus praecox	40	4,5	friable	Branchy	3,5	E	Cupressus macrocarpa	160	34	8
Viburnum tinus	30	3,5	friable	inversely egg-shaped	1	N	Juglans regia	30	10	3
					2,5	NE	Gleditschia triacanthos	60	22	3,5
Cornus mas	40	5	friable	inversely egg-shaped	0	0	*Cornus mas*	40	5	2
Laurocerasus officinalis	30	5	friable	Branchy	4,5-3,5-3,5	NE-SE	Tilia cordata	60	17	5
					5	S	Aésculus	60	15	5,5
Aucuba japonica	30	3	friable	inversely egg-shaped	5-5-4-4	SE-S-SE-SW	Holm oak	80	20	6-7
					5	E	Sequoiadendron	50	20	3
					2,5	W	Platycladus	30	10	3

Under plantation crown spatial variations of the most meteoparametres, first of all solar radiation and precipitation, are quite big and depend upon architectonics of crowns. Temperature condition of air and soil as the determining factor of plant activity depends upon solar radiation that gets the plants and ground surface. Plants, bedded under high trees of various breeds, occur in different insolation conditions. Change of illumination, temperature and moisture level is the most obvious evidence of plant effect on the environment. Having simultaneous observations, illumination power, measured on the open areas non-shaded by tree crowns, in the fair weather on August the 28th reached 47000-50000 lx. Under tree crown under intensive shadowing conditions, illumination power value getting under crown space of lower layer bushes ranges from 200 till 550 lx. Under crown space of Cornel has much more illumination - 1450lx. In the end of August in 2014, because of long period with lack of precipitation, plants of Arboretum in Nikitsky Botanical Gardens growing on non-watering areas and suffering of high air temperature were under water deficit conditions. Parallel measurements of illumination, air temperature and humidity, soil temperature and moisture level were made to compare the phytoclimatic characteristics under canopy of lower layer study plants (table 3).

Table 3

Phytoclimatic characteristics under canopy of lower layer plants (28.08.2014, 10:30 MSK)

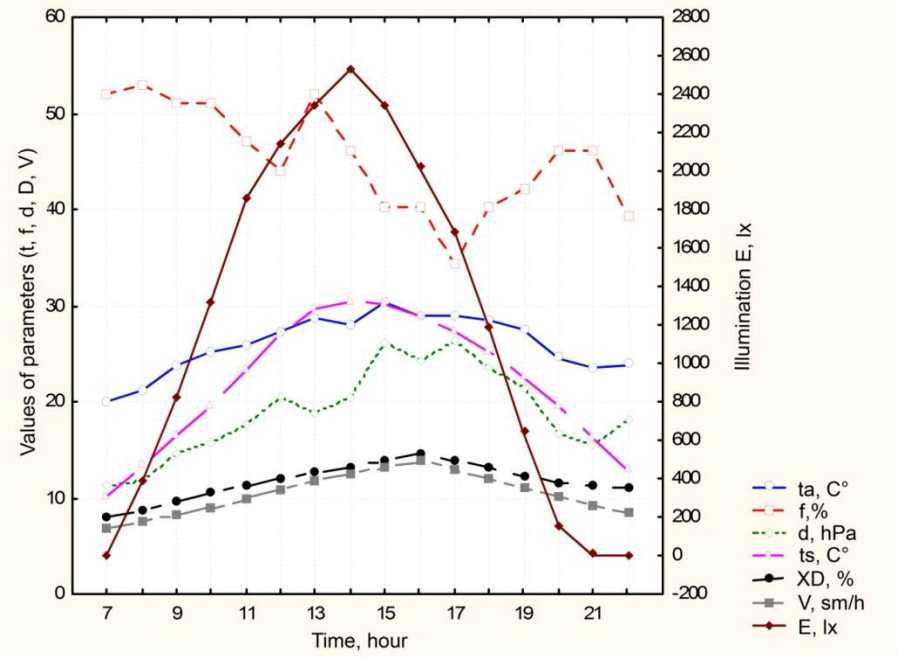
Cultivar	Environmental parameters					
	Air temperature, °C	Relative air humidity, %	Soil temperature, °C	Soil moisture at depth point of 20sm, %	Illumination, lx	Transmittance, %
Pittosporum heterophyllum	25,0	49	20,0	7,2	500	1,94
Buxus sempervirens	24,5	57	20,0	13,7	200	0,68
Euonymus japonica	25,0	54	20,0	7,2	200	0,58
Chimonanthus praecox	25,7	48	20,1	14,8	370	0,64
Viburnum tinus	27,1	49	21,0	12,4	550	1,11
Cornus mas	25,5	50	20,5	11,4	1450	3,18
Laurocerasus officinalis	25,2	52	20,0	10,2	300	0,69
Aucuba japonica	25,2	57	20,5	14,9	450	1,14

It is a well-known fact, that a cultivar drought-resistance is determined by plant response to changing of environmental conditions. Under effect of extreme factors, plants, adapted to certain environment, during evolutional process, are able to response in different way. Having low moisture potential of the root zone, plants of diverse ecological groups decrease their water exchange parameters (xylem stream velocity), whereby main physical and biochemical processes in plants are under control. Plants with different ecological and physiological characteristics possess diverse intolerance degree to soil moisture potential, what effects on plant metabolic rate as a response to water stress condition. Character of response to negative impact, its velocity and depth, displays the plant tolerance to an actual stress. Having water deficit more resistant cultivars are characterized by reduction of their metabolic rate (xylem stream velocity), that protects from excessive dehydration, and long-term turgor preservation [3]. Such a type of response is inherent for plants from dry areas and for xerophytes in general (fig.1 a, b). Nonresistant plant cultivars activate their mass exchange and power interchange to support water relationships on necessary high level;

(fig.2b) as a result we have an active water discharge in the system soil – plant – atmosphere, and incapacity to support level of water retentivity of tissue on maximum point. These differences can be useful for development the criterion of relative physiological drought-resistance degree of various plant cultivars based on investigation of water transport mechanism.

Applying the phytomonitoring methodological base makes it possible to determine general regularities of water exchange variations, that characterizes qualitative and quantitative correlations between physiological characteristics of plant water regime (xylem moisture deficit and linear velocity of xylem stream) and environmental parameters (air temperature and humidity, air humidity deficit, soil temperature and illumination). Pictures 1 and 2 present a dynamics of these parameters during the daylight for 4 studied plant cultivars with different degrees of drought-resistance. They occupy extreme (*Cornus mas* and *Viburnum tinus* – xerophytes, *A. japonica* – mesophyte) and intermediate (*E. japonica* – xero-mesophyte) places in our relative drought-resistance lines.

a)



b)

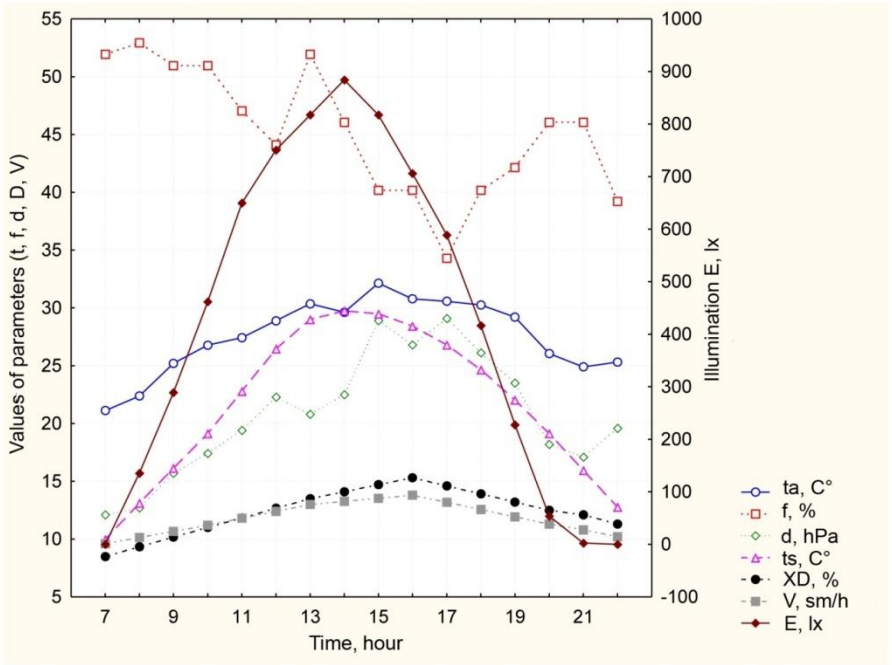
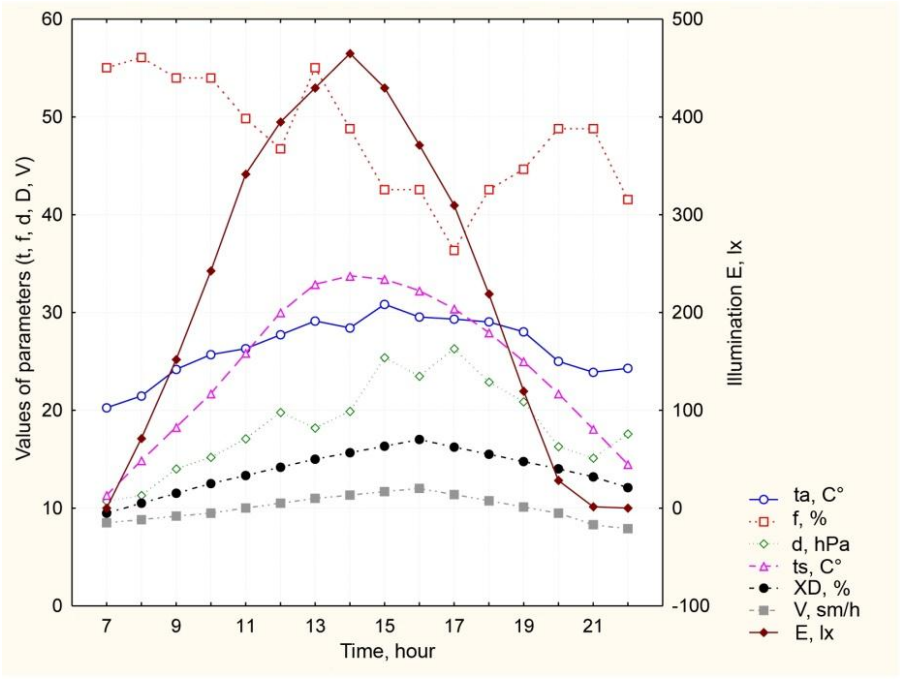


Fig. 1 Diurnal variation of environment and water regime parameters
а) *Cornus mas*
б) *Viburnum tinus*

at – air temperature, °C; f – relative air humidity, %; d – air humidity deficit, hPa; st – soil temperature, °C; E – illumination, lx; XD – xylem deficit, %; V – Linear velocity of xylem stream, sm/h)

a)



b)

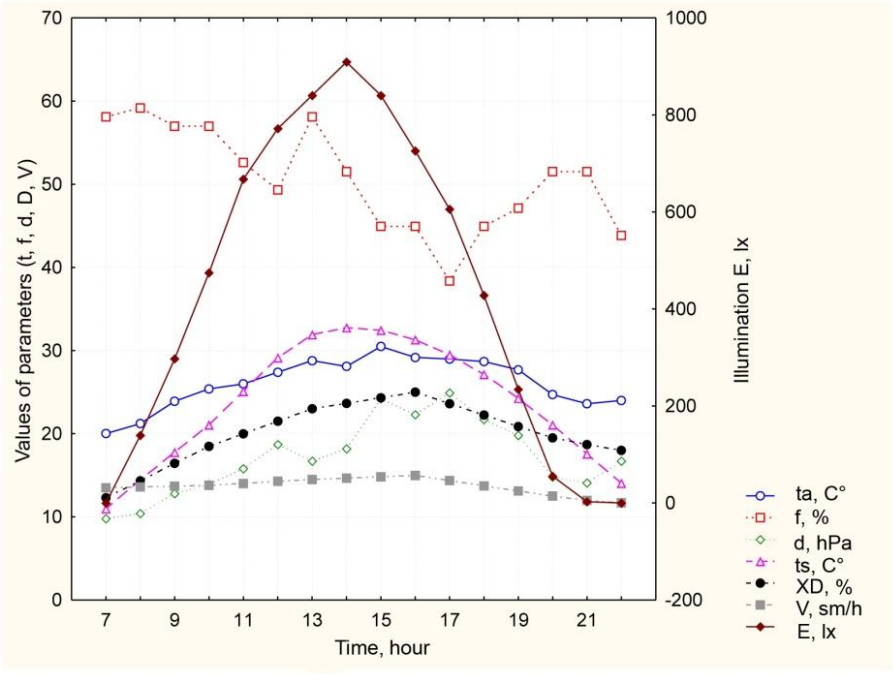


Fig. 2 Diurnal variation of environment and water regime parameters
a) *Euonymus japonica*
b) *Aucuba japonica*

at – air temperature, °C; f – relative air humidity, %; d – air humidity deficit, hPa; st – soil temperature, °C; E – illumination, lx; XD – xylem deficit, %; V – Linear velocity of xylem stream, sm/h)

A number of methods to define relative drought-resistance of plants was developed before. [3,4,5]. In regard to described investigations of 8 plant cultivars xylem moisture deficit is calculated by formula (1):

$$XD = (1 - \frac{A}{Amax})100\% \qquad (1)$$

XD – xylem moisture deficit, %;

A – Current value of heat pulse variation, rel.unit;

Amax – maximum value of heat pulse variation, rel.unit.

In our researches xylem moisture deficit value of study cultivars rates from 14,6% - 25,0% and it was reported as an equal value in decreasing order: *A. japonica*– 25 %, *P. heterophyllum*– 18,9 %, *L. officinalis*– 17,5 %, *E. japonica* - 17,3 %, *B. sempervirens*– 16,6 %, *Ch. praecox* – 16,2 %, *V. tinus* - 15,7 %, *C. mas* – 14,6 %.

To determine common mechanism of variation of water exchange parameters, characterizing quantitative correlations between physiological features of plant water relationships and environmental parameters, it was attempted to create a simulator that allows prognosticate studied correlations extremely accurately. To this purpose equalization of linear multiple regression was equated, where independent variable is environment $X_1 – X_5$ (see below), dependent variable is xylem moisture deficit in the plant stem – XD, %.

1 – air temperature, °C (X_1);

2 – Relative air humidity, % (X_2);

3 – air humidity deficit, hPa (X_3);

4 – temperature of soil surface, °C (X_4);

5 – illumination, lx (X_5).

The equalization:

$$XD, \% = a_0 + a1 \times X_1 + a2 \times X_2 + a3 \times X_3 + a4 \times X_4 + a5 \times X_5.$$

Having substituted environmental parameters (received on 28.08.2014) into equalization, following values for study plant cultivars were obtained:

P. heterophyllum:

$XD, \% = 2{,}8089 - 0{,}5040 \times X_1 + 0{,}1802 \times X_2 + 0{,}5464 \times X_3 + 0{,}2528 \times X_4 - 0{,}0001 \times X_5$,

$R^2 = 0{,}9895$ – coefficient of determination.

B. sempervirens:

$XD, \% = 11{,}69 + 0{,}1636 \times X_1 - 0{,}1478 \times X_2 - 0{,}1888 \times X_3 + 0{,}3488 \times X_4 - 0{,}0065 \times X_5$

$R^2 = 0{,}9479$.

E. japonica:

$XD, \% = 8{,}1090 + 0{,}1101 \times X_1 - 0{,}0806 \times X_2 - 0{,}0304 \times X_3 + 0{,}3594 \times X_4 - 0{,}0070 \times X_5$

$R^2 = 0{,}9793$

Ch. Praecox:

$XD, \% = 3{,}7516 - 0{,}0502 \times X_1 + 0{,}008 \times X_2 + 0{,}1714 \times X_3 + 0{,}4227 \times X_4 - 0{,}0057 \times X_5$

$R^2 = 0{,}9812$

V. tinus:

$XD, \% = 8{,}4648 + 0{,}0994 \times X_1 - 0{,}0986 \times X_2 - 0{,}0369 \times X_3 + 0{,}3651 \times X_4 - 0{,}0034 \times X_5$

$R^2 = 0{,}9717$

Cornus mas: XD, % = 9,0391 + 0,1964×X_1 - 0,1268×X_2 - 0,0837×X_3 + 0,2667×X_4 - 0,0008×X_5

R^2 = 0,9626

L. officinalis:

XD, % = 14,3608 + 0,4695×X_1 - 0,2416×X_2 - 0,3897×X_3 + 0,3380×X_4 - 0,0047×X_5

R^2 = 0,9726

A. japonica:

XD, % = 16,7119 + 0,8671×X_1 - 0,3375×X_2 - 0,6430X_3 + 0,4076×X_4 - 0,0019×X_5

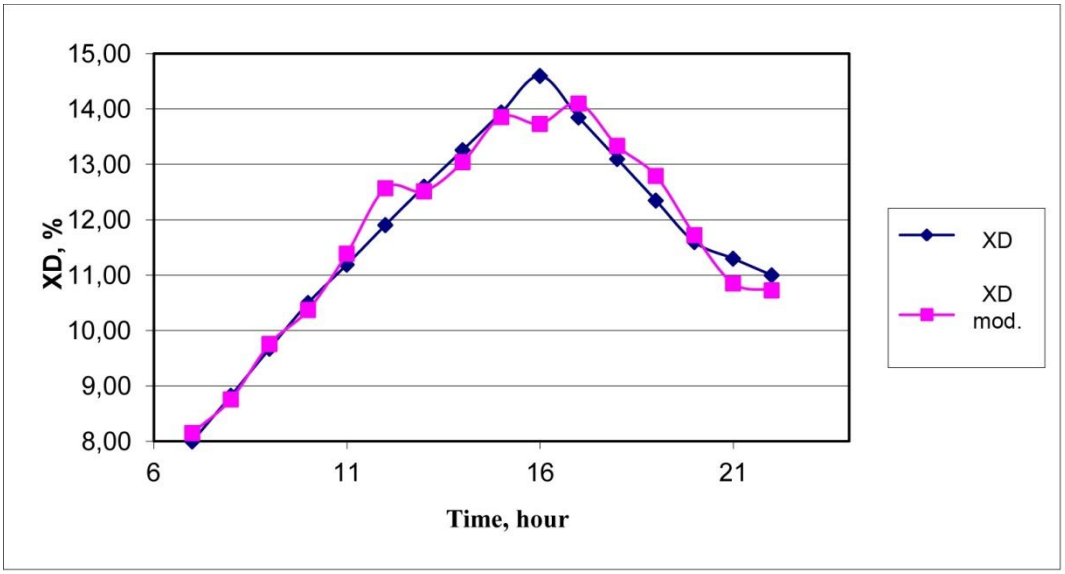
R^2 = 0,9814

Due to analysis of received results, it`s possible to approve a high accuracy of this simulator, range of coefficient of determinations is 0,9479 – 0,9895, what is permissible for biological objects.

Received correlations due to this way allow calculate xylem moisture deficit of the study cultivars in accordance to environmental parameters at any time with frequency of 1 hour.

Figures 3 and 4 present experimental and calculated values of xylem moisture deficit for *C. mas*, *V. tinus*, *E. japonica* and *A. japonica*.

a)



b)

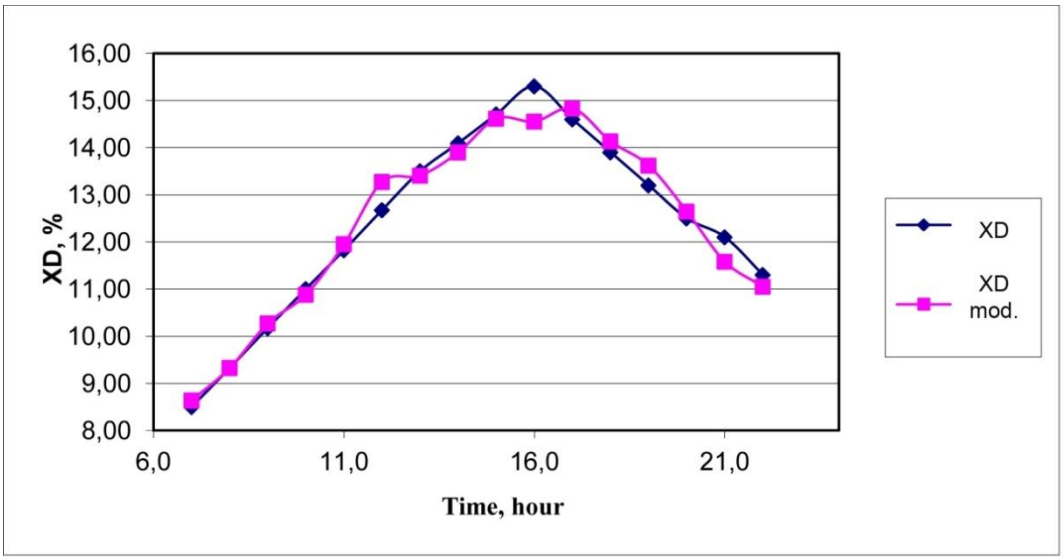
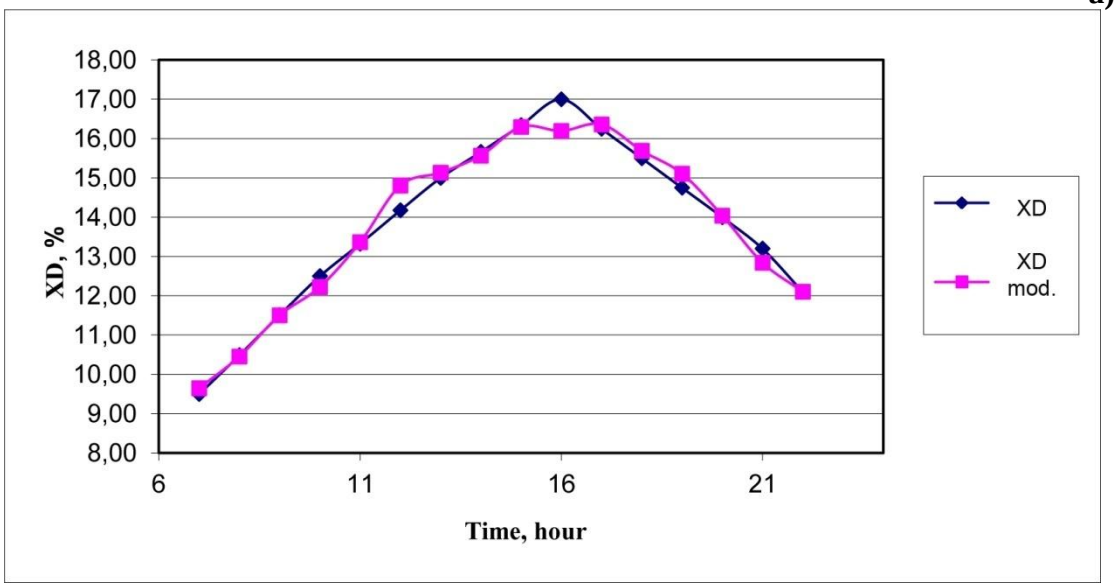


Fig. 3 Diurnal variation of xylem moisture deficit
a) *Cornus mas*
b) *Viburnum tinus*

XD – experimental curve
XD mod. – theoretical curve

a)



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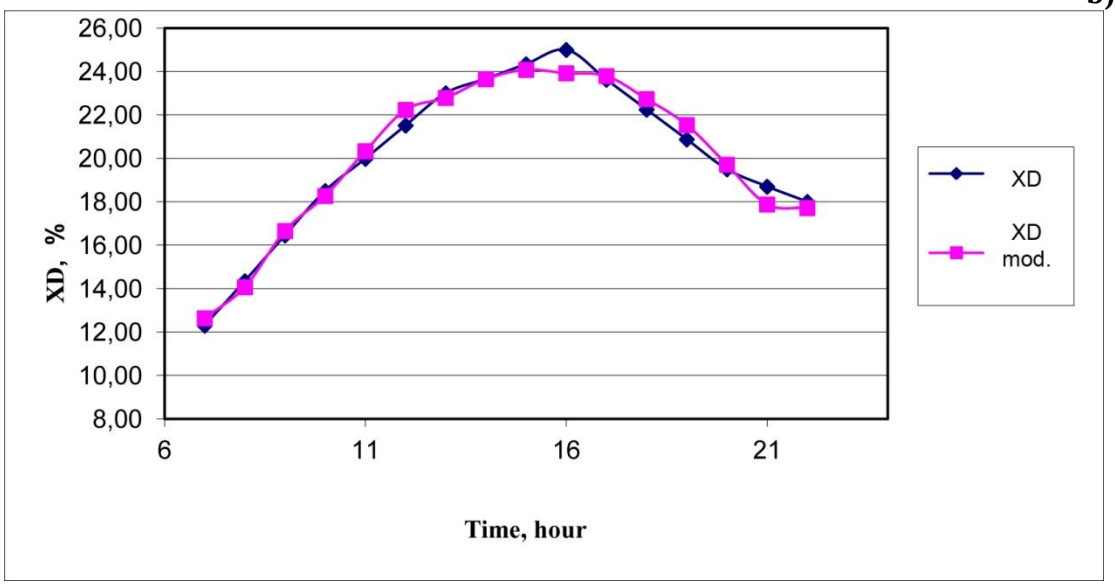

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Fig. 4 Diurnal variation of xylem moisture deficit
a) *Euonymus japonica*
b) *Aucuba japonica*

XD – experimental curve
XD mod. – theoretical curve

Taking into consideration the highest sensitivity of the method for determination of water stress coefficient while researching peculiarities of water regime and draught-resistance, at the same time determining xylem moisture level applying this sensor, we were measuring linear velocity of xylem stream [3]. This parameter allows find out correlation between coefficients of water stress and draught-resistance of study cultivars. The linear velocity of the stream was calculated by formula (2):

$$V=K/t_0 \qquad (2)$$

V - linear velocity, sm/h

K - constant coefficient with a definite configuration of sensor elements;

to – time of pulse advancing between heater and microthermocouple of the sensor (h).

Dependence of xylem stream linear velocity (Vst) to environment (X_1 – X_5 denotations are the same as in equalization of xylem moisture deficit XD) is denoted by regression equation:

For *P. Pittosporum heterophyllum*:

Vst, sm/h = -1,40379 - $1,0550\times X_1 + 0,3832\times X_2 + 0,9943\times X_3 + 0,0893\times X_4 - 0,0010\times X_5$,

$R^2 = 0,9728$ – coefficient of determination

B. sempervirens:

Vst, sm/h = $11,37 - 0,0732\times X_1 - 0,0410\times X_2 + 0,0447\times X_3 + 0,1124\times X_4 + 0,00003\times X_5$

$R^2 = 0,8875$.

E.japonica:

Vst, sm/h = $-0,6976 - 0,5293\times X_1 + 0,2158\times X_2 + 0,5982\times X_3 + 0,1379\times X_4 - 0,00002\times X_5$

$R^2 = 0,9658$

Ch. Praecox:

Vst, sm/h = $2,5629 - 0,4506\times X_1 + 0,1933\times X_2 + 0,5415\times X_3 + 0,0368\times X_4 + 0,0008\times X_5$

$R^2 = 0,8866$

V. tinus:

Vst, sm/h = $6,542 - 0,1909\times X_1 + 0,0549\times X_2 + 0,1934\times X_3 + 0,1945\times X_4 - 0,0004\times X_5$

$R^2 = 0,9873$

C. mas:

Vst, sm/h = $2,2083 - 0,3830\times X_1 + 0,0759\times X_2 + 0,4031\times X_3 + 0,3779\times X_4 - 0,0010\times X_5$

$R^2 = 0,9785$

L. officinalis:

Vst, sm/h = $2,1884 - 0,6604\times X_1 + 0,2669\times X_2 + 0,7351\times X_3 + 0,0761\times X_4 + 0,0019\times X_5$

$R^2 = 0,9573$

A. Japonica:

Vst, sm/h = $5,1957 - 0,6370\times X_1 + 0,2482\times X_2 + 0,6446\times X_3 + 0,0207\times X_4 + 0,0024\times X_5$

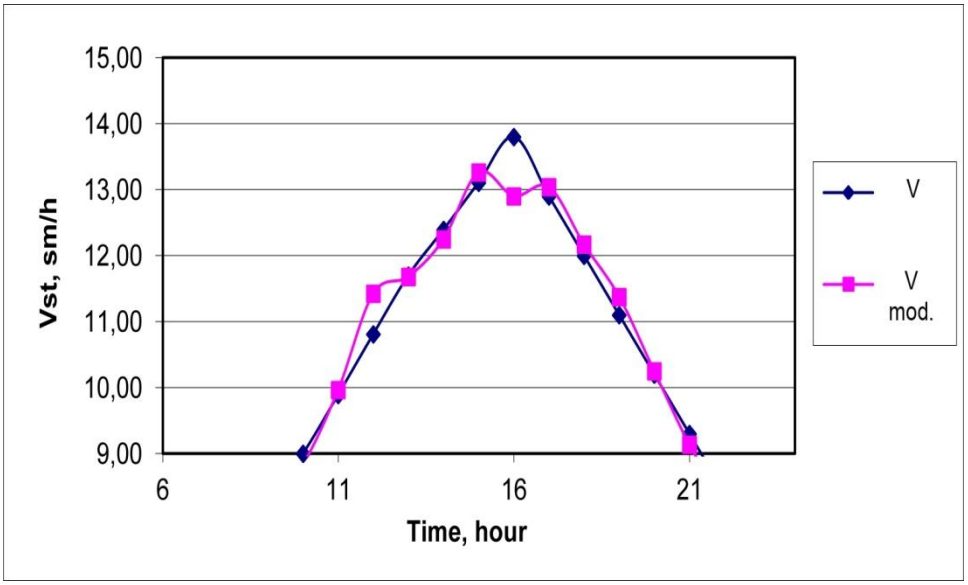
$R^2 = 0,9446$

Water stress coefficient of these cultivars under conditions of water moisture deficit (see linear regression equalization above) ranged from 0,68 (*C. mas*) till 0,90 (*A.japonica* and spread in decreasing order: *A. japonica* – 0,90; *Ch. praecox* – 0,83; *L. officinalis* – 0,80; *B. sempervirens*– 0,72; *E. japonica* – 0,708; *P. heterophyllum* – 0,703; *V.* tinus - 0,69; *C. mas* – 0,68.

Making conclusion of experiments at researching the correlation of xylem stream linear velocity to environmental factors it was denoted that genotypically the most drought-resistant cultivar out of study cases is C.mas, the least drought-resistant is Aucuba japonica.

Figures 5 and 6 show experimental and calculated variations of xylem stream linear velocity during the daylight for C.mas, *V.* tinus, E. japonica and A. japonica.

a)



b)

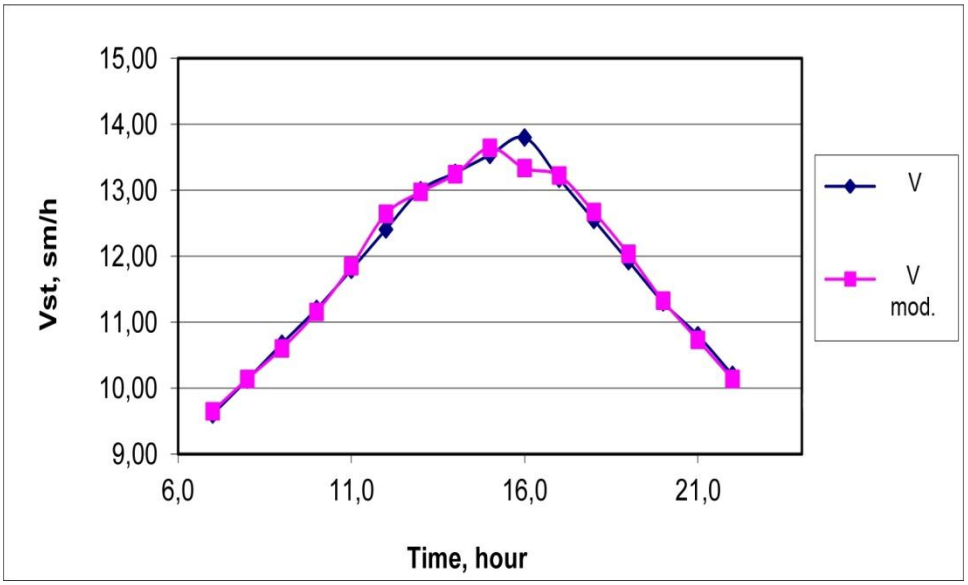
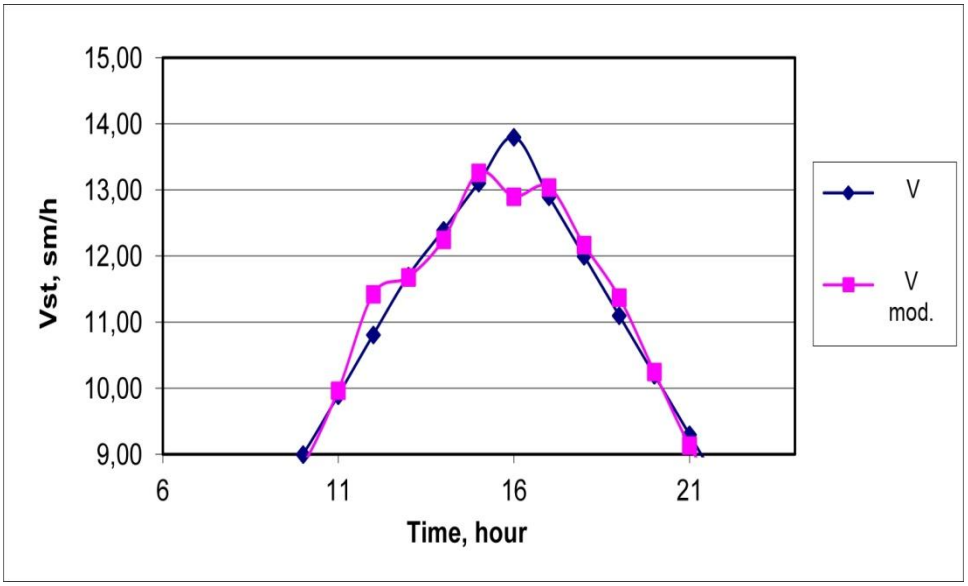


Fig. 5 Diurnal variation of xylem stream linear velocity
a) *Cornus mas*
b) *Viburnum tinus*

Vst. – experimental curve
Vst. mod. – theoretical curve

a)



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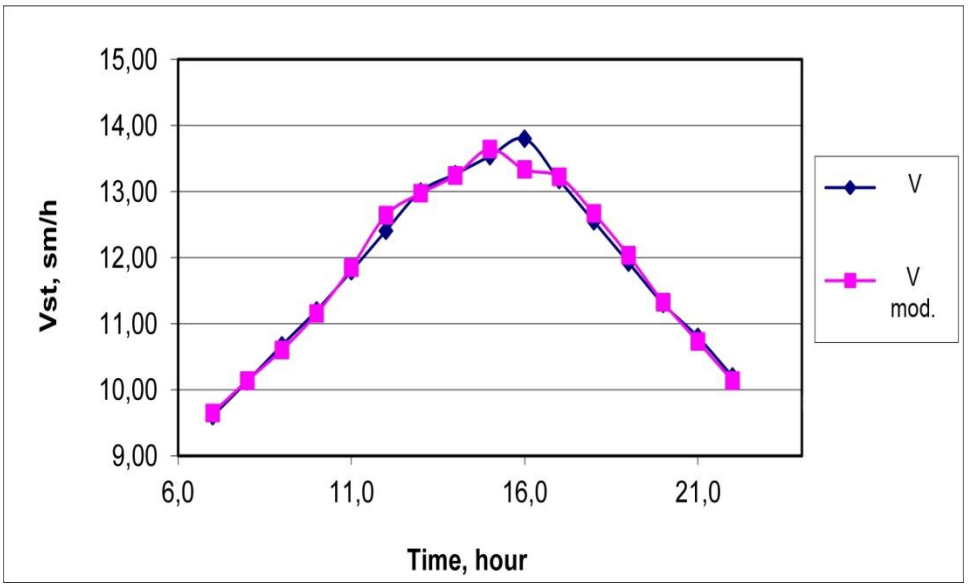

Fig. 5 Diurnal variation of xylem stream linear velocity
a) *Cornus mas*
b) *Viburnum tinus*

Vst. – experimental curve
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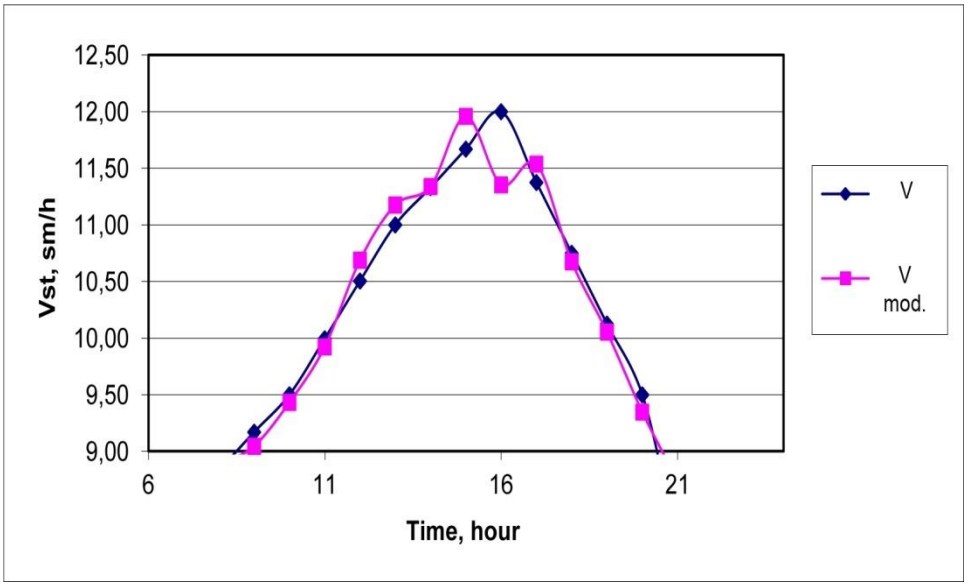

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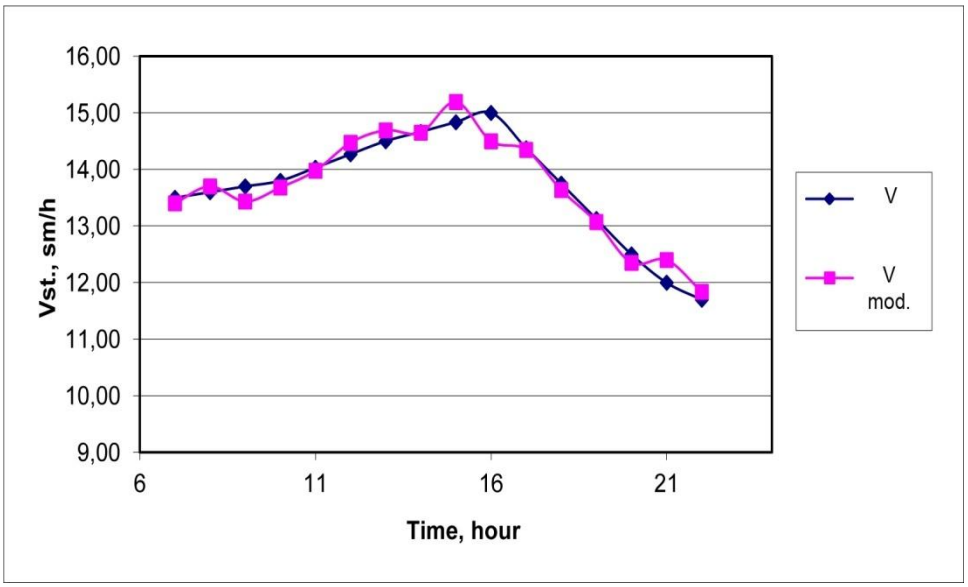

Fig. 6 Diurnal variation of xylem stream linear velocity
a) *Euonymus japonica*
b) *Aucuba japonica*

Vst. – experimental curve

Vst. mod. – theoretical curve

Analysis of curves displays, that difference between experimental and calculated values isn`t larger than 10-15% that is quite acceptably for calculation of biological objects` simulator.

Researches results allow specify well-known scientific facts about peculiarities of water regime and drought-resistance of study plant cultivars and further recommend them for cultivation in a definite geographical region.

Conclusions

1. Assessment of environmental factors changes under crowns of study bush cultivars was carried out (illumination, air temperature and humidity, soil moisture level).

2. Applying phytomonitoring methods allowed investigate physiological characteristics of water regime of the study cultivars and reveal common regularities in their water regime changes correlated to the principle environmental factors. It was presented possibility of phytomonitoring methods appliance in organization of systems analysis of water stress and development of life-time diagnostics methods for researching of plant relative drought-resistance.

3. Rapid methods were suggested to prognosticate water regime and relative drought-resistance peculiarities of study cultivars, one of them was patented (Method for determination of xylem deficit moisture).

4. Correlation between ecophysiological characters of the study cultivars and principal environmental factors were researched, developed dynamic models of these correlations were developed. Difference between experimental and calculated values doesn`t exceed 10-15%, what is quite acceptable for prognostic purposes in Biology.

5. Research results play a considerable role for Biology in general and can be a source of extra information while making a comparative assessment of plant drought-resistance, moreover they can be applied to develop assessment criterion of genotypical drought-resistant cultivars and sorts, what allows differentiate them during future certification.

6. Developed prognostication methods of relative drought-resistance are advisable for applying to assess properties and select the most adaptive species for certain conditions of cultivation.

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Plugatar Yu.V., Ilnitsky O.A., Kovalyov M.S., Korsakova S.P. A dynamic model of the water relationships of some bush cultivars growing in lower layer under conditions of park phytoclimate on South Coast of the Crimea // Works of the State Nikit. Botan. Gard. – 2014. – V. 139 – P. 11 – 28.

This article covers analysis of peculiarities of water relationships and drought tolerance of eight bush cultivars in Arboretum of Nikitsky Botanical Gardens, which grow under phytoclimate conditions of the lower layer. With this purpose two rapid methods of phytomonitoring were applied to get their ecophysiological characteristics. As a result of researches the dynamic models of dependence between ecophysiological characteristics of study plant cultivars and main environmental factors were developed, some peculiarities of their water relationships and drought tolerance were detailed. Revealed dependence makes it possible to differentiate these plant cultivars in accordance to drought tolerance that will allow recommend them for cultivation in the conditions of a particular region on South coast of the Crimea, taking into consideration its microclimatic features.

Key words: *rapid methods, the peculiarities of the water relationships, xylem moisture deficit, relative drought resistance, simulator.*

UDK 582.661.56:581.524.2(477.75)

DISTRIBUTION PECULIARITIES OF *OPUNTIA HUMIFUSA* (RAF.) RAF. WITHIN SEVASTOPOL AREA

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Introduction

Opuntia humifusa (Raf.) Raf. is one of the preferable plants in landscape architecture. It belongs to a group of cactus with American origin, being protected in natural environment on origin land [42, 54], but tending to running wild in many regions of the world. For today there is information about widespread occurrence of this cultivar in Australia and South Africa. In Europe *O. Humifusa* is characterized as an invasive cultivar for Spain, Italy, France, Croatia, Switzerland, Germany, Bulgary [57,58]. On the territory of Russian Federation this running wild cultivar was registered in Northwest Caucuses [22, 23, 34, 35] and in the Crimea [1-2, 5-6, 10,13,14,24,25,27,31,37,40,41 etc.]. As to Sevastopol area data about naturalized opuntia populations was recorded in single publications [5-8, 10, 27, 40] and it can be found online as well [15,32].

O. humifusa was brought under cultivation in the beginning of XIX century by scientists of Nikitsky Botanical Gardens early in its term [1,5-7 and etc.]. Ecological and biological characteristics, that caused its introduction success, made it dangerous and alien cultivar in the Crimean flora after 200 years [5,6]. It is one of the most popular cactus cultivar in the Crimea, often reveals transformer characteristics being an ergasiophyte by introduction way and agrophyte by its adaptation level [5].

Nowadays biological invasions are considered as one of factors causing species diversity reduction [12, 16 and etc.]. Investigation of this problem is extremely urgent. At the same time *O. humifusa* distribution and effect on aboriginal phytocenosis of Sevastopol area were hardly studied. Thorough analysis of its spread peculiarities and dynamic behavior will make it possible to develop scientifically grounded recommendations in planting and population control of this invasive cultivar along the city line.

Objects and research methods

Sevastopol is situated in southwest of the Crimean peninsula, its total area makes 107,96 thousand ha. This region is characterized by diversity of geological, geomorphological, hydrographical and climatic conditions. Sevastopol is an area of different lithologic and stratigraphic complexes covering periods of Mesozoic and Neogene; they include sedimentary and volcanogenic formations [33]. Relief consists of gullies, deep valleys and canyons (Chernorechensky, Sukhorechensky and etc.) [21]. According to agroclimatic zoning [11] Sevastopol region covers seven climatic districts, which are characterized by dry and intermediately hot climate with mild winter. At the same time considerable differences in the coastal zone, intermontane kettles, lowlands and midlands are marked due to altitude above the sea level and prevailing wind effect, that determines temperature regime and rainfall distribution. The soil cover consists of some genetic soil types: brown, rendzina, brown mountain-forest soil, meadow brown, meadow and alluvial soils. The most popular soil type is carbonaceous subtype of brown soil of dry forests and bushes [19, 28].

According to the botanical and geographical zoning scheme, territory where researches took place belongs to Sevastopol region of Gornokrymsky district, its vegetation cover consists of low-stemmed forests and sibljak (*Carpinus orientalis* Mill., *Quercus pubescens* Willd. and *Q. petraea* Liebl. (58% of the area) combined with herbaceous vegetative groups: steppes and savannahs. Light forests with *Juniperus excelsa* M. Bieb. occupy 8,5 % of the total territory and create the largest forest tracts in the Crimea, that determines the region specific [18,33]. Besides there are forests and open woodlands where mainly *Fraxinus excelsior* L., *Pinus brutia* Ten. and *Pistacia mutica* Fisch. et C.A. Mey. grow. Allocation of plant cover has a clearly defined zoning character [33]. Within residential area vegetation has been extremely changed due to anthropogenic factor. Concerning the studied region here are cenoses of following plant categories of ecologofloristic classification: *Quercetea pubescenti-petraeae* (Oberdorfer 1948) Jakucs 1960, *Festuco-Brometea* Br.-Bl. et R. Tx. in Br.-Bl. 1949, *Thero-Salicornietea* R. Tx. in R. Tx. et Oberdorfer 1958, *Festuco-Puccinellietea* Soó 1968, *Juncetea maritimi* Br.-Bl. (1931) 1952, *Asteretea tripolii* Westhoff et Beeftink 1962 in Beeftink 1962, *Bolboschoenetea maritimi* Hejny in Holub et al. 1967, *Phragmito-Magnocaricetea* Klika in Klika et Novak 1941, *Crithmo-Limonietea* Br.-Bl. 1947, *Cakiletea maritimae* Tx. et Preising 1950, *Stellarietea media* R. Tx., Lohmeyer et Preising in R. Tx. ex von Rochow 1951, *Plantaginetea majoris* Tx. et Preising in Tx. 1950, *Artemisietea vulgaris* Lohmeyer, Preising et R. Tx. in R. Tx. 1950, *Onosmato polyphyllae-Ptilostemonetea echinocephali* Korzhenevsky 1990 [3, 9, 17, 36, 38, 49].

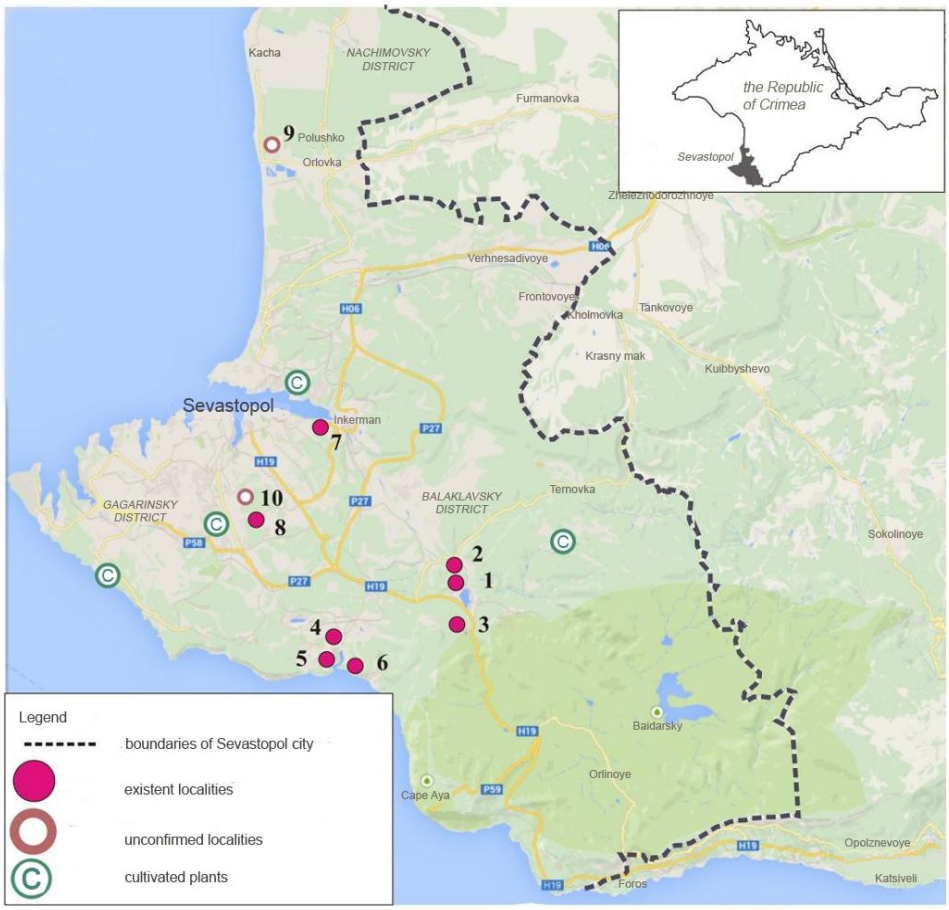
Sevastopol is an area of landscapes joint: Piedmont, the main Range and South Coast of the Crimea. As on Gryshankov G.E. conception four of five Mountain Crimea native zones were marked out: piedmont (three belts), mountain (three belts), mountain meadow yaila (one belt) and South Coast half subtropical (one belt) [33]. As a result of anthropogenic influence present landscapes of Sevastopol are a sophisticated combination of natural non-affected, constructive and derivative landscapes. This territory has got a highly landscaping diversity, where 59 types of locality were found out [33].

Researching of *O. Humifusa* allocation trend across Sevastopol region was carried out during route studies in 2012-2014. Each locality was investigated according to its area occupied by opuntia cenopopulation, number of clumps, morphological plant peculiarities, renewal way. Base characteristics of biotope were described as well (altitude above the sea, slope angle and its exposition, soil type, phytocenosis). Geobotanical inspection was carried out according to J.Braun-Blanquet approach [30,45]. Number of plants was determined on the ground of geobotanical description, made by authors, in accordance with a real classification of the Crimean [9,26,49] and European [56] vegetation. Syntaxon names are presented as on phytosociological nomenclature demands [61]. Taxons names correspond to the Crimean flora checklist [20].

Digital cameras Sony DSC-H1, Sony DSC-H7, Sony DSC-HX200 were applied for photographic surveying. Collected herbarium specimens were transferred to possession of NBG-NSC (Yalta), living plants are preserved in the succulent plant collection of Nikitsky Botanical Gardens.

Results and discussion

As a result of researches in Sevastopol region eight different by size *O. humifusa* localities were described. Their location is presented on the map-scheme (Fig.1). Data from literary sources about opuntia growth around vil.Orlovka [40] and verbal directions about its growth in Sarandinaki gully weren`t confirmed during the field researches.



1-Gasphorta mountain; 2-Telegraphnaya mountain; 3- the Orta-Kaya range; 4-Kadokoiskiye highlands; 5-Tavros mountain; 6-Asketi mountain; 7-Georgiyevskaya gully; 8-Khomutovaya gully; 9-vil.Orlovka; 10- Sarandinaki gully

Fig.1 Map-scheme of *Opuntia humifusa* allocation in Sevastopol region

Base characteristics of opuntia biotopes and cenopopulations spread across different localities of Sevastopol are presented in table 1.

Table 1

Characteristic of *Opuntia humifusa* (Raf.) Raf. localities along the city line of Sevastopol

Locality (1)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Common projective plant cover, %	50 – 90	35 – 70	70 – 75	50 – 90	40 – 90	40 – 90	40 –60	50 – 80
Opuntia projective cover, %	10 – 40	10 – 60	5 – 30	40 – 75	30 – 50	25 – 30	5 – 10	<5
Number of clumps in a locality (2)	+++	+++	+	+++	+++	+	+	+
Renewal characteristic (3)	v,s	v,s	v,s	v,s	v,s	v	v	v
Bearing fruits characteristic (4)	p	p	a-p	p	p	a	a	A
Biotope characteristic								
Locality area, m²	5000	7000	1 – 30	3000 – 5000	from 1 till 1000	10	100	5
Slope angle, degree	0 – 20	10 – 35	0 – 10	20	25	10	0 – 5	0 – 5

Locality (1)	1	2	3	4	5	6	7	8
Slope exposition, degree	135	100, 180 – 200	15 – 20	90	150	180	10	180
Altitude above the sea level, m	205 – 210	30 – 45	185	49	20 – 50	318	85	125
Soils (5)	B, JCh	B, JCh	B, JCh	B, JCh	B, JCh	B,B	B, SCh	B, JCh
Class of vegetation (6)	*Q.pub.-petr. Art.v. K.-Cor.*	*Q.pub.-petr., F.-Br. K.-Cor.*	*Q.pub.-petr. F.-Br. K.-Cor.*	*F.-Br. K.-Cor.*	*Q.pub.-petr. F.-Br. K.-Cor.*	*Q.pub.-petr.*	*F.-Br. K.-Cor.*	*F.-Br. Art.v.*
Modern anthropogenic effects (7)	G, L, AE	AE	AE, F, R	F, G, L	L, B	R,L,F	R ?	G, R
Area exploitation before	+	+	?	+	+	?	+	+

Notes.

1. Localities site: 1- Gasphorta mountain; 2 – Telegraphnaya mountain (villages Khmelnitskoye, Chernorechiye); 3 – the Orta-Kaya range, "Toropova dacha"; 4 – the Kadikoiskiye Highlands, 5 – Tavros mountain; 6 – Asketi mountain (Balaklava); 7 – Georgiyevskaya gully; 8 – Khomutovaya gully, "Maksimova dacha" (Sevastopol).
2. Number of clumps in a locality: + single (1-5); ++ - numerous (6-50); +++ - over 50 clumps.
3. Renewal characteristic: v – vegetative; s – seed.
4. Bearing fruits characteristic: a – average (1-3 fruits on a section); p – plentiful (5-12 fruits).
5. Soil: B, JCh – brown, rubbly, on Jurassic limestone; B, SCh – brown, rubbly, on sarmatian limestone; B, C – brown, on conglomerates.
6. Plant class: *Q.pub.-petr. – Quercetea pubescentis-petraea; F.-Br. – Festuco-Brometea, Art.v. – Artemisietea vulgaris, K.-Cor. – Koelerio-Corynephoretea.*
7. Type of anthropogenic effect: G – grazing, L – littering, AE – unauthorized excavation, F – fire, R – recreation, B – disorder of the plant cover as a result of building.

Almost all localities were exploited with various economical purposes (according to the evidences). The economical activity was changing gradually or stopped for a long period. During the Crimean war (1853 - 1856) on Telegraphnaya mountain there was an optical telegraph station belonged to the Italian army, not far from vil.Kadykoj and Kadikojskiye Highlands (today it`s Kadykovka, a part of Balaklava district, Sevastopol city) military base of the British Expeditionary corps was situated, Georgiyevskaya gully was in use by the French and English armies. In the beginning of XX century Tavros mountain and Khomutova gully were the location of country cottages, both slopes were terraced. In XIX century Khomutova and Georgiyevskaya gullies were quarried. But only one locality Gasphorta mountain was noted as a place of *O. Humifusa* planting during Italian cemetery reconstruction in 1882 [27,39]. Origin of *O. Humifusa* spread in other localities is still unknown.

Various sorts of opuntia were brought to Europe from America in the end of XV and by middle of XIX century it had got a widespread over European collections and gardens. In VII – XVIII centuries they started cultivation of the opuntia in South Africa and Australia, in XX century most sorts became invasive in South Africa and Australia, countries of Central and Western Europe [48,50,51,57,58]. Most probably *O. Humifusa* was carried in Sevastopol due to foreign soldiers during the Crimean war regardless of introduction works of Nikitsky Botanical Gardens. They could plant it as an easy growing ornamental evergreen plant on military burial areas or cultivate it with nutritional and medicine purposes. Useful properties of this opuntia cultivar were approved by modern scientists [47].

Study of opuntia growing in different cenopopulations of Sevastopol region made it possible to reveal some peculiarities in the species morphology and biology under conditions of secondary natural habitat. *Opuntia humifusa* is characterized by diffusive and contagious

allocation in phytocenoses; either a single plant or its group can form clumps from 1-3,5m across diameter, created by related specimens with vegetative and generative origins. In time some clumps can join and create dense growth.

O. Humifusa plants grow on the ground surface or rise 5-10 sm above it. Articulated shoots consist of flat and pulpy segments (cladodes). The leaves are small, pulpy, round a little pointed 03, - 0,5 sm by length and about 1 mm by thickness; they develop in areolas at the beginning of vegetative and generative plant shoots and soon fall off. Segments have various form: flat, from almost round (2-8sm across diameter) till inversely egg-shaped ((5)7 – 10(15) sm by length, (3)5 – 6(7) sm by width, 0,5 – 0,8 sm by thickness; their color is light green or subdued green, often with diametrically rugose surface (fig.2, 3). Oval areolas with light grey stands of wool are up to 4 in one diagonal row. A number of glokhidiums in areolas is not very large, irregularly spaced on a crescent-shaped place at the top of areola, along the perimeter or by bunches along the areola; its length is 1-2 sm, but close to the lower segments their number and length increase; glokhidiums are fawn-colored. Barbs, as a rule, are absent; they are located at the top of the segment and along its border (fig.3). Flowers of opuntia are bright yellow or lemon-colored (Fig.4), petals are orange at the bottom, 2-3 sm with ovary, 7-8 sm across diameter. Its blossoming occurs from the end of May till the middle of June. Fruits ripen in October – November. Having ripened most of fruits fall off, but late set fruits often stay on the plant during the whole winter till the next blossoming period. Fruits form is various from almost round pear-shaped till oblong top-shaped (fig.2:4), but fruit form of the same plant is quite stable, that proves genetic determinancy of this characteristics. Fruit size depends upon inherited peculiarities and environmental conditions; they can range significantly: from 2 till 5 sm by length (average size 3-3,7sm) and from 1-2,3 across diameter (on average1,4 – 1,7sm) in the thickest part. The fruit top is a little foveate (deeping size is 2-3 sm). 10 fresh fruits weight 30-60 g. Fruit color ranges from grey-pink till purple and bordeux; pulp color is various as well from light-pink till dark pink or pink-purple. Fruits are eatable, pulpy, sticky sweet or sour-sweet. Each fruit contains 5-40 seeds (av.14-17). A plant consisting of 20-25 segments can bear till 70-100 fruits. In some populations (Gasphorta mountain, Kadykoiskiye Highlands) proliferous fruits were found out (Fig.2:5). The seed characteristics: comparatively big-sized, 3,8 – 5,0 mm across diameter; 2,4 – 2,6 mm by thickness; grey and beige-colored; rounded kidney-shaped, slightly flattered with a narrow border (fig. 2:6); successful sprouting in Sevastopol environment.

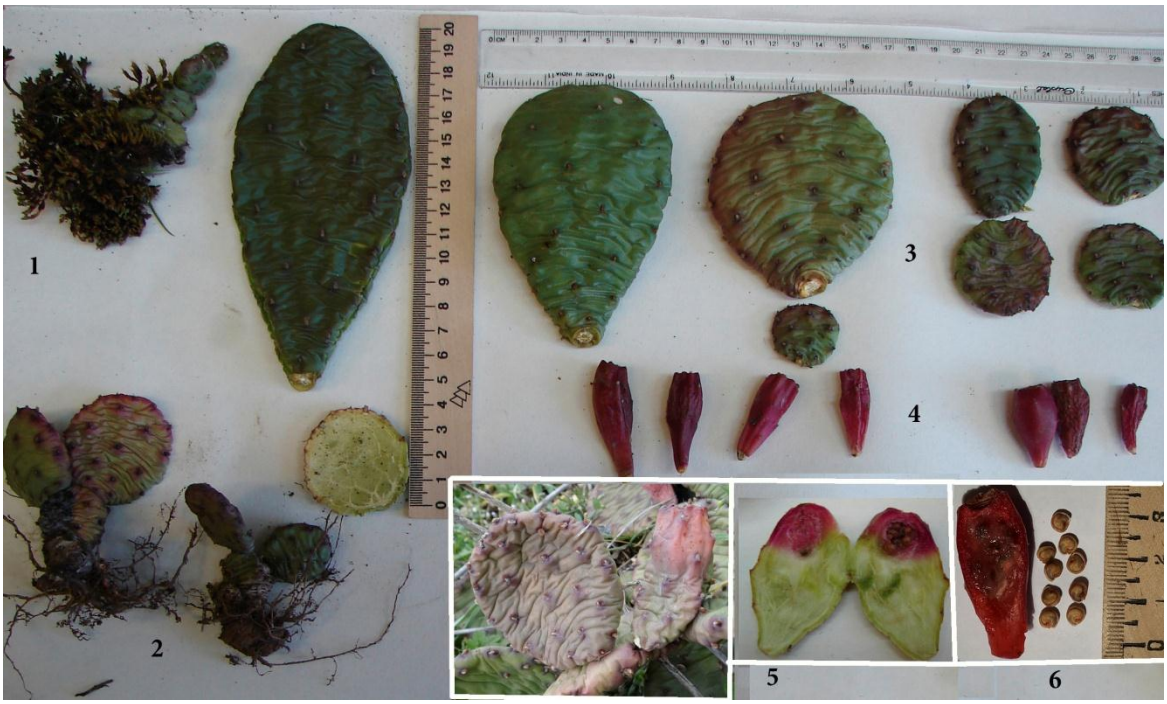


Fig.2 Morphological characteristics of *Opuntia humifusa* plants from cenopopulations growing on Telegraphnaya mountain within outskirts of vil.Khmelnitskoye and Gasphorta mountain within outskirt of vil.Oboronnoye

1 –seed origin plants; 2 – vegetative origin plants; 3 – cladode (segments); 4 – fruits; 5 – proliferous fruits, 6 – seed and fruit in section

Morphological parameters of described plants modify according to environment. In open biotopes the most part of cladodes has a rounded shape and small size (on average 5-6sm across diameter). Plants blossom and fructify intensively (on average 7-10 flowers and the same number of fruit on a segment). As a rule fruits are numerous, ripening simultaneously, rounded pear-shaped 2,5 – 3,5 sm by length and 1,7 – 1,8 sm across diameter; correlation of the length and diameter is 1,2 – 1,6. In close herb assemblages or under tree and bushes crown segments become much longer till 11 – 15sm, have inversely egg-shaped form and narrowed at the bottom. Blossoming and fruit-bearing process is marked out only on some segments which have about 1-4 fruit sets on average. Fruits have a long form 3,2 – 4,5 sm by length and 1,5 – 1,8 sm across diameter, correlation of length and diameter is 2,1:2,6. It`s a common case to meet still green fruits in November – February..

Having investigated almost all localities it hasn`t been found any barb on the plant segments. But in a cenopopulation on Telegraphnaya mountain along with typical *O. humifusa* plant, which shoots don`t have barbs, there are some plants having barbs. Barbs are strong and straight, till 2,5 – 3 sm by length, white and yellow colored with a dark top, located on the upper areolas in one instance. Segments of these plants are oval, thicker in comparison with segments of barbless plants growing next to them under the same conditions (Fig.3). In our opinion, these specimens can be referred to the close taxon *O. pollardii* Britton et Rose, which is sometimes [44] considered as synonym with *O. humifusa*, but mostly it is distinguished as a separate species [46,53,55,59,60] or a subspecies of *O. humifusa* subsp. *pollardii* (Raf.) Majure [52]. Plants with such characteristics weren`t found out in any other locality of Sevastopol city and the Crimean peninsula in general, moreover they are not presented in the succulent collection of Nikitsky Botanical gardens. To clarify points of taxonomy and origin of non-typical form opuntia plants in area of Telegraphnaya mountain, extra investigations are necessary, including genetic studies.

Fig.3 Morphological peculiarities of *Opuntia humifusa* plants from cenopopulation on Telegraphnaya mountain within outskirts of vil.Khmelnitskoye
A – plant habit view (12.04.2014); B,C – blooming (19.06.2014) and deflored (25.06.2014) plants; D – seeds (26.01.2015)

Biology of the same cultivars in different condition has some distinctions. A vegetative renewal, blossoming and bearing fruits are noted in all cenopopulations, but in spite of a high seed productivity, sprouts and juvenile specimens are revealed in small number and only in 5 localities (see table 1, fig. 2:1; fig.4). Obviously, there is a tendency of weak seed sprouting and considerable plant death rate on different stages of ontogenesis, probably caused by dry conditions and high temperature of soil surface.


1


2

Fig.4 *Opuntia humifusa* plants with seed origin
1 – immature specimen, Tavros mountain; 2 – sprouts and juvenile specimens, Kadykoiskiye Highlands

Having summarized all data it`s possible to conclude, populations of wild *O. humifusa* are found mainly in southwest of Sevastopol region in biotopes of two types. The first type is in localities № 1-6: Gasphorta and Telegraphnaya mountains, the Orta-Kaya range, the Kadykoiskiye, Highlands, Tavros and Askety mountains (table 1, fig.1,5). They are situated on western spurs of the main Crimean mountain range (on the Balaklava Highlands) in hypsometric scope from 20 – 320 m above the sea level on the upper Jurassic rocks – limestone and occasionally conglomerates, on watersheds and by watershed surface, on

abrupt and middle angled prominent and ladder-shaped slopes of different expositions, mostly of southern rhumbs, as a rule well-illuminated, in juniper and oak light forests on the rock and stone expositions and skeletal carbonaceous variations of brown soils. It is well-known, a big concentration of the limestone of Main mountain range, connected with their metamorphic characteristics, determines peculiarities of soil forming process, water regime and surface flow of water [28]. Limestone fracturing degree causes accumulation of a strong thick layer with stony and detritus weathering products at the bottom of rocks and cliffs, also screes are common phenomenon on slopes, gravitational flows and other factors, which obviously forward favorable conditions for cladodes establishment and seed renewal of *O. humifusa.*

Cenopopulations of localities № 1-6 (see table 1) occupy area from several till seven thousand of square meter with opuntia projective cover from 5 till 75%. On the slopes of Tavros mountain there are seven different by area groups. Population size is determined due to favorable for cactus growing areas and probably occurrence term of this invasive cultivar there.

The most typical habitat for *O. Humifusa* in Sevastopol region is ecotone zone covered by melkozyom or a thin layer of skeletal soil between rock expositions of the upper Jurassic limestone and glacis or middle-angled slopes with well-developed soil cover, where petrophyt-steppe and forest vegetation grows. As a rule this zone is occupied by plants of phytocenoses of *Alysso alyssoidis-Sedetalia* Moravec 1967order *Sedo-Scleranthenea* (Br.-Bl. 1955) Dengler in Dengler et al. 2003 subclass *Koelerio-Corynephoretea* Klika in Klika et Novák 1941 class. A distinctive feature of the assemblages is a succulent considerable role in their floristic structure and projective cover that certifies favorable characteristics of such ecotopes for this living form. In phytocenoses of *Alysso-Sedetalia* order, succulent family Crassulaceae J. St.-Hil. dominates, but obviously for Cactaceae Juss. cultivars and *Opuntia* genus particularly these conditions are quite congenial.

The Kadykoiskiye Highlands

Tavros mountain

The Orta-Kaya range (by Svyrina S.A.)

Asketi mountain

Gasphorta mountain

Khomutovaya gully

Telegraphnaya mountain

Georgiyevskaya gully

Fig. 5 *Opuntia humifusa* in different localities of Sevastopol region

At the bottom of the slopes where opuntia segments occur due to gravitational process and sometimes establish, its cenopopulation is a part of other phytocenoses: petrophyte

steppes of *Festuco-Brometea* class with prevailed *Bothriochloa ischaemum* (L.) Keng. and *Asphodeline lutea* (L.) Rchb. or forest and light forest phytocenoces of *Quercetea pubescenti-petraeae* class including juniper light forests belonged to the union *Jasmino-Juniperion excelsae* Didukh, Vakarenko et Shelyag 1986. In extremely damaged plant cenoses typical cultivars of *Artemisietea vulgaris* class is considerably marked out. Moreover in these phytocenoses besides aboriginal cultivars, invasive ones of the Crimean flora are frequently found [4] : *Ailanthus altissima* (Mill.) Swingle и *Rhamnus alaternus* L. Here due to lack of illumination and competition with arboreal and bush breeds and large polycarpic herbage, characteristics of specimens vitality in *O. Humifusa* populations are lower: their blossoming is not so intensive, less fruits and seeds, not all fruits ripen completely. Populations within Asketi mountain possess the worst vital parameters, probably because of considerable altitude above the sea level and peculiarities of the underlying rocks – conglomerates with a low-level of water permeability in comparison with limestone rocks; all these factors create not quite favorable hydrothermal conditions for opuntia.

Another biotope including localities № 7 and 8 (Georgiyevskaya and Khomutova gullies) (table 1, fig.1,5) is situated in the piedmont zone on the true altitude 85 – 125 m. This biotope is presented by glacises of gullies inside of Sarmatian limestone deposits, covered by more or less well-developed rendzina or brown soils. Specific characteristic of soil-forming geological material is clayey gravelly texture of soil, not high consistency and stable alkali reaction [28]. Vegetative cover was formed by petrophytes and typical stipa-fescue steppes, belonged to *Festuco-Brometea* class. Opuntia populations are small-sized on this area, they don`t occupy large territories and possess low projective cover, the blossoming and bearing fruits is not so intensive, only vegetative propagation is noted. To our opinion, such vital parameters of area caused by competition with components of steppe phytocenoses first of all, by considerable isolation of these assemblages for alien cultivars and probably less favorable edaphic and climatic condition for opuntia growing.

Perhaps other landscapes of Sevastopol territory are not quite favorable for opuntia as well. In this way location of cenopopulation, on border with steppe zone where winters are more severe, in addition to anthropogenic factor (building of a cottage village) could make a contribution to the population vanishing, that was known before by literature sources from village Orlovka outskirts [40].

No matter that at present most of well-developed cenopopulations in Sevastopol region are marked out in degraded cenoses, characterized by different invasion level, otherwise an ability to adapt alien cultivars in their composition, invasion of *O. Humifusa* into natural phytocenoses represents considerable danger for keeping of vegetative cover parameters in general and populations of certain rare and protected cultivars. Almost in all cenoses it competes with being in need of protection *Asphodeline lutea* in the Crimea. Moreover spreading of opuntia can cause decreasing and even vanishing of cenopopulations, typical for these cenoses cultivars, included into Red Data Book of Russian Federation [29], such as *Paronychia cephalotes* (M. Bieb.) Bess., *Genista albida* Willd., *Iris pumila* L., *Anacamptis pyramidalis* (L.) Rich., *Asphodeline taurica* (Pall.) Endl., endemic taxons of the Crimean flora as well (*Stipa eriocaulis* Borb. subsp. *lithophila* (P. Smirn.) Tzvelev, *Dianthus marschallii* Schischk., *Satureja taurica* Velen., *Asperula supina* M. Bieb. subsp. *caespitans* (Juz.) Pjatunina, *Veronica taurica* Willd. subsp. *taurica*).

Conducted researches permitted to find out, that opuntia plants growing in Sevastopol regiongot over geographical, ecological and biological barrier, possess highly adapted abilities, in most localities have highly vital parameters, clumps size reaches 1,5 – 3,5 m across diameter, in places create unbroken growth. The highest invasive potential was revealed in cenoses with lighted plant cover on well-drained, but regularly moistened by precipitation or condensate moisture steep slopes and glacises of southeast or southern

exposition on altitudes 30-200 m above the sea level in juniper light forests and petrophyte steppe phytocenoses. In both cases, intended and random introduction of *O. humifusa* into favorable conditions will result its spreading and adaptation in other places in Sevastopol area, partly in juniper light forests in Baidarskaya valley, Rodnoye and Rezervnoye villages, in petrophyte and steppe cenoses of Fiolent cape territory and other places of the Gerakleisky peninsula.

Conclusions

1. At present eight localities of *O. humifusa* were found out in Sevastopol region: on Gasforta, Telegraphnaya, Tavros, Asketi mountains, Orta-Kaya mountain range, Kadykoiskiye Highlands, in Georgiyevskaya and Khomutovaya gullies.
2. Opuntia cenopopulations are found in biotopes of two types: on upper Jurassic rocks in juniper light forests in cenoses of *Koelerio-Corynephoretea*, *Festuco-Brometea* and *Quercetea pubescenti-petraeae* classes and on Sarmatian limestone in steppe phytocenoses of *Festuco-Brometea* class. Conducted biomorphological and populational researches revealed the first mentioned biotype is more favorable for *O. humifusa* growth.
3. *O. Humifusa* plants in the investigated region have higher vital parameters. All researched populations keep up blossoming, bearing fruits and vegetative propagation, in five localities seed propagation is marked out.
4. An additional study is essential to specify taxonomic location and origin of some atypical forms of *O. Humifusa* in Sevastopol region.

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Bagrikova N.A., Bondareva L.V., Ryff L.E. Distribution peculiarities of *Opuntia humifusa* (raf.) Raf. within Sevastopol area // Works of the State Nikit. Botan. Gard. – 2014. – V. 139 – P. 29 – 42.

This work presents data about distribution of *Opuntia humifusa* (Raf.) Raf. within Sevastopol territory. During this research eight localities were described, the characteristics of the plant morphological parameters and some biological features were revealed. Analysis of the current status of the populations, description of various communities with opuntia permitted to determine its highest status of naturalization and refer it to the transformer cultivars. This species successfully self-renews by vegetative and seed ways and trends to further dissemination.

Key words: *invasive cultivars, Opuntia humifusa, Sevastopol, the Crimean peninsula.*

UDK 582.661.56:581.524.2 (477.75)

INVASIVE CULTIVAR *OPUNTIA LINDHEIMERI* ENGELM. GROWING IN SOUTH CRIMEA

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Introduction

Genus of *Opuntia* Mill. is one of the largest genera of cactaceous, including from 90 till 250 cultivars according to different authors [7, 9, 20, 39 – 41, 43, 44]. No less than 27 cultivars are characterized as invasive in different parts of the Earth [44]. The first cultivation of opuntia in the Crimea happened in the beginning of XIX century in Nikitsky Botanical Gardens. The first adaptation of the opuntia on the peninsula occurred in the middle of the same century. Taking into consideration that till the middle of last century wild cactuses were found only on areas of foreign army burials dated by the Crimean War period and on old German cemeteries, these facts are obviously connected not only with considerable introduction in the parks, but a real tradition of some European peoples to bed these evergreen attendance free plants on cemeteries. The first data about opuntia running wild in the Crimea are presented in works of the first half of XX century [1, 2, 15]. Afterwards opuntia naturalization was noted by other authors. The most part of publications deals with *O. humifusa* (Raf.) Raf (or *O. vulgaris*, *O. opuntia*) [1, 4, 15 – 17, 27, 28, 35, 37, 38 and etc.] and single works note spontaneous sprouting of some other cultivars [4, 5, 8 – 12, 14, 25, 26, 31, 38]. In 2012-2013 four cultivars of opuntia were marked out as alien plants in the Crimean flora [3, 23], in 2014 – eight taxons [6], in accordance with researches of 2014 within grant RFFI, we can say about naturalization from nine to ten genus representatives, but nomenclatural position of some taxons demands some clarification.

Research objective is to specify systematical location, assess population present state, character and spreading tendencies of one of the most dangerous invasive cultivars in South Crimea.- *Opuntia lindheimeri* Engelm

Objects and research methods

Research objects are cenopopulations and single specimens of *O. Lindheimeri*, spontaneously sprouting on South Coast of the Crimea within the following areas: Foros, Simeiz, Gaspra, Cape Martiyan, Gurzuf, Artek, Ayu-Dag piedmont, Cape Plaka, outskirt of Lazurnoye, Solnechnogorskoye. Some parameters and characteristics were taking into consideration in introduction populations in Simeiz, Livadiya, Nikitsky Botanical Gardens, Solnechnogorskoye, Malorechenskoye. Investigation of *O. Lindheimeri* population on areas of Artek, Cape Plaka was launched in late 1980 – 1990, further study of the cultivar naturalization in South Crimea was conducted in 2013-2014.

To find out places of opuntia populations method of route and reconnoitering studies was applied, literature and Internet sources and verbal communication of other specialists were in use as well. Morphological parameters of plants were researched applying traditional methods as in natural as under conditions of laboratory. Surveying was conducted by digital cameras Canon PowerShot SX130 IS, Sony DSC-H1, Sony DSC-HX200. Identification of opuntia was carried out due to standard reports, accepted for Cactaceae Juss. family [39 – 41], analysis of modern on-line sources [45]. Nomenclature corresponds to international database IPNI [47]. Native flora was defined using “Identification guide for Crimean higher plants” [32] and other sources. Their nomenclature corresponds to accepted nomenclature for

last checklist of the Crimean flora. [23]. Plant syntaxon names are presented according to international requirements [48].

Most of study localities of wild O. Lindheimeri are in the central part of South Coast of the Crimea; according to system of physical and geographical zoning this territory belongs to the western district of the Crimean South Coast of the Submediterranean [34]. The Local climate is Mediterranean, subtropical, dry, hot, with moderately warm winter. Average annual air temperature is 12-14°C. Temperature in the warmest month (July or August) makes 23-25°C, in the coldest months (February) is +2,5 – +4,5^{0}C. Absolute minimum makes –15–17^{0}C. Annual precipitation is 550mm, the most part falls in cold season [13]. Soils on weathering product of clay slate, limestone and igneous rocks are brown. Single localities were found out in the eastern part of South Crimea, belonged to the Eastern district of the Crimean South Coast of the Submediterranean, where winters are a little colder and annual precipitation is less. In accordance to floristic zoning this area belongs to Krymsko-Yuzhnoberezhny region of Yuzhnokrymsky district of Krymsko-Novorossijskaya subprovince of Evksynskaya province of Sredizemnomorskij region [23]. According to geobotanical zoning Mountain Crimea belongs to Sredizemnomorskij region of sclerophyllous forests, maquis, sibljak, phryganas and tomillares [21]. Being in the system of high-altitude zonation South Coast of the Crimea is in the belt of maritime xerophytic juniper and oak forests and shrubs [34], or in the lower forest-steppe belt of hemixerophilous forests, xerophilous lighted forests and savannahs of southern macroslope of Crimean mountains [19].

Research results and discussion

While researching present situation of biological diversity in the Crimea, it was found out that invasive activity of some representatives of *Opuntia* genus has been increased in recent years. Till the middle of XX century there were notes about only one cultivar running wild - *O. humifusa* (Raf.) Raf. (syn. *O. rafinesquii* Engelm, *O. humifusa* Raf., *O. vulgaris* Haw. non Mill., *O. mesacantha* Raf., *O. opuntia* (L.) Karsten, *O. caespitosa* Raf., *O. compressa* McBride, *O. intermedia* Salm.-Dyck, *O. nana* Vis., *C. compressus* Salisb. *Cactus humifusus* Raf., *Cactus opuntia* L. with different varieties [9, 20, 24, 41, 43, 45 and etc.]). After scientific researches (1930-1960), conducted in Nikitsky Botanical Gardens, in cultivation and adoption of different winterhardly cultivars of opuntia in green building within parks of sanatoria and populated localities of South Coast of the Crimea, introduction populations of these plants occurred there. Further concerning some localities the cultivars of opuntia adopted and started their distribution outwards the cultivated places, invaded into natural biotopes as well. This fact refers opuntia to adventive or invasive plants on occasion.

At present one of the most popular opuntia species either cultivated or wild in the Crimea is *O. lindheimeri. (Fig.1)*

Fig.1 Explored places of introduced and spontaneously sprouted plants and cenopopulations of *Opuntia lindheimeri* in South Crimea

Brief morphological description of this species, cultivated in Batumi, was presented by Zamyatnin B.N. in Russian [24], but we suppose it`s necessary to characterize peculiarities of some plants naturalized in the Crimea. Under conditions of South Coast of the Crimea *O. Lindheimeri* is a large succulent plant, height of 0,5 – 1m. Due to more intensive shoot growing horizontally, inherent for this species, in comparison with growth rate verticall , a specimen can make a clump of 1-2m across diameter (fig.2A). With time separate clumps are able to unite and create growth; populations occupy any available free of trees and shrubs territory. Shoot segments are rounded or inversely egg-shaped, large, (10) 17 – 30 (39) sm by length, (9) 15 – 20 (23) sm by width, 0,7 – 1,0 sm by depth, light green or dull green, occasionally with lighted dove-coloured shade in autumn and winter (after fall of temperature close to 0^0C) often gain yellowish and reddish anthocyanin shade (Fig. 2F). Leaves, typically for all opuntia genus representatives, are pulpy, slightly pointed and round reaching 1 sm by length and 2 mm by depth; leaves develop in areolas at the beginning of vegetative and generative shoots regrowth, quite fugacious. Areolas are large, oval sized 5 – 6 x 3 – 4 mm, with brown stands of wool with spacing place of 3-4 sm from each other, one diagonal row has 4-5 areolas. Barbs are all over the whole segment excluding bottom areolas on the flat surface – (1) 2 – 3 on each areola, on the border there can be 3-5. Barbs are quite large (till 4,7 sm by length, 1 mm by depth), hard, sometimes have perpendicular disposition according to segment surface, but more often bent at an angle of (30) 45 – 60 ° towards it, often slightly arched, flatted at the bottom, quite often twisted round at an angle of 90 - 180°. Barb color at the bottom of segment is red and brown (“rusty-coloured”), barbs located higher are yellow and amber-coloured, that become lemon-coloured at the point; there are dark ring-shaped structures. Glochidia are not numerous, placed on crescent-shaped line at the top of areola, 1 – 2 (4) mm by length, green and brown with reddish (rusty) shade (Fig. 2. B,F; 3.C). Flower size is 5 – 7 sm by length (with ovary), 7 – 9 sm across diameter, perianth leaves (petals) number is 12 – 15, 15 – 18 mm by length, 15 – 21 mm by width. They are bright-, dark – or orange-yellow (Fig.2. E; 3.A), rarely orange-red, before wilting periods flower colour gets a bit darker. Blossoming period under conditions of South Coast of the Crimea is in the end of May – beginning of June. Fruits ripen in October – November, typically for this cultivar after getting ripened fruits fall off. Fruit size is (2,5) 4,5 – 5,5 (7,2) sm by length,

(2,3) 3 – 3,2 (3,8) sm across diameter. Form rate is from almost round till pear-shaped, but mostly they are oval or barrel-shaped with rounded bottom, that resembles percussion instrument timpani and conga (fig. 2. F; 3.B). One population can include plants with different by fruit form and size.

Fig. 2 Morphological characteristics of *Opuntia lindheimeri under conditions of South Crimea:*
A – common plant habitus; B – vegetative renewal; C – sprouts; D – juvenile specimen; E – flowers; F – fruits; G – cut along fruit and its seeds.

Fruit top has a slight basin sized from 4 – 7 mm. Fruit color is bordeaux or crimson and bordeaux, pulp is bordeaux and purple (bordeaux). Fruits are quite soft, juicy, slightly fibrous, sour-sweet, tasty. One segment can contain from 1 till 12 fruits, average number is 5-7. One plant divided into 30-40 segments yields about 150-200 fruits. Each fruit contains 90 – 265 seeds (usually 180 – 220). Seed color is yellow-light grey; its size is 3,2 – 4,3 mm across diameter, 1,5 – 2 mm by depth, form is irregular round and heart-shaped with wide yellowish border, flattened at the top and bottom (Fig.2. G). These plants are able to grow under conditions of South Coast Crimea (Fig. 2.C).

As systematics of Cactaceae family on the whole and *Opuntia* genus particularly is still conflicting, to determine properly systematic position of cultivated and running wild representatives of this genus in the Crimea is a difficult task. In Nikitsky Botanical Gardens study opuntia was cultivated as *O. engelmannii* Salm-Dyck [1, 2, 7, 45]. Wild opuntia on cape Plaka has the same name in publications [8 – 12, 38] and some internet sites [18, 33 and etc.]. Though according to modern concepts its morphological characteristic mostly corresponds to *O. engelmannii* subsp. *lindheimeri* (Engelm.) U. Guzmán et Mandujano, often taken as a separate cultivar *O. lindheimeri* Engelm. [45, 47], but not to *O. engelmannii* subsp. *engelmannii* (or *O. engelmannii* s. str.). It differs from typical opuntia *O. Engelmannii* by glochidia and barbs (yellow with red-brown bottom and well-emphasized rings, but not white with chalky shade and umber almost black bottom with non-expressed rings) color, fruit form and color, and some other distinguish features (Fig.3).

In the Crimea *O. Lindheimeri* is successfully cultivated in the coastal zone of South Coast (cape Sarych, Foros, Goluboj Zalyv, Simeiz, Alupka, Miskhor, Gaspra, Livadiya, Yalta, Nikita, Gurzuf, Partenit, Alushta, Solnechnogorskoye, Malorechenskoye, Morskoj and etc.), piedmont and steppe areas (in Sevastopol, Saki, Yevpatoriya). Last decades cases of this cultivar running wild and invasion into phytocenoses were registered. For the first time it was included into checklist of adventive plants by Bagrykova N.A. and Yena A.V. [3, 23]. In scientific literature there is detailed information about *O. Lindheimeri* population only (named by *O. Engelmannii)* on cape Plaka [8, 12, 38]. Although quite adapted populations, which have vegetative or seed renewal, were described during field researches in 2014 in Foros, Gaspra, on cape Martyan, Gurzuf, Atek and other places.

The largest and obviously oldest population of *O. Lindheimeri* grows 2 km to East from village Partenit on the territory of sanatorium “Utyos” on the seaside slope of Plaka cape. This cape is a rocky cliff by 50m height, composed by intrusive igneous magmatic geological materials – diabase porphyrites dated by Middle Jurassic period, the cape top is covered by clay slates dated by Triassic period and hornstoned rocks. Population of this cultivar is mostly located on steep slopes (angle slope rates from 15° till 60°) of the southern and southeast exposition at altitude range 10 - 45m, total area is 1,5 ha. Opuntia is found in biotopes of two types.

The first biotopes concerns expositions of porphyrite rocky monolith, divided by horizontal and vertical cracks into separate layers and blocks; it occupies southeast and southwest parts of the cape and down slope area in its central (southern) part. *O. lindheimeri* specimens inhabit in rock cracks and being the largest and strongest among local vegetation, become dominants of khazmophyte groups of the following classes: *Asplenietea trichomanis* (Br.-Bl. in Meier et Br.-Bl. 1934) Oberdorfer 1977 and *Koelerio-Corynephoretea* Klika in Klika et Novák 1941.

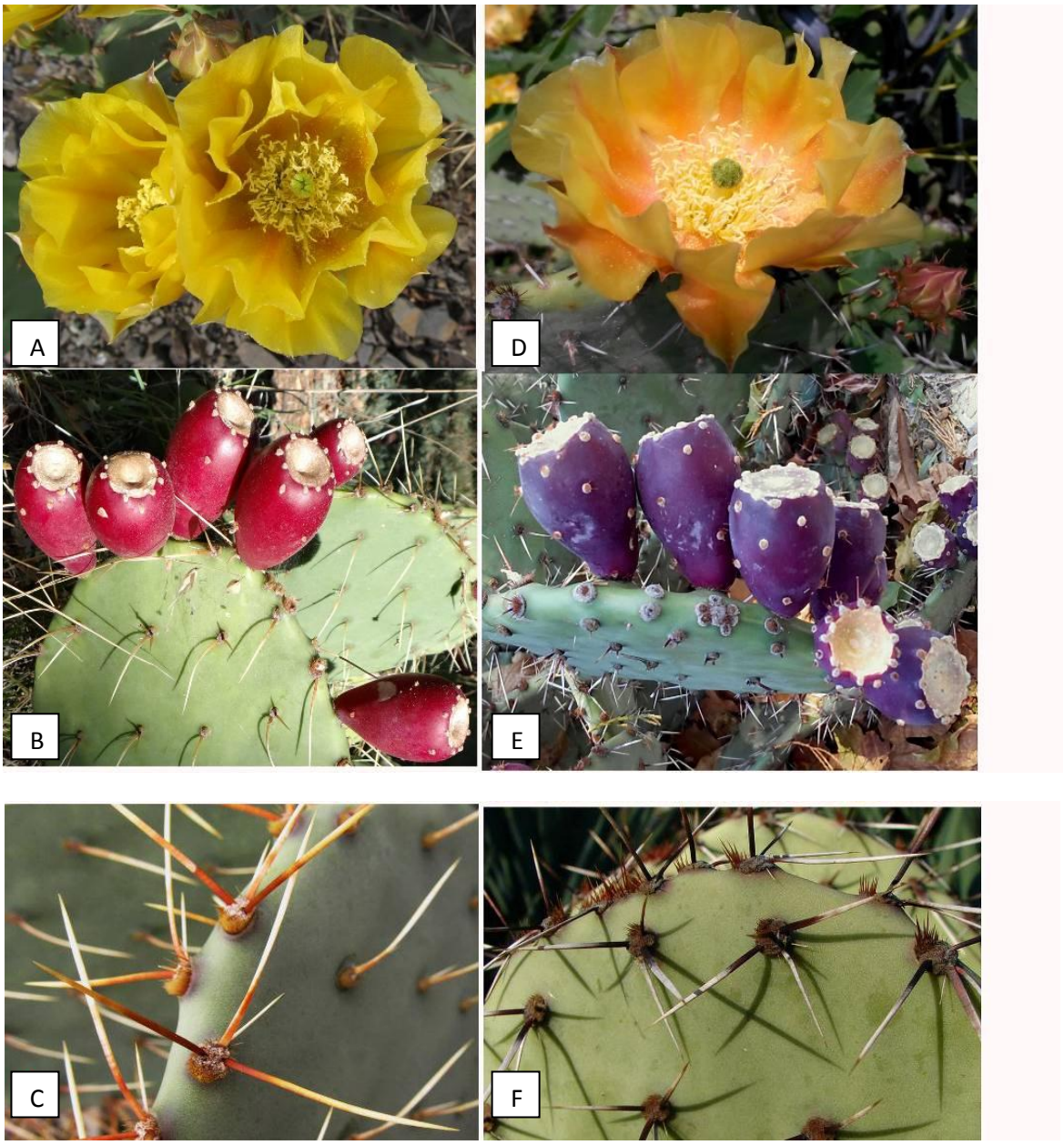

Fig.3 Comparative characteristics of some morphological parameters of *Opuntia lindheimeri* (A,B,C) and *O. Engelmannii* (E,F,G).
A,D – flowers; B,E – fruits; C, F – barbs and glochidia

Common vegetative projective cover of this biotope makes from 10 till 90%. Opuntia projective cover ranges from 3 till 90%, besides it creates single-species growth at the bottom of the southern slope (Fig.4), but it hasn`t been found on edge western and edge eastern areas yet. Here is a list of other cultivars typical for rocky cenoses of Plaka cape: *Parietaria judaica* L., *Sedum hispanicum* L., *S. pallidum* M. Bieb., *Allium saxatile* M. Bieb. s. l., *Ceterach officinarum* DC., *Galium mollugo* L., *Veronica cymbalaria* Bodard, *Avena barbata* Pott ex Link subsp. *barbata*, *Misopates orontium* (L.) Raf., *Cheiranthus cheiri* L. (two last cultivars are adventive).

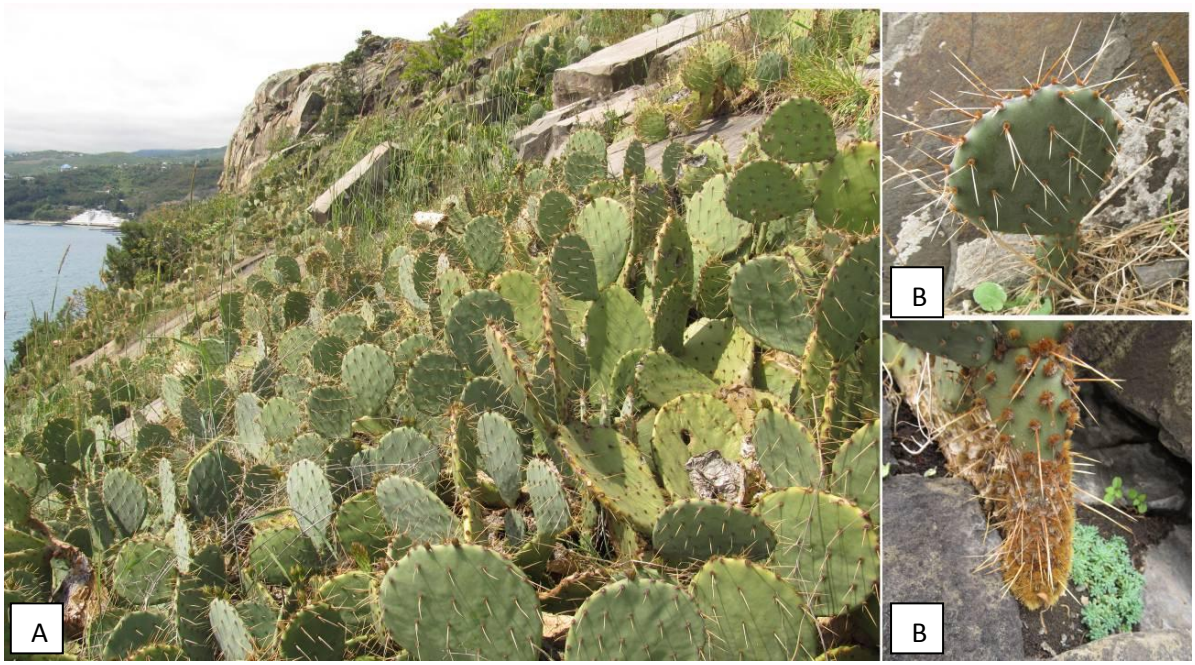

Fig. 4 Cenopopulation of *O. Lindheimeri* on Cape Plaka:
A – general view of opuntia growth; B,C – seedlings

The second biotope is rubbly and clay screes and slide slopes in the middle of the cape area. A large number of ceramic fragments and other artifacts are found here. It proofs historical information about anthropogenic development of the territory opened up many years ago. Along with exogenous geological processes, man economical activity became dangerous for natural vegetative cover, degradation process was launched. Nowadays vegetative of this biotope consists of three layers. The first layer includes small cenoses of short trees (1,5 – 4 m) *Pistacia mutica* Fisch. et C.A. Mey., *Juniperus excelsa* M. Bieb., *Cupressus sempervirens* L., they create lighted forests with closing of leaf canopy from 0,1 till 0,5 (av. 0,2) in the middle and upper parts of the slope, young specimens of *Ailanthus altissima* (Mill.) Swingle are presented on this area as well. Shrub layer was created by *Jasminum fruticans* L. with projective cover from 0 – 30% (on average 5 – 10%). In the herb layer, which is presented by groups of Mediterranian class *Thero-Brachypodietea* Br.-Bl. ex O. de Bolós y Vayreda 1950, dominant plant are *Asphodeline lutea* (L.) Rchb., *Ephedra distachya* L., *Teucrium chamaedrys* L., *Taraxacum hybernum* Steven, *Convolvulus cantabrica* L., *Hordeum bulbosum* L., *Aegilops biuncialis* Vis. and etc. A considerable role of agrestal species was marked out: *Rapistrum rugosum* (L.) All., *Tribulus terrestris* L., *Portulaca oleracea* L. The common projective cover ranges from 45 till 90%, while *O. Lindheimeri* cover makes 10 – 90% of the total area. Among *O. lindheimeri* growth in this locality there are single specimens of another opuntia cultivar naturalized on South Coast of the Crimea, beforehand referred to *O. tunoidea* Gibbes (it is registered in catalogues of Nikitskiy Botanical Gardens as *O. pseudotuna* Salm-Dyck). Phytocenosis of cape Plaka contains a number of plants, being protected in the Red Data Book of Russian Federation (RDBRF) [30], the Red Data Book of Ukraine (RBU) [36] or candidates for the Red Data Book of the Crimea (RDBC). They are *Juniperus excelsa, Pistacia mutica* (included into RDBRF, RDBU, candidates for RDBC), *Asphodeline lutea* (included into RDBU, candidate for RDBC), *Avena barbata*, *Hedypnois rhagadioloides* (L.) F.W. Schmidt, *Dianthus marschallii* Schischk. (included into the checklist of plants being in need of protection on the territory of Autonomous Republic of the Crimea, 2013, they are candidates for RDBC as well), *Avena sterilis* L. subsp. *ludoviciana* (Durieu) Nyman (included into checklist of plants,

being in need of special protection in the Crimea). A highly invasive activity of O. *Lindheimeri* becomes a considerable danger for plant populations of rare cultivars on area of the Cape Plaka.

According to data of previous researches in 1990th opuntia population growing on the cape Plaka consisted of 40 groups (clumps) after the fire in 1998, its size ranged from 0,5m till 3,0m across diameter [8]. By present number of groups has increased till 500. Generative specimens occupy about 90% of the whole population. Percentage of juvenile specimens is approximately 8%. Senile specimens and several old dead plants were revealed. Propagation of *O. Lindheimeri* is mainly vegetative; it occurs due to anthropogenic factor (segments are cutting off), hurricanes, raging along the cape during winter, or falling down rock debris. All these factors stimulate opuntia further movement down the steep slope being under gravity action and rootage. From 5 till 10% of the population are specimens with seed origin. The most popular areas for seed sprouting are cracks in a rock monolith, places not favorable for segment rootage; owing to moisture high level, storage of humus and more stable substrate. In narrow cracks, bent vertically mainly specimens of seed origin survive.

O. lindheimeri displays an obvious invasive activity in another habitant - 1 km to west of Ayu-Dag mountain on the coastal slope between camp complexes of "Morskoj" and "Pribrezhny" of the International Children`s Centre "Artek" (fig. 5, 2B – F, 3A). These conditions are similar to the cape Plaka conditions – rocky expositions of clay slate of Triassic period in an altitude range of 5 – 15 m above the sea level. Populations occupy slopes of southeast, south and southwest expositions with slope angle from 5 - 10° in the near watershed zone it reaches 30 - 40° on steep lands. Natural vegetation of the territory is a composition of shrub growth of Mediterranean class (class *Cisto-Micromerietea julianae* Oberdorfer 1954) with a few short arboreal breeds, fragments of bluegrass, nodular and wheatgrass petrophyte and steppe cenoses (*Festuco-Brometea* Br.-Bl. et R. Tx. ex Br.-Bl. 1949) and calciphobous ephemeretum (Koelerio-Corynephoretea). In floristical composition the principle role belongs to *Paliurus spina-christi* Mill., *Pyrus elaeagrifolia* Pall., *Cistus tauricus* J. Presl et C. Presl, *Jasminum fruticans* L., *Bothriochloa ischaemum* (L.) Keng, *Elytrigia caespitosa* (K. Koch) Nevski subsp. *nodosa* (Nevski) Tzvelev, *Convolvulus cantabrica* L., *Clinopodium nepeta* (L.) Kuntze, *Eryngium campestre* L., *Taraxacum hybernum*, *Prospero autumnale* (L.) Speta, *Anisantha tectorum* (L.) Nevski, *Aegilops biuncialis* Vis., *Vulpia ciliata* Dumort., *Alyssum umbellatum* Desv., *Helianthemum salicifolium* (L.) Mill., *Sedum caespitosum* (Cav.) DC. and etc. There were revealed some synanthropic (*Echium italicum* L. subsp. *biebersteinii* (Lacaita) Greuter et Burdet, *Tribulus terrestris* L.) and adventive (*Rhamnus alaternus* L., *Olea europaea* L., *Bupleurum fruticosum* L., *Foeniculum vulgare* Mill.) plants.

In accordance with data of Volokitina Yu.S. (oral report) and our observations in 1970-1980th on area described above only 1-3 specimens of this opuntia cultivar grew there, obviously carried in from the neighbor territories of camps "Morskoj" and "Pribrezhny"; on the camp territory it was planted in late 60th and early 70th of last century with the purpose of area reconstruction and landscaping. Nowadays population consists of no less than 70 large specimens, being mainly on generative stage. Most plants propagate by vegetative way, but there are specimens with seed origin. There were fixed approximately twenty seedlings of last and current year. Area size of a population makes about 1000 m². Percentage of *O. Lindheimeri* makes 10 – 40% relative to the common vegetative projective cover (70-90%). On the border of populations single specimens prevail, as a rule centers are occupied by clumps of some plants, their size reaches 1,5 – 3m across diameter. In this locality *O. Lindheimeri* grows close to another naturalized opuntia cultivar *O. macrorhiza* Engelmann. Mostly opuntia colonizes exposed stony, rubbly or clay areas on slate slopes with vegetative deficit or occupied by efemeretum cenoses. Though their adaptive capacity is striking; these plants take root successfully under crown of small xerophytic especially therophyllous trees, even among

rockrose growth, supplanting it gradually. Therefore opuntia colonization of this area is a direct threat for three rare and protected plant cultivars - *Cistus tauricus* (included into RDBU, candidate for RDBC), *Vitex agnus-castus* L., *Sedum rubens* L. (included into checklist of plant cultivars, being in need of special protection on the territory of AR Crimea, 2013, candidates for RDBC) and a number of Crimean, Crimean and Caucasian endemics as well.

Fig.5 General view of *O. Lindheimeri cenopopulation* in Artek.

Next locality of *O. Lindheimeri* is situated 3 km to west in southeast part of Gurzuf village. Simultaneously with other sorts of opuntia (according to preliminary data: *O. tunoidea* and *O. laevis* J.M. Coulter) *O. Lindheimeri* grows on rocky limestone slopes between Geologov and Krymskaya streets above musical school. This area includes southern spurs of Bolgatur cliff, composed of Massandra formation limestone, that is a tongue of ancient Pliocene landslide. Opuntia plants are spread along watershed ridge, precipitous slopes of southeast and southwest expositions on an altitude of 60 – 70 m above the sea level. Residential zone has taken place here for many centuries, but in spite of it on stony slopes difficult to access there are well-reserved natural complexes, typical for limestone expositions of South Coast. Though in late 1900-th – early 2000-th anthropogenic effect on reserved natural landscapes increased a lot as a result of a new cottage development in the upper part of the slope. First spontaneously sprouting plants of opuntia on this area are dated to the same period. Their first sprouting place was refuse dump, where separate segments occurred after pruning of plants used in landscaping of house territory. Later opuntia spread down the watershed ridge probably because of accidental mechanical damage caused by man. At present populations consists of no less 5 large (height – 40-50 sm) specimens, propagated by segment establishment. Three of them have got the stage of active blossoming and bearing fruits, but another two specimens are on pregenital stage. There is also a young plant with seed origin, inhabited in the line of a limestone rock. Rest of specimens grows either on rocky outcrops of limestone or clay-rubbly and scree slopes, either on open up places or in the shadowed, partly under the crown of not high deciduous trees. Area of *O. Lindheimeri* growing in this locality makes approximately 100 m². The total projective vegetative cover is 60%, but study opuntia cultivar occupies no more than 3 % of the area, but on the upper part

of the slope this value gets 15%. Natural vegetative cover of the area is composed of fragments of pistachio lighted forests with cenoses of limestone rocks, stony floral chaos and elements of petrophyte variants of Mediterranean steppes (classes of *Quercetea pubescenti-petraeae* (Oberdorfer 1948) Jakucs 1960, *Asplenietea trichomanis* и *Thero-Brachypodietea*). A dominant is *Pistacia mutica*, *Celtis glabrata* Steven ex Planch., *Rhus coriaria* L., *Jasminum fruticans, Clinopodium nepeta*, *Taraxacum hybernum*. An important role belongs to adventive plants (*Prunus cerasifera* Ehrh., *P. dulcis* (Mill.) D.A. Webb, *Lonicera etrusca* Santi) and a weed component (*Ballota nigra* L., *Carduus pycnocephalus* L. subsp. *albidus* (M. Bieb.) Kazmi, *Galium aparine* L.). Considerable increasing of anthropogenic effect on this area last decades, including pollution by costruction waste and household rubbish, trampling down, more frequent fire cases, destroying of natural vegitation as well as possible sources of plants in close proximity favor spreading of alien plants, such dangerous potential species-transformers as opuntia.

One more locality of adapted *O. Lindheimeri* population is a territory of nature reserve "Mys Martiyan" [4,29], bordered to buildings of a former military unit, situated 200 – 250 m to East from the village of Nikitsky Botanical Gardens, on 180-190 m altitude above the sea level (Fig.6).

Source of invasion is vegetation planted on the military unit area in 1950 – 1970. At present opuntia renews by vegetative and seed ways as in residential zone as in natural phytocenoses of the reserve. This habitat is characterized by outcrops of limestone on the daylight surface, as a result the soil cover consists of carbonate varieties of brown soils in dry forests and brushes. Growth and single specimens of opuntia on the reserve territory belong to highly juniper and bushy oak cenoses (class *Quercetea pubescenti-petraeae*), total projective cover makes 50 – 70%, *O. lindheimeri* occupies till 15 –30%. Woody shrub layer presents *uniperus excelsa* (till 25 – 30%), *Quercus pubescens* Willd., *Pistacia mutica, Juniperus deltoides* R.P. Adams, *Carpinus orientalis* Mill. и *Fraxinus angustifolia* L. (till 10 – 15%), *Jasminum fruticans* (от 5 до 30%), *Cistus tauricus* (от 10 до 30%), *Clematis vitalba* L. (до 25 – 30%), *Ruscus aculeatus* L. (до 45%), *Hedera helix* L. (до 10%), single instance *Cornus mas* L. Dominants in the herb layer are *Dactylis glomerata, Elytrigia repens* (L.) Nevski, *Taraxacum erythrospermum* Besser, *T. hybernum*, *Geranium robertianum* L., *Teucrium chamaedrys*, highly stable but not having a high projective cover: A*egonychon purpureocaeruleum* (L.) Holub, *Prospero autumnale, Fibigia clypeata* (L.) Medik., *Melandrium album* (Mill.) Garcke., *Achnatherum bromoides* (L.) P. Beauv., *Crepis pulchra* L., *Stachys velata* Klokov, *Melilotus neapolitanus* Ten. And etc. Synanthropic species: *Diplotaxis tenuifolia* (L.) DC., *Galium aparine, Chenopodium album* L., *Marrubium peregrinum* L.

Fig. 6 Population of *O. Lindheimeri* in the natural reserve "Mys Martiyan":
A – general cenosis view with opuntia; B – a flower; C – a juvenile plant; D – an immature specimen with seed origin

Naturalized population occupies territory more than 150 m². It has some groups sized from 2 till 4,5m (each group has 8-12 plants), besides along the perimeter of growth there are specimens of generative, virginal and juvinille plants. Total number of plants makes more thn 80. Percentage of generative specimens is more than 80% plants, in opuntia growth it was found more than twenty seedlings of last and current years. Most of plants fructify abudantly, segments have 1-10 fruits, an average number is 5-6. Segments are big 25-30sm by length, fruit form is oblong, characteried by quite big size (till 7 sm by length and 3,3 sm acrose diametre). In most cases opuntia plants grow on gaps between trees, where has an abundant bearing fruit. Basically virginal speciemens are noted under tree crowns.

Several running wild cactuses including opuntia, were found on area of village Gaspra, on the seaside slopes of western spur of Cape Aj-Todor (Mayachny) within area of sanatorium "Dnepr" (former estate "Kharaks"). Five groups and separate specimens of *O. Lindheimeri* (total number is 75 – 85 specimens) are spread on the plot of 550-600 m on precipitous seaside slopes (slope angle ranges from 15 till 60°) of southern and southeast expositions with altitude range of 20 – 45 m above the sea (fig.7A), as well as on the park clumps (fig.7C). Natural vegetative cover combined of bush growth of *Cisto-Micromerietea julianae* class and fragments of petrophyte and steppe vegetation of *Festuco-Brometea* class, is extremely damaged, as in 1970-th a considerable part of slopes was covered by antilandslide layer made of armature and concrete (fig.7A). At present such ground has its dominants: naturalized introduced cultivars (*Petrosedum reflexum* (L.) Grulich, *Bupleurum fruticosum* L., *Centranthus ruber* (L.) DC., *Jacobaea maritima* aggr. (=*Senecio cineraria* DC.), *Antirrhinum majus* L., *Cheiranthus cheiri* L.), where occurance of opuntias was fixed. The total projective cover of these synantropic cenoses makes 5-20%. Majority of *O. Lindheimeri* plants on slopes, most probably were planted in 1970 – 1980-th. [22], young specimens on terraces at flights of stairs might have been planted recently (last years) together with other representatives of *Opuntia* genus from Cactaceae family.

Self-renewed separately growing specimens of opuntia or their groups, consisting of 3-14 adult specimens, are found in the lower and middle parts of slopes, or on the rocky outcrops of limestone or rubbly and clay slopes in a composition of half-natured cenoses among single growing trees of *Quercus pubescens*, *Pinus brutia* Ten., *Jiniperus excels* (fig. 7B). Bushy and herb layers contain *Paliurus spina-christi*, *Cistus tauricus*, *Jasminum fruticans*, *Elytrigia caespitosa* subsp. *nodosa*, *Taraxacum hybernum*, *Prospero autumnale*, *Alyssum obtusifolium* Steven ex DC. and etc. Besides them among adventive cultivars there were revealed mentioned-above introduced plants, and *Rhamnus alaternus*, *Ailanthus altissima* as well. The total projective cover makes 30-40%, *O. Lindheimeri* occupies 5-10%. Inspection of present population state was carried out in late autumn period – November 2014, that didn`t allow reveal the floristic composition completely. Diameter of adult opuntias or their groups makes 1,2 – 4,5m, height of plants is 60-90sm. Mostly populations include generative plants, which had abundant bearing fruits, number of fruits on segments is 2-10, average number is 7-8. Fruits have diverse form and size. Sometimes (single cases) juveniles with seed origin and immature specimens with vegetative origin were found on park clumps and slopes (fig. 4C). In western part of the park on damaged by man slope separate specimens or clumps of *O. tunoidea.*were marked next to O. *Lindheimeri* plants. Cultivated and naturalized plants of the second type of opuntia occur more often on clumps and seaside slopes in the central and east parts of sanatorium "Dnepr".

Fig.7 Opuntia on the territory of sanatorium "Dnepr":
A – two cultivars of opuntia in anthropogenically converted biotopes on the seaside slopes; B – virginal specimen of *O. lindheimeri* in a half-natured cenosis; C – juvenile, immature and generative *O. lindheimeri* plants on a park clump

Other localities of South Coast of the Crimea contain single spontaneously growing *O. lindheimeri* plants. One of them was found in 2014, within outskirts of Alushta – 1 km to west from village Lazurnoye on the steep seaside slope of Khalikin-Tepesi mountain, composed of clay slates of Triassic period so-called "Granilnya Golovkinskogo". Opuntia (bearing fruit specimen) grows on the altitude of about 10m above the sea, on the slope of east exposition with the slope angle of 20° on site of landslip, zone of short-sized oak and

pistachio light forests (class *Quercetea pubescenti-petraeae*), among growth of *Ruscus aculeatus* and *Cotinus coggygria* Scop. As in the nearest locality there is no any seed sources, perhaps *O. Lindheimeri* specimen has an ornithogenic origin, otherwise seeds were brought by birds presumably from cape Plaka, 3 km to the east.

A small *O. Lindheimeri* population, to our opinion, of 2-3 years, was revealed by Belych T.V (oral report) on the watershed range, adjoined from North to Ayu-Dag mountain, district of the cottage village "Novaya derevnya". This locality is situated at altitude about 225 m above the sea, it`s the highest point out of all localities containing this cultivar plants on South Coast of the Crimea. Opuntia grows two meters from macadamized road on a glacis of the northwest exposition of clay slates in herbaceous cenoses which include *Bothriochloa ischaemum*, *Hordeum bulbosum* L., *Poa bulbosa* L., *Clinopodium nepeta*, *Prospero autumnale, Taraxacum hybernum*, *Plantago lanceolata* L., small annual plants as well (a vegetable variant with anthropogenically damaged cycle of *Koelerio-Corynephoretea* class). The population occupies about 1m², includes three vegetative fruitless specimens of 20sm height; it consists of 2 – 10 segments and one separate established cladode. All specimens have vegetative origin. Plants are in the depauperated condition, probably caused by unfavorable hydrothermal regime (excess humidity and too low air temperature in winter) and anthropogenic effect. The population origin is a sequence of plant rubbish dump being next to its emergence. Cultivated opuntia specimens were found on territories of neighboring housing estates.

The eastern point of cultivated and naturalized opuntia *O. lindheimeri* (data of 2014) is situated to east of village Solnechnogorskoye (Fig.8). The total area where opuntia was found in this locality makes 1,5 ha; it was revealed more than 50 specimens, what makes less 3% of the given territory, dominants are introduced plants on purpose. Cultural plantations of opuntia were found on terraces, adjoined to housing estates (Fig. 8, C, D) down the highway Alushta-Sudak and up the area of geological monument of nature "A part of the seaside territory between Solnechnogorskoye vil. and Malorechenskoye". Most part of vegetation are generative, but there were found immature specimens with vegetative origin as well. Besides opuntia there are other cultivars on terraces (*Juniperus excelsa, Cedrus deodara* (Roxb.) G. Don, *Pinus brutia*). Opuntia plants have been cultivated in half-natured cenoses that cover seaside slopes. Natural vegetative cover of this area is probably composed fragments and derivatives of blue grass and knotty coach grass, wormwood petrophyte steppe assemblages of *Festuco-Brometea* class, pistachio light forests and efemeretum of *Koelerio-Corynephoretea* and *Thero-Brachypodietea classes. Orijective cover of steppe cenoses makes 30-50 %. In floristical composition the principle role goes to Bothriochloa ischaemum, Elytrigia caespitosa* subsp. *nodosa, Artemisia lerchiana* Stechm., *Alyssum umbellatum, Convolvulus cantabrica, Eryngium campestre, Taraxacum hybernum, Poterium polygamum* Waldst et Kit and etc., in arboreal and bush layer: *Pistacia mutica, Jasminum fruticans are emphasized. Some synatrope plants were revealed* (*Echium italicum* L. subsp. *biebersteinii, Tribulus terrestris, Malva erecta* J. Presl. et C. Presl.). Opuntia plants are mainly represented by virginal specimens consisting of 2 – 8 segments.

Fig.8 Cenopopulation of *Opuntia lindheimeri* growing on area between villages Solnechnogorskoye and Malorechenskoye: A,B – single plants of opuntia on the seaside cliff; C,D – general view of the slope with plantations, opuntia specimen is on the house territory.

Cultivated younge specimens and spontaneously sprouting single plants with vegetative origin were found on the edge and stony epositions of the cape, composed of quartzitic sandstone (Fig. 8 A,B), deeply cracked and spotted with small and round hollows. At the bottom of the cape there is a disorderly bulk of boulders; rocks rise above the sea in a seaside zone. Cenoses located on the seaside and steep slopes (slope angle from 20-30 till 60-70°) of souteast expositions with the common projective cover of herbage 10-40% the followings have been frequently found: *Ephedra distachya* L., *Artemisia lerchiana, Kochia prostrata* (L.) Schrad., *Galatella linosyris* (L.) Rchb.f., *Teucrium polium* L. *Elytrigia caespitosa* subsp. *nodosa, Capparis herbacea* Willd.

Generally the percentage of spontaneously sprouted opuntia plants for counted specimens what is no more than 10%; they grow in the middle part and bottom of the steep seaside slopes at altitude 10-15 m above the sea level among bulks of boulders and stones. It is possible to presume that further opuntia can spread the cape territory and its adjacent zone.

Lately wild *O. Lindheimeri* was found in the western part of South Coast of the Crimea, settlements Foros and Simeiz. Foros park contains 5 localities with 19 sprouted opuntia specimens. 8 specimens having vegetative origin consist of 10-20 cladodes, six specimens have 1-7 segments and 5 specimens with seed origin (3 of them are juvenile plants) have from 2 till 4 segments. All plants weren`t in a bearing fruit period at the moment of inspection (23-24.11.2014). Opuntia is mainly spread out on the seaside limestone slopes of the southern and southeast expositions at altitude 10-15m above the sea level, slope angle is 10-35°. Vegetation cover is damaged significantly in most localities, common projective cover makes 20-40%, *O. Lindheimeri* occupies no more than 5%. Large specimens of *Pistacia mutica* are found rarely; dominant species are *Rhus coriaria, Jasminum fruticans, Hordeum bulbosum, Dactylis glomerata, Taraxacum hybernu.* Plant cultivars which mostly grow in synathrope localities are found here as well: *Carduus pycnocephalus* subsp. *albidus,*

Rhagadiolus edulis Gaertn., *Chondrilla juncea* L., *Lepidium draba* L., *Galium aparine*. Adventive plants are also revealed there: *Ailanthus altissima, Sonchus oleraceus* L., *Jacobaea maritima* aggr., *Cheiranthus cheiri*. Some juvenile and immature *O. Lindheimeri* specimens grow on artificial stone hill (Fig.9 B) 3,5 m height with more than 25 m² of total area. Dominants of this cenosis is *Opuntia humifusa*; while *Sedum pallidum, Anizantha sterilis* (L.) Nevski, *Geranium robertianum, Galium aparine, Lathyrus* sp. are found frequently; and adventive plants - *Setaria viridis* (L.) P. Beauv., *Ailanthus altissima, Cheiranthus cheiri*. Separate juvenile and virginal specimens are found on the park clumps (Fig. 9C).

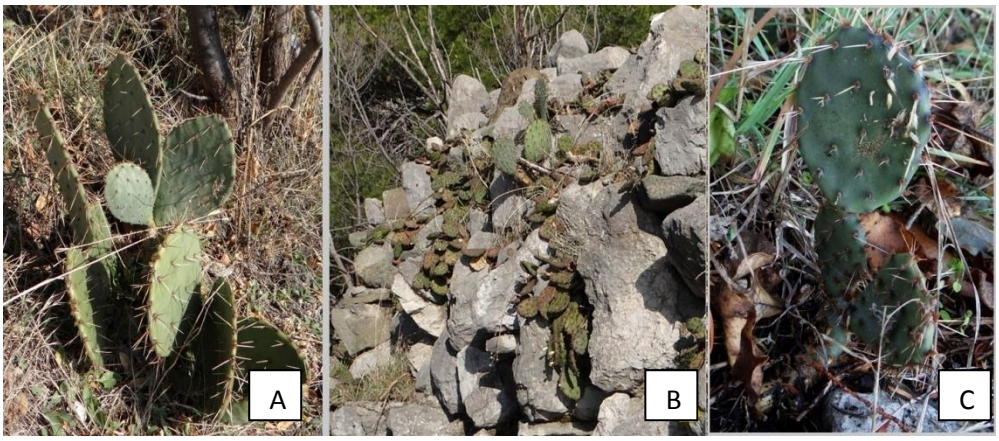

Fig.9 ***Opuntia lindheimeri*** **in Foros park:**
A – pregenerative specimen with vegetative origin on the seaside slope; B,C – juvenile specimens on the stone hill and park clumps

In Simeiz, Krasnomayachnaya street, at altitude of 75 m above the sea level on the steep rubbly scree limestone slope of the southern exposition it was found a fruit bearing specimen of *O. lindheimeri* with vegetative origin. This slope is covered by natural plant cenosis – light forest of *Quercus pubescens* and *Juniperus excels* with *Prunus dulcis* and *Rhus coriaria*. Dominant in the herb layer is *Euphorbia rigida* M. Bieb. Vegetation is an ecotone derivate cenosis of *Quercetea pubescentis-petraea* and *Thero-Brachypodietea* classes. The most popular anthropogenic effect on this area is constructional waste and household rubbish. Obviously opuntia segments occurred here with rubbish some years ago. According to still unproved oral reports, single specimens of opuntia grow on the area of natural monument "Cat Mountain". Favorable biotopes and opuntia cultivation stimulate further invasion of this cultivar in Simeiz and its outskirts.

Morphological peculiarities of plant, growing in all revealed localities correspond to *O. lindheimeri*. description mentioned above. Wild plants of *O. engelmannii* s. str.,cultivated in Nikitsky Botanical Gardens and some other parks of South Coast of the Crimea, haven`t been registered.

Conclusions

According to the research, wild plants of large opuntia growing in South Crimea belong to *O. lindheimeri* (*O. engelmannii* subsp. *lindheimeri*), but not to close taxon *O. engelmannii* (*O. engelmannii* subsp. *engelmannii*) as it was supposed before.

In period 2013 – 2014 it was investigated known before and new-found localities of naturalized *O. Lindheimeri* population in South Crimea. At present following points of this opuntia cultivar spontaneous sprouting have been revealed and studied: Foros, Simeiz, Gaspra, Cape Martiyan, Guruf, Artek, piedmont of Ayu-Dag mountain, Cape Plaka, outskirts of Lazurnoye, Solnechnogorskoye. For the first time nine localities have been described.

Population of *O. Lindheimeri* has been found in lower zone of South Coast of the Crimea at altitude 5-225 m above the sea level (characterized by subtropical climat of mediterannian type) on diverse skeletal brown soils, on glacis and steep slope of different expositions of various rocks (limestone of Massandra formation, clay slates of Triassic period, intrustive igneous rocks), on rocks, landslide and scree rubbly and clay slopes among high juniper, pistachio and pubescent oak light forests, bush growth and herb cenoses, composed of petrophyte dwarf subshrubs, perrenial and small efemers in anthropogenically effected and more natural biotopes.

Almost all localities have plants chracterized by high vital parametres with plentiful blossoming and bearing fruits often with seed propagation, besides population at the northern piedmont of Ayu-Dag mountain. Considerable increasing of some populations size and occupying territory has been noted for last 20-30 years. New localities of this cultivar naturalization and intensive invasion into anthropogenically effected and natural cenoses have been found as well. These facts certify a high-level invasive activity of *O. Lindheimeri* in South Crimea, its further spreading, that`s why monitoring of the cultivar tends is essential for this region.

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Bagrikova N.A., Ryff L.E. Invasive cultivar of *Opuntia lindheimeri* Engelm. growing in South Crimea // Works of the State Nikit. Botan. Gard. – 2014. – V. 139 – P. 43 – 62.

The article concerns assessment of the current state of *Opuntia lindheimeri* Engelm. cenopopulations, one of the most dangerous invasive plants of the region, and distribution of this cultivar across South Crimea. Systematic position of the taxon previously identified as *O. engelmannii* was clarified. Morphological description of the form running wild in the Crimea was provided. Ten localities of spontaneous growth of opuntia cultivar were revealed and characterized. Its further spread trends are discussed as well.

Key words: *invasive cultivars, morphological description, Opuntia lindheimeri, Opuntia engelmannii, the Crimea, Nature Reserve "Cape Martyan".*

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PLANTS IN GLAREOPHYTON OF MOUNTAIN CRIMEA UPPER BELT AND THEIR SHOOT FORMATION TYPES

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Introduction

Scree formation, as an original form of mountain landscapes is a result of rocky surface weathering, denudation of the rocks with further shift and accumulation of fractions (colluvium) on adjoined slope. Scree is considered as a layer of loose colluviums, though such slopes include more or less sloping plots with a thin colluviums layer, but stony soil or rock outcrop. Instability of fractions is caused by excess of colluviums inclination over natural angle slope. Speed of fractions accumulation depends upon rock weathering [10].

Peculiar relief, special ecological regime and original vegetation of colluvium covers are generalized in "scree phenomenon" in Botany field. As a rule vegetation of screes consists of separate lighted groups and single plants. Mosaicism and nature of scree vegetation composition, presented by a few similar living forms, permit to consider these conditions extreme for formation of vegetation. Extreme ecological factors for vegetation of screes are marked out as follows: lifelessness and looseness of rocks, low-level fertility of buried fine earth, abnormal range of temperature indices in diurnal and annual seasonal cycles, distinctive illuminate, thermal and water regimes and etc. [6, 12, 13, 15, 17].

Nevertheless, it`s obviously scree area is occupied by plants capable to adapt to this special environment [14]. These plants are denoted as glareophytes, general definition for scree vegetation is "glareophyton" [14] or "unstable petrophyton" [4]. According to ecological habitat glareophytones are classified into facultative cultivars, spread out the screes and obligate – plants, capable to grow in unstable fraction covers only [14]. Facultative glareophytes are constant and sporadic elements of scree vegetation. These cultivars have different coenotic belonging and bioecological genesis: a huge number of plant diasporas from vegetation cenoses of belt types, petrophyte groups within the nearest rocks and rubbly slopes, occurs on heterogeneous by ecological conditions scree plots due to water, wind, animals and sliding down soil. As a result of ecological selection screes are inhabited by plants with special characteristics. Adaptive capacity of facultative glareophytes to scree environment lies in renewal ability after covering the overground organs by rubble, development of strong root systems or rhizome, drought-resistance [2, 6, 12, 14, 4, 15]. Adaptive characteristics of obligate glareophytes have never been studied before.

Objects and methods of the research

Research object is plants growing in colluviums covers on screes at piedmont of Shagan-Kaya rock (1436 m above the sea level), southeast slope of Gurzufskaya yaila, and at the bottom of Eklizi-Burun rock on southeast slope of Chatyr-Dag (1527 m above the sea level). During the life cycle of following plants-glareophytes, formation of root system, hypocotyl, shoot system, propagation way and dissemination were under observation: *Rumex scutatus* L. (*Polygonaceae*), *Sobolewskia sibirica* (Willd.) P.W. Ball (*Brassicaceae*), *Lamium glaberrimum* (K. Koch) Taliev (*Lamiaceae*) и *Viola oreades* Bieb. (*Violaceae*). Renewal buds were the ground to reveal appropriate functional zone of monaxonic shoots and plants in general, bud position relative to rubble surface was analyzed as well. Peculiarities of renewal zone of plant specimens were interpreted as an adaptive characteristic of definite cultivar

relative to unstable colluviums and buried fertile substrate. According to findings, key adaptive characteristics of glareophytes were determined, difference between facultative and obligate glareophytes were also indentified.

Results and discussion

A surface layer of colluvium is a barrel stony rubbly cover, 5-15 sm in deep, more or less fertile substrate (fine earth) is buried under it (Figure). Soil profile of fine earth making 15-20 sm is characterized by highly skeletal degree (90% of rubble), diffusivity of plant fertile and nutrition elements, water permeability, optimal aeration and thermal conductivity. Buried fine earth layer has a special hydrothermal regime. On the surface extreme contrast of temperature conditions and wind regime are caused by sharp difference of altitude (rock top - piedmont), inside the cover this factor is not so important. In frost-free season during the daylight colluviums surface is getting warm maximum, but at night it`s getting cold dramatically. It causes water condensation out of atmosphere. Percolating water through the fractions isn`t able to evaporate. That`s why in summer there is a special humid and cool environment inside the cover.

As to similar construction of inhabitant stages on unstable screes by various biomorphological plant groups, it`s taken into consideration "initial lifelessness" of colluviums covers [1, 6, 7, 12, 14, 15, 16, 17], cultivars with underground rhysome, hypogeogenic rhizome, are pioneer elements of glareophyton [3, 4, 6, 12, 16], [11]. In Mountain Crimea herbaceous perennial-microtherm *V. oreades* is characterized by such a kind of rhizome. At early phase of ontogenesis main root of V. oreades take hypocotyls and buds cotyledonary node in the ground, where rhizomatous axis with cataphyll, renewal buds and secondary root are formed [3] (Figure A).

Established in the soil or fine earth axis bring vegetative buds to the surface, which develop into overground shoots with green leaves and generative organs. Underground rhizome functions as renewal and vegetative colonization element. Low seed renewal caused by insect damage of fruitcases is compensated by extremely vegetative mobility. Having constant axis branch, a specimen quickly occupies an optimal for its growing area.

Mentioned type of rhizome favors *V. oreades* adaptation to conditions of stony soils and lighted vegetation cover, and being of its overground organs under any loose substrate. As a rule plants with underground rhizome belong to forest ecosystems [11]. *V. Oreades* plants of the upper belt of Mountain Crimea mainly develop on moisture and cool northeast slopes under *Pinus sylvestris L*. crown. *V. oreades* of Mountain Crimea isn`t in a group of glareophytes.

One more biomorphological type of glareophyton plants is perennial with deep scape and root system. In Mountain Crimea this group is presented by *R. scutatus*, evergreen plant with seed propagation. Ripened in summer fruits fall off around the maternal plant and get through the rubble into fine earth layer. Seeds sprout in spring. Plantlets have hypocotyl of 10 sm and more which brings the main shoot on the rubble layer level. The primary shoot system is formed, the main cone-shaped root grows vertically into fine earth. Plant shoots are extremely lengthened (till 40 sm), creeping with rising tops. By winter lengthened parts with generative and vegetative shoots die, but basal shortened parts with numerous accessory buds stay on the rubble surface. In time plagiotropic parts of shoots form caudex. Scape and root system of adult plants consists of a strong main root and a number of lateral roots, thickened hypocotyl gets through the rubble layer, caudex with renewal buds are situated at rubble surface, wintering shoot parts are on the rubble level (Figure B). During the plant life ground zone of renewal keeps a stable position relative to colluvium surface.

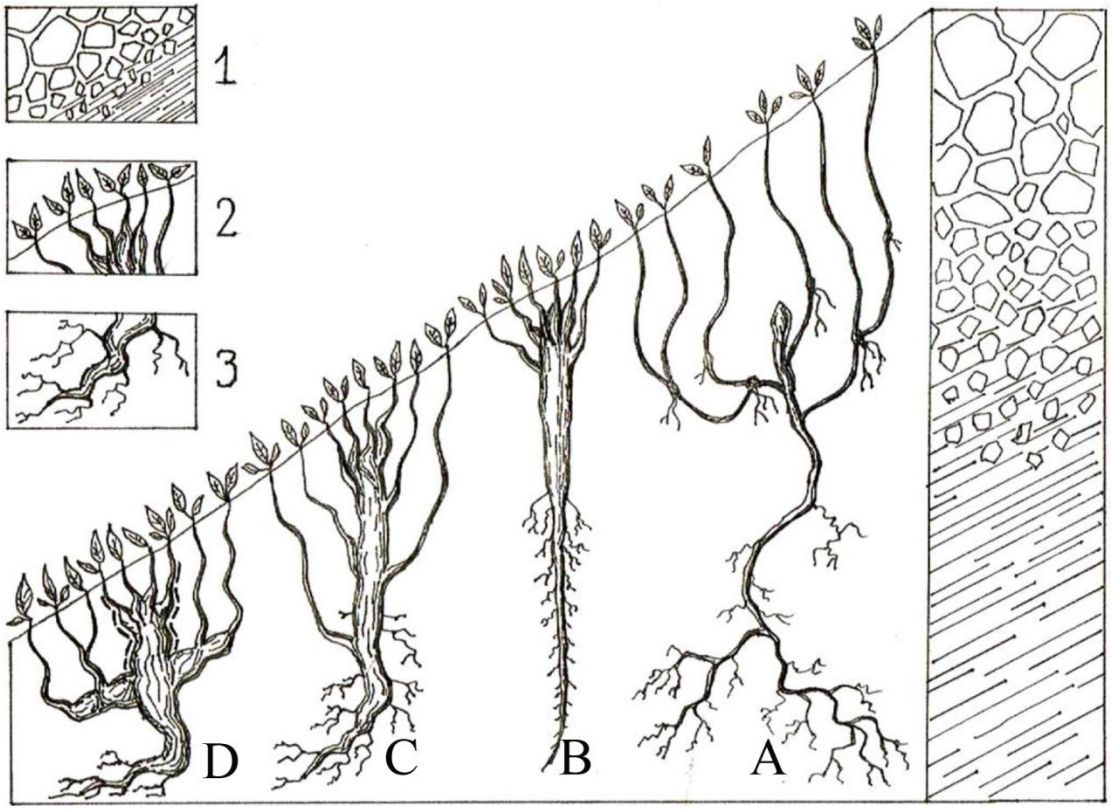
Adaptive characteristics of perennial plants belonged to this type, favor their colonization not only on buried under rubble fine earth, but any other more or less fertile and

moistened in summer, aerated loose solid substrates with deep profile. Fruit of *R. scutatus* is alate. Due to aerodynamic features dried on rubble or on the shoots fruits are shifted by wind within different distances as inside the screes as on the stony-rubbly and rocky ecotopes, non-typical habitat for this cultivar. According to complex of bioecological characteristics *R. scutatus* genetically is not connected with either screes (though it`s often found here), or stony habitat in general. This species belongs to rather facultative petrophytes, consequently to facultative glareophytes.

Plant cultivars, found only on scree areas are of particular interest; their characteristics reveal a special bioecological nature of obligate glareophytes.

Fruits of herbaceous biennial-monocarpic *S. sibirica* at the second year of a living cycle – one-month silicles – fall off around maternal plants and get through rubble into the fine earth. Seeds sprout in spring. Plantlets have hypocotyl of 10 sm and more which brings the main shoot on the rubble layer level. The main root grows vertically into fine earth 7 sm deep. Some strong lateral roots spread along horizontal plane. Primary shoot system consists of ground and overground rosellate vegetative shoots: main shoot and laterals out of accessory buds. Shoot development keeps out of hypocotyls adventitious buds and root system [8]. Renewal zone of this glareophyte living cycle consists of ground-overground and underground components (Figure B).

Unstable covers of colluviums are the ground for herbaceous plant with short-life cycle *L. glaberrimum*. Nut-shaped erems of *L. glaberrimum* fall off in summer, seeds sprout next spring. Plantlets have hypocotyl of 10 sm and more which doesn`t get through the rubble layer yet and doesn`t bring the main shoot on its surface. The main root grows vertically into fine earth 5 sm deep. Primary system of overground organs consists of the main and two monaxonic laterals, growing out of cotyledonary node buds [9]. At the first year of living cycle plant renewal zone is on the rubble level and limited by nodes of lower shortened internodes of the first shoots. In the end of the first season in its living cycle after dying of shoot parts 30 sm by length, established in the fine earth main root involves survived shoot parts with renewal buds into rubble layer. In spring the second generation of monaxonic shoots develops out of renewal buds. Their lengthened deceleration area gets through buried cool in summer rubble layer, enrichment zone is close to maximum warmed in a daylight surface. Length of parakladii increases in basipetal order, that`s why all of them get through the rubble layer and bring lateral inflorescences above its surface. In the living cycle of *L. glaberrimum* renewal zone changes its position relative to rubble surface: its function includes primary near-surface and secondary deeper phases (Figure D). Mesophilia and quickly lengthened organs (hypocotyls, monocarpic shoots, underground rhizomatous axis) are main distinctive features of facultative glareophytes. Original characteristics of glareophytes are following: short living cycle, short scape and root system, spreading flexibility in the renewal zone relative to rubble layer due to adventitious renewal buds or renewal zone involving into substrate. This complex of features is able to adapt study plants to fine earth, buried under unstable rubble. Due to these properties obligate glareophytes develop in spite of fraction accumulation speed and their regular crumbling. Ecological connection of obligate glareophytes with scree covers is revealed by the only way of propagation – dissemination and the only way of dissemination – barochory: fruits and seeds falling off close to the maternal plant.



Facultative glareophytes, gradually occupying unstable scree, are able to change dynamic and other landscaping parameters dramatically, even transform it into stable rubble slope with soil and meadow vegetation [1, 6, 7, 12, 14, 15, 16, 17]. Obviously, paucity and rarefaction of scree vegetation completely exclude any biotical effects on this landscape. Vegetation effect is not comparative with geomorphological processes. Obligate glareophytes, particular plant group by its genesis and bioecological nature are not considered in these schemes.

S. sibirica is wide spread on Chatyr-Dag scree, but screes at Shagan-Kaya piedmont are not occupied by this cultivar; *L. glaberrimum* populations are found on all large screes of Mountain Crimea. Accordingly, most likely isolation of obligate glareophytes happened simultaneously with Crimean mountains orogenesis and formation of scree landscapes here. Theoretical transformation of unstable colluviums covers (the only possible ecological environment for obligate glareophytes development) into stable slopes with developed vegetation cover would cause extinction of dedicated obligate glareophyte groups.

Conclusions

Therefore obligate glareophytes have genetic, ecological and biomorphological distinctions in comparison with other petrophyte groups being a part of glareophyton.
Biological complex of obligate glareophyte characteristics completely corresponds to environmental conditions of their development. Unstable colluviums covers are eternal as an ecological niche for development of obligate glareophyte populations.

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Nikiforov A.R., Korzhenevsky V.V. Plants in glareophyton of Mountain Crimea upper belt and their shoot formation types // Works of the State Nikit. Botan. Gard. – 2014. – V. 139 – P. 63 – 67.

The screes vegetation, various according to its bioecological nature and coenotic value, is glareophyton. Glareophyton mainly consists of facultative petrophytes, which get on screes from phytocenosis of zonal types and rock aggregations, so-called facultative glareophytes. Besides it glareophyton contains plants ecologically belonged to unstable debris covers – obligate glareophytes. Obligate glareophytes, petrophytes and other ecological origin types have quite a number of biomorphological distinctive features. These distinctions take place due to complex of genetic characteristics and environment where plants of diverse ecological group grow. If unstable debris covers are the only habitat for obligate glareophytes, for petrophytes these conditions are extreme.

Key words: *Mountain Crimea, scree, glareophytes, life form*.

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NEW SPECIES OF MYKOBIOTA INHABITING THE NATURE RESERVE "CAPE MARTYAN": MACROMYCETES

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Introduction

Fungi being a part of biogeocenosis are components of phytocenoses by way of mycosynusias. It is an organic and inherent element of any phytocenosis, one of the components in heterotrophic biota block. Monitoring of mycobiota is a part of general monitoring on reserve area. It is an essential step in collecting data about mycobiota, as most species of macromycetes are characterized by regular bearing fruits, a number of species possesses such a property as uncontrolled fruictification. Long-term monitoring gains a particular importance concerning rare species, making Red lists and creation of regional Red books.

On territory of the State nature reserve "Cape Martyan" (SNRCM) within long-term monitoring, lists of fungi species for every year are made, that permits to enrich and correct data of the whole researching period. SNRCM is situated in the central part of the Crimean Submediterranean, its total area makes 240 ha: 120 ha of land and 120 ha of water area. Since 1981 traditionally Records of reserve nature have been added by lists of macromycetes inventory. Since 1996 section "Calendar of nature" has been replenished by annual lists of macromycetes. Besides these Records, information about species composition, ecological groups, dynamics and fructification peculiarities, rare and protected species are kept in a number of publications and summaries [4, 7, 8, 10, 13 and etc.]. The list that generalized study results of macromycetes in NR "Cape Martyan" for 1980-2010 was published in 2010. [9]. It includes 298 taxons of species and intraspecies range: Ascomycetes – 9, Basidiomycetes – 289. According to data of 2011-2014 and results of herbarium treatment of early collections, 23 more new species for the reserve, are presented in our article.

Objects and methods of the research

Research object were Basidiomycetes and Ascomycetes, collected within the territory of SNRCM during stationary and itinerary inspections. Methodology of material collection and treatment corresponded to common approaches in investigation of macroscopic fungi (macromycetes) functioned as elements of phytocenoses [1, 2]. Abundance and species sociability were rated by Haas scale, ecological status was determined due to works of Kovalenko A.E. [5]. Investigation of morphological characteristics of fruit bodies and microstructures was realized applying light microscope MBI-11.

Classification of macromycetes is presented according to the nineth edition of «Ainsworth & Bisby's Dictionary of the Fungi» [14]. Latin names of species and genera correspond to nomenclatural database «Index Fungorum» [17].

Results and Discussion

New species revealed in SNRCM are quite heterogeneous by their taxonomic composition, living forms, ecological status, seasonality, cozological importance. They are representatives of two classes, 7 orders, 15 families and 18 genera. There are 8 mycorhiza creators and 15 saprotrophes of different appropriation, 10 species with bazidiomas development during warm and (April – October) and cold (November – March) periods, 3

species are polyseasonal. For Crimea peninsula there are 10 new species, 14 rare and protected species. 19 species are referred to overground mycosynusias (12 species are in mycosynusias of juniper and oak cenoses, 7 species are a part of pine and oak cenoses), out – of-layer mycosynusias include 4 species. Besides presented macromycetes, one type of myxomycetes was found there, new in phytocenoses of the reserve.

Below there is an annotated systematized list of new species. While working out this list following symbols and abbreviations were used: Ecological groups. *Mr* – symbiotrophs, saprotrophs: *Hu* – humic, *St* –on mat, *Le* – on wood, xylotroph, *M* – on moss. Phytocenoses. OHb – oak (*Quercus pubescens* Willd.) with oriental hornbeam layer (*Carpinus orientalis* Mill.), OHb(Jp) – oak with juniper (*Juniperus excelsa* M.B.) and oriental hornbeam layer, JpO – juniper and oak, JpOHb – juniper and oak with oriental hornbeam layer, OJp – oak and juniper, OJpHb – oak and juniper with oriental hornbeam layer, OHb(Jp,P) – oak with oriental hornbeam layer, partly juniper and pine *Pinus pallasiana* D. Don), JpO(P) – juniper and oak, partly pine, JpPO – juniper, pine and oak, ArbOJp(P) – arbutus (*Arbutus anrachne* L.) oak and juniper, partly pine. Abundance/Sociability. 4 – in many places, 3 – unevenly, scattered, 2 – scattered a lot, + – single place (one specimen or one group, assemblage) / 3 – by big groups, 2 – by small groups, 1 – single specimens. Degree of species novelty. (*) – new in the reserve (the first find in the reserve),* – new in the Crimea (the first find in the Crimea).

A S C O M Y C O T A
A S C O M Y C E T E S
PEZIZALES

Pezizaceae

PEZIZA Fr.

(*)*Peziza badia* Pers. – *Hu*, moistened ecotope, on stone with *Adiantum capillus-veneris*, +/3, 05.06.2012, 13.07.2012.

(*)*Peziza violacea* Pers. – *Hu*, the same place, +/3, 14.02.2013, 14.03.2013.

Tuberaceae

TUBER P. Micheli

(*)*Tuber aestivum* Vittad. – *Mr*, block № 3, JpPO, 1 specimen, 05.05.2014. During a long-term period reliable information about *T. aestivum* spreading in the Crimea were added up to data of Khrystyuk P.M. (1961), which were included into "Ocherk o sjedobnyh i nesjedobnyh grybah Kryma" [12]. Last decade *T. aestivum* was registered on South Coast (05.06.2006) and in Karadagsky reserve (16.11.2007) – the first of the Crimean reserves where this fungus was found [11]. It was included into the Red Data Book of the Russian Federation (RDBRF), as a species, reducing its number, and Ukraine (RBU), as a rare species with underground fruit bodies, European Red Data List of threatened fungi (ERDLTF) as well [6, 13, 16].

B A S I D I O M Y C O T A
B A S I D I O M Y C E T E S
A g a r i c o m y c e t i d a e
AGARICALES

Coprinaceae

COPRINUS (Fr.) Gray

(*)*Coprinus xanthothrix* Romagn. – *Hu*, OJp, block № 14, 1 specimen., 03.04.2013.

PSATHYRELLA (Fr.) Quél.

***Psathyrella spadiceogrisea* (Fr.) Maire.** *Hu*, moistened ecotope, at the stone with *Adiantum capillus-veneris*, +/3, 3 specimens., 02.06.2011.

(*)*Psathyrella piluliformis* (Bull.) P.D. Orton [syn. *Psathyrella hydrophila* (Bull.) Maire]. *Hu*, the same place, +/2 (3+3 specimens.), 17.06.2011.

Cortinariaceae

CREPIDOTUS (Fr.) P. Kumm.

(*)*Crepidotus cesatii* (Rabenh.) Sacc. [syn. *Crepidotus sphaerosporus* (Pat.) J.E. Lange]. *Le*, block № 10, OHb(Jp), on the oak wood, 4/2-3, 13.01.2012.

Entolomataceae

ENTOLOMA (Fr. ex Rabenh.) P. Kumm.

****Entoloma nidorosum* (Fr.) Quél. [syn. *E. rhodopolium* (Fr.: Fr.) Kummer f. *nidorosum* (Fr.) Noordel.].** *Mr*/*St*, southern part block № 10, OHb(Jp,P), +/2 specimens., 04.11.2010. This species was included into the RBU as a rare species, found sporadically [13]. The most favorable European regions are southern and southeast.

Hygrophoraceae

HYGROPHORUS Fr.

****Hygrophorus arbustivus* Fr.** *Mr*, block № 12, JpO(P), 2-3/1-2, 06-22.02.2013.

Pluteaceae

PLUTEUS Fr.

****Pluteus hiatulus* Romagn.** *Le*, block № 10, JpPO, 1 specimens., 07.10.2013.

Tricholomataceae

ARRHENIA Fr.

Arrhenia rickenii* (Hora) Watling [syn. *Leptoglossum rickenii* (Hora) Singer]. *St*, blocks №№ 10, 13, OJpHb and JpOHb, 2(+)/2, 24.02.1010, 10.12.2012.

MYCENA (Fr.) GRAY

(*)*Mycena purpureofusca* (Peck) Sacc. *St*/*Le*, block № 10, JpPO, 3/2-3, 22.01.2013.

(*)*Mycena zephirus* (Fr.) P. Kumm. *St*, block № 10, JpPO, 3/2-3, 07.10.2013.

RICKENELLA Raithelh.

****Rickenella fibula* (Bull.) Raithelh.** *M*, block № 14, JpO, +/2, place of growth is at a path, in moss 22.01.2013.

BOLETALES

Boletaceae

BOLETUS L.

****Boletus depilatus* Redeuilh.** *Mr*, block № 6, OHb, 1 specimen., 05.07.2006. A rare species, included into ERDLTF [16] is a candidate for RBU [3]. Its localities are Eastern and Central Europe, the Mediterranean, Balkan Peninsula.

****Boletus lupinus* Fr.** *Mr*, blocks №№ 6, 10; JpPO, 5 specimens., 21-25.10.2013. warm-requiring xerophilous species, mostly found in southern, especially in the Mediterranean and European regions, but isn`t adapted in the northern regions, can be found in Israel. It was included into ERDLTF [16].

****Boletus queletii* Schulzer.** *Mr*, block №№ 2; 4, JpOHb, 3/1-2, 03-09.07.2014. Thermophilous species. It is mostly spread in plain and deciduous forests (Oak-Hornbeam, Oak, Beech (Fagus). It was included into ERDLTF [16], a candidate for RBU [3].

Suillaceae

SUILLUS Gray

****Suillus collinitus*** (Fr.) Kuntze. *Mr*, blocks №№10, 11, JpPO и ArbOJp(P), 1-2/1-2, 24.12.2010, 28.11.2012. Thermophilous species, mostly spread in Southern Europe, creating mycorhiza in connection with some species of pine (*Pinus halepensis* Miller, *P. nigra* J.F.Arnold, *P. pinea* L., *P. sylvestris* L.), well-known in Iran, favorable environment is a lime soil. It was included into ERDLTF [16].

PHALLALES

Geastraceae
MYRIOSTOMA Desv.
(*)*Myriostoma coliforme* (Dicks.) Corda. *Hu*, block № 5, OHb(Jp), three groups consisted of 3-5 specimens, 22.10.2013. In the Crimea *M. Coliforme* was actual for old parks only. It was included into addition to the Bern Convention, ERDLTF, RBU (as a rare species), Appendix to RDBRF [6, 13, 15, 16].

POLYPORALES

Ganodermataceae
GANODERMA P. Karst.
(*)*Ganoderma lucidum* (Curtis) P. Karst. *Le*, block № 4, OHb(Jp), place of growth is on a tree of pubescent oak, 2 specimens 20.08.2014. It was included into RDBRF, as a rare species, and ERDLTF [6, 16].

RUSSULALES

Russulaceae
RUSSULA Pers.
(*)*Russula torulosa* Bres. *Mr*, blocks №№ 6, 10, 11, 12, JpPO and phytocenoses with pine plants, 2-3/1-2, 21.10-12.11.2013.
Stereaceae
STEREUM Hill ex Pers.
(*)*Stereum hirsutum* (Willd.) Pers. *Le*, OHb and OHb(Jp), +/2-3, summer-autumn. Before it wasn`t included into the species list, as by mistake it was denoted as syn. *Trametes hirsuta* (Wulfen: Fr.) Pilát instead of *Coriolus hirsutus* (Wulfen) Pat. [9].

THELEPHORALES

Bankeraceae
PHELLODON P. Karst.
(*)*Phellodon melaleucus* (Sw.: Fr.) P. Karst. *Hu /Le*, block № 11, JpPO, place of growth is a gully, +/2-3, 05.12.2010.

M Y C E T O Z O A
M Y X O M Y C E T E S

Ceratiomyxaceae
CERATIOMYXA J. Schröt.
(*)*Ceratiomyxa fruticulosa* (O.F. Müll.) T. Macbr. OHb(Jp), block №10, place of growth is on semidecomposed oak wood, 19.06.2006.

Conclusions

Therefore by present number of macromycetes taxons of species and intraspecies rank in the State nature reserve “Cape Martyan” reaches 321: Ascomycetes – 12, Basidiomycetes – 309.

Since last generalized published list (2010) it has been found 23 more species, new for this reserve, 10 of them are presented in the Crimea for the first time, 1 species of myxomycetes was new for the nature reserve.

Among new species there are 14 rare including 3 protected species in Russian Federarion (*Ganoderma lucidum*, *Myriostoma coliforme*, *Tuber aestivum*) and 3 – in Ukraine (*Entoloma nidorosum*, *M. coliforme*, *T. aestivum*), 7 species are included into ERDLTF (*Boletus depilatus*, *B. lupinus*, *B. queletii*, *G. lucidum*, *M. coliforme*, *Suillus collinitus*, *T. aestivum*), and 1 species is in the Addition to Bern Convention (*M. coliforme*). These facts

emphasize long-term monitoring importance, carried out on the territory of the SNR "Cape Martyan".

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Sarkina I.S. New species of mykobiota inhabiting the nature reserve "Cape Martyan": macromycetes

// Works of the State Nikit. Botan. Gard. – 2014. – V. 139 – P. 68 – 73.

The article includes data of 23 new species of macromycetes in the State nature reserve "Cape Martyan". They were obtained during the field collection in 2011-2014 years and treatment of the herbarium. 14 species of the given number are rare and protected, 10 species are presented for the first time in the Crimea Peninsula. There is an annotated list of new species. Generally by now 321 taxons of macromycetes of specific and intraspecific class are known for this natural area of preferential protection (120 ha).

Key words: my*kobiota, macromycetes, the State nature reserve "Cape Martyan", monitoring.*

UDK 551.515.9(477.75)

THE REVIEW OF NATURAL HYDROMETEOROLOGICAL PHENOMENA WITHIN REGION OF NIKITSKY BOTANICAL GARDENS

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Introduction

Hydrometeorological phenomena are characterized as natural (elemental) hydrometeorological phenomena (NHP) if they reach definite values of intensity, duration, emergence time and can cause damage for economy and threat to human security. Natural hydrometeorological phenomena are the most dangerous result of climate instability. Being climatic extrema, they influence on the long-term weather patterns of a region. In recent years due to considerable climate fluctuations, a number of NHP in Russia and Ukraine has increased and mostly they become catastrophic with large-scale material losses for a state economy and even human victims [6, 7]. As a rule, NHP emerge in complex, what aggravates their negative effect: heavy showers are accompanied by storm wind, squall, hail; blizzards are accompanied by snowfall and high wind, sleet and ice formations and etc. Each month, season, period and year is characterized by a definite type of the natural phenomenon or a complex of phenomena, caused by anomalous circulation processes taking place in atmosphere and meteorological conditions [4].

South Coast of the Crimea isn`t a high risk area, though elemental hydrometeorological phenomena are registered annually here. Some of such phenomena occur almost every year (abundant rainfall, wind). Probability of others is too low (tornado, blizzards). Nevertheless integrated study of dangerous weather phenomena dynamics and analytical summarizing of NHP expectancy is a base to assess meteorological risk in this area.

At present agrometeorological station “Nikitsky Garden” possesses observation data for 85 years period, which will make it possible to generalize, retrace dynamics, some regularity and peculiarities of NHP emerge on South Coast of the Crimea in the region of Nikitsky Botanical Gardens.

The research objective is a complex study of dynamics, NHP mapping during a year and probability of their occurrence in the region of Nikitsky Botanical Gardens with further assessment of meteorological risks on this territory.

Objects and methods of the research

Data of agrometeorological station “Nikitsky Garden” (situated on the territory of Nikitsky Botanical Gardens), including a number of observations, were used during this research. Observation period for principle meteorological elements makes 85 years (1930 – 2014). To describe frequency and time-space distribution of storm winds on the area of Big Yalta, information of 1869 -1961 period (generalized by staff of marine hydrometeorological station “Yalta” and presented in the work “Hydrothermal regime of South Coast of the Crimea”) was used [2].

Documents of Goscomhydromet USSR, Ukrainian GMC and Roshydromet served as the base of criteria for natural hydrometeorological phenomena (RD 52.3.2.03.-13, RD 52.04.563 – 2013).

Phenomena reaching definite values, capable to cause damage for economy and material loss are characterized as natural (elemental) hydrometeorological phenomena

(NHP): 1) wind (squall) with velocity of 25 m/s and more, 2) shower or an intensive rain, ≥30mm per 1hour or less, 3) heavy rain, ≥30mm per 12hours or less, 4) heavy snowfall, ≥20mm per 12hours or less, 5) air temperature pulldown till -10°C and below within South Coast of the Crimea (SCC), 6) hail, 20mm across diameter and bigger, 7) worsening of horizontal visibility (because of fog, blizzard) till 100m for 12 hours and longer, 8) hot dry wind, 9) blizzard or duststorm with wind velocity of ≥15 m/s for 12 hours and longer, 10) increasing of the air temperature on SCC till 40°C and higher, 11) ice-covered ground with formation of ≥20 мм, 12) sleet formation of ≥35 мм.

Physiographic position of South Coast of the Crimea and the Crimean Mountains underlie formation of synoptic processes, causing dangerous natural phenomena. On Nikitsky Botanical Gardens area since 1930-2014 it was registered 330 cases of weather phenomena that reached criterion of NHP, set for this territory. Average number of such phenomena annually is 4. The most frequent phenomena as follows: heavy rains, strong wind and air temperature pull down till -10°C (Fig.1). For this period, 137 cases (42% of total NHP, emerged on the area of Nikitsky Botanical gardens) of heavy rain and 132 cases (40%) of heavy wind were registered (table 1). Air temperature pull down till -10°C were fixed 35 times (11%). In recent 85 years of meteorological observations in Nikitsky Botanical Gardens it was registered 5 cases of heavy shower and 7 showfalls (see table 1). In this period on South Coast of the Crimea, regular air temperature increasing in summer causes precipitation reduction in June and August. In combination with high summer temperature it calls more dry phenomena, decreasing crop capacity of SCC agriculture. Concerning above-mentioned period, hot dry wind was registered in August 1971, July 1999 and July-August 2007. Heavy hail emerges rarely: July the 8th 1977, July the 15th and August the 29th in 2006. Hailfall was lasting for about some minutes, but economy of SCC got huge losses. Size of hailstones reached 20-25 mm across diameter; some of them were 30mm each. For last 25 years on the area of SCC long-term cases of fog were registered with horizontal visibility of 100m/12 hours and longer: March the 25th 1990 and May the 8th 2006. Long-lasting blizzards with horizontal visibility of 100 m lasting for 12 hours weren`t registered on South Coast of the Crimea. Since 1930 till present only watersprouts were fixed (13.08.1987). Cases of tornado weren`t recorded.

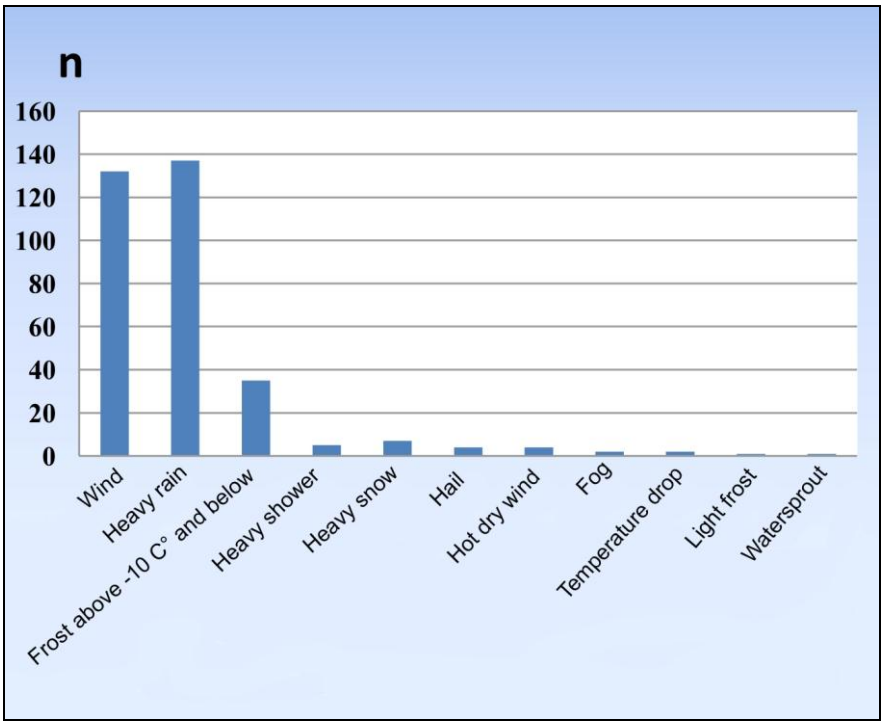


Fig.1 Types and number (n) of natural hydrometeoroplogical phenomena, registered on the area of Nikitsky Botanical Gardens during 1930 – 2014 period.
(Not considering anomalously hot/cold weather and extremely fire hazard)

Table 1

Number of natural hydrometeoroplogical phenomena during 1930 – 2014 period and decades separately on the area of Nikitsky Botanical Gardens

Years	1930-1940	1941-1950	1951-1960	1961-1970	1971-1980	1981-1990	1991-2000	2001-2010	2011-2014	1930-2014
Wind ≥ 25 m/s	1	5	2	18	30	21	20	28	7	132
Heavy rain ≥30 mm per ≤12 h	20	9	15	17	15	20	19	19	3	137
Heavy shower ≥30 mm per ≤1 h	0	0	0	1	0	0	0	4	0	5
Frost ≤-10◦C	7	7	3	4	4	4	0	3	3	35
Heavy snowfall ≥20 mm per ≤12 h	0	0	0	2	0	1	1	1	2	7
Hail ≥20 mm	0	0	0		1	0	0	3	0	4
Fog <100 м during ≥12 h	0	0	0	0	0	1	0	1	0	2
Hot dry wind	0	0	0	0	1	0	1	2	0	4
Watersprout	0	0	0	0	0	1	0	0	0	1
Hard frost	0	0	0	0	0	0	0	1	0	1
Temperature drop	0	0	0	1	1	0	0	0	0	2

Sharp reduction of daily average air temperature per a day within 10°C occurred twice: February the 26th and 27th in 1968, January the 12-13th in 1972.

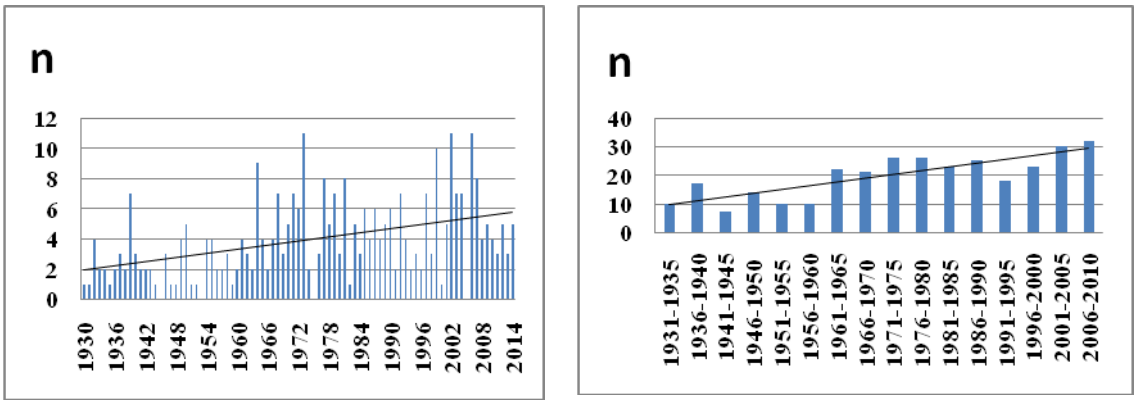
In recent 85 years phenomena of hard frost, caused losses, was registered only once on the area of Nikitsky Botanical Gardens – at the beginning of April, 2004. Due to advection of cold polar air on South Coast of the Crimea air temperature below zero was keeping during 10 hours in April the 3rd. The frost was accompanied by northeast wind with velocity of

16m/s (by wind gust) and low relative humidity (35-39%). At night (April the 3-4th) advective frost was intensified by radiation cooling, minimal air temperature fell till -5,5°C, temperature of soil surface went down till -8,5°C.

Such intensive frosts hardly ever occur on the coast in the beginning of April. Before 2004 they were registered only in April the 3-4th in 1965, when minimum air temperature fell till -5,7°C. The following should be mentioned there weren`t any damages for fruit plants after this phenomena in 1965, as all cultures were in state of bud swelling or budbreak. In spring 2004, due to extremely warm weather almond, apricot and peach trees were in blossom, alycha shedded its blossom, fig tree was on the stage of budbreak and forming of infructescences of the first generation, grape vine was on the stage of mass bud swelling, some cultivars of grape vine had a budbreak. That`s why frost in April the 3-4th in 2004 damaged all blossoming fruits completely. That year damage of grape vine reached 30-45%, yield of apricot, peach and alycha on South Coast of the Crimea was small or wasn`t at all.

In period of 1930-2014 blizzard and duststorm with an average wind velocity of ≥15 m/s lasting for 12 hours or longer didn`t occur. Maximum air temperature on the territory of Nikitsky Botanical Gardens wasn`t higher than 39°C. Observation for ice-covered ground and sleet formation are not carried out.

To specify nature of elemental hydrometeorological phenomena changes, trends of all phenomena case number, recorded on Nikitsky Botanical Gardens area, were diagramed and analyzed. These diagrams include annual and 5-years periods data (Fig.2), and dominant phenomena (heavy wind and heavy rain,) information as well (Fig.3 – 4). The linear trend permits to investigate fluctuations in the middle of time line, tracing their interannual dynamics, which is characterized by alternation of increasing and decreasing periods. Increasing direction of the linear trend is caused by sophisticated interaction of atmosphere circulation, natural and anthropogenic aerosols, solar activity level, greenhouse gases [4]. Linear trend was counted in complex for all phenomena. It presents a tendency to increasing of the total number.



a) **b)**

Fig. 2 Number of natural hydrometeoroplogical phenomena cases (n) annually (a) and by 5-years periods in the region of Nikitsky Botanical Gardens (b)

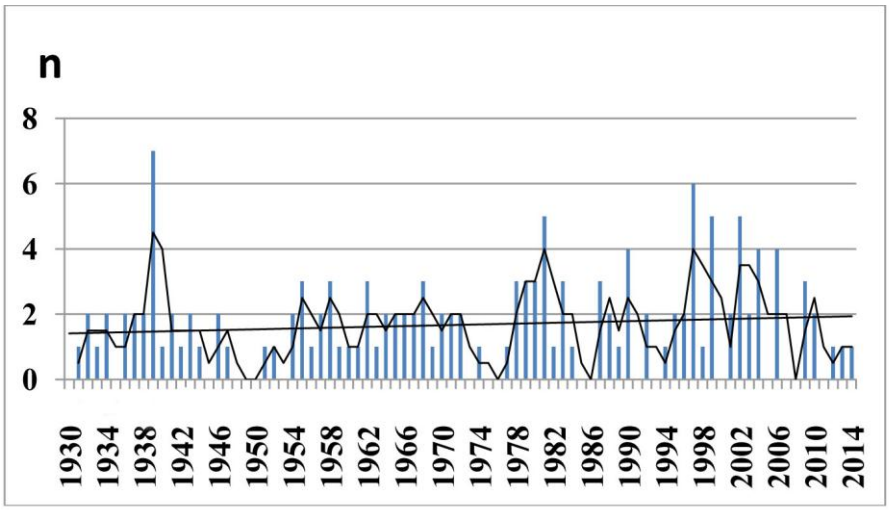


Fig. 3 Number of heavy rain cases (n), ≥30mm per 12 hours and less in the region of Nikitsky Botanical Gardens

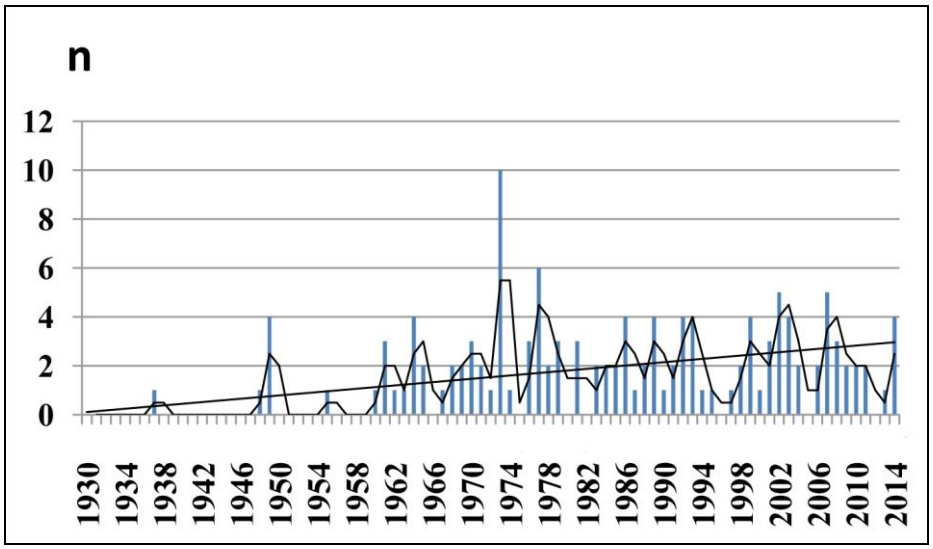


Fig. 4 Number of wind cases (n) of 25 m/s and more in the region of Nikitsky Botanical Gardens

Different phenomena have their own tendency, but in recent 15 years a positive tendency prevails due to global warming. According to this trend total number of phenomena increases. The biggest number was marked in five-year periods: 2001-2005 and 2006-2007 (30, 32) NHP (fig.2); it can be caused either by intensive anomalous synoptic situations or more thorough registrations. There is a tendency to more frequent heavy rain and strong wind (Fig. 3-4). According to interannual dynamics of heavy rain phenomena (Fig.3), 11-years cycle of solar activity is traced in alternation of occurrance increasing and decreasing periods. Connection between rainfall and solar activity cycle was mentioned in works of some scientists [1, 5, 6]. But to prove this hypothesis and create a model for prognostication, more in-depth studies are necessary. Predictable regularity of solar activity cycles permits to forecast humidity and possible activation of unfavorable phenomena. Cycles of atmospheric precipitation have grave consequences: 1. Water supply in the Crimea, capacity of reservoir replenishment, social problems, economical losses; 2. Landslide activization or damping, underflooding, duststorms, erosion by water and etc. [1, 6].

These trends present status of NHP for 1930 – 2014, what is quite stable for this period. Within new data, tendency direction can change.

On South Coast of the Crimea the most widespread natural hydrometeorological phenomenon is heavy rain, which causes catastrophic showers, mud-and-stone flows, inundation, floods of agricultural territory, living and production areas, even results changes of landscape, particular in the Crimean Mountains.

Rains, ≥30 мм per 12 hours and less, occur quite frequently on South Coast of the Crimea and in the region of agrometeorological station, about once or twice a year. Total number of NHP with liquid precipitations (heavy rain, shower), occurred on the territory of Nikitsky Botanical Gardens for 1930-2014, is 142. In recent 30 years frequency of rain (with such a level of precipitation) has considerably increased. (table 2).

Table 2.

Frequency of heavy rain (number) with different precipitation level, occurred in the region of Nikitsky Botanical Gardens for 1930-2014.

Years	Amount of precipitation, mm						
	≥30	≥50	≥100	≥150	≥200	≥250	≥285
1930-1940	20	4	2	1			
1941-1950	9	2					
1951-1960	15	2					
1961-1970	16	6	2	2	2	1	1
1971-1980	15	2					
1981-1990	21	3					
1991-2000	19	3					
2001-2010	23	2					
2011-2014	3	1					
1930-2014	142	26	4	3	2	1	1
Notes: Hereinafter: Empty table cells are for no occurrence.							

Peculiarities of atmospheric circulation, the Crimean Mountains and the Black Sea effect on seasonal distribution and type of precipitation on South Coast of the Crimea. Amount of precipitation in cold season is more than in warm, due to Mediterranean cyclones reached the Black Sea. Precipitations are more prolonged but not so intensive. In frost-free season the Crimean Mountains favor intensive orderly upward movement of air and convection [3]. Above mountains there are favorable conditions for activization of weather fronts, accompanied by intensive showers and thunderstorms, squalls, sometimes mud-and-stone flows, causing huge damage in agriculture. Annual variation of heavy rain frequency presents a seasonal periodicity. Out of 142 cases, registered in Nikitsky Botanical Gardens, heavy rains mainly occurred in summer (44) and autumn (54) months in recent 85 years. Such a seasonal periodicity was usually traced in decade periods (Fig.5). In winter month rain occurrence, ≤30 mm per 12 hours or less, is marked for 4 years per 10; spring period: 1-2 years per 10; summer time: 5-6 years/10; in autumn – 6-7 years/10 (table 3).

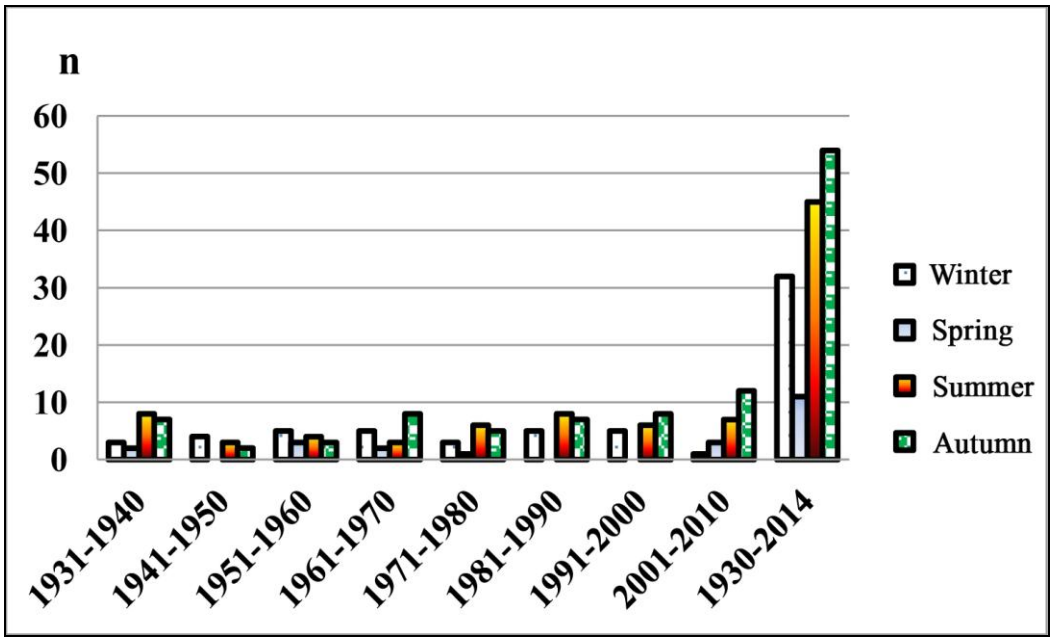


Fig.5 Heavy rain (number,n), ≥30 mm per 12 hours and less in the region of Nikitsky Botanical Gardens

Season	Amount of precipitation, mm						
	≥30	≥50	≥100	≥150	≥200	≥250	≥285
Winter (XII-II)	34	7	0	0	0	0	0
Probability, %	40	8	0	0	0	0	0
Spring (III-V)	12	0	0	0	0	0	0
Probability, %	14	0	0	0	0	0	0
Summer (VI-XIII)	44	11	2	1	0	0	0
Probability, %	52	13	2	1	0	0	0
Autumn (IX-XI)	54	8	2	2	2	1	1
Probability, %	64	9	2	2	2	1	1

In recent 85 years 26 cases of rain, ≥50 mm, were registered. Probability of such phenomenon on Nikitsky Botanical Garden area makes 3 years per 10. Heavy rains, ≥100 mm, were fixed here only 4 times (On August the 11th and 12th in 1939, on September the 5-6th in 1968). Rainfall of ≥150 mm has occurred 3 times (1939, 1968), 285 mm within one rain has been recorded only once. For the whole term of meteorological observations at agrometeorological station, the heaviest rain, in fact heavy shower occurred in September of 1968. It began raining at 14.30 on 4th of September and finished on 6th of September at 23.50 (in meteorology in pointed years conventional 1 day lasted since 21:00 till 21:00). That rain consisted of several heavy showers; then for 1 hour or less amount of precipitation reached 85,6 mm: since 21:42 till 22:42 it was 40 mm. In period from 04:30 on the 5th of September rain let up and till 10:10 30mm were added, since 13:40 till 14:30 – 37mm. At 17:45 rain let up. Total amount of precipitation in period since 12:30 on the 4th of September till 17:45 on the 5th of September made 270 mm. Since 17:45 till 19:20 it was raining slightly with breaks. At 19:20 on the 5th of September heavy rain began, lasting till 23:50 on the 6th of September (MSK), more 14,8 mm fell. In total for 3 days (the 4, 5 and 6th of September) amount of precipitation reached 284,8 mm. This rain was registered by hyetograph (rain grapher) of agrometeorological station "Nikitsky Garden". Tape of hyetograph registered 24 discharges on the 5th of September. Till 2007 amount of precipitation fell on the 5th of September, 240 mm per day, was considered an absolute maximum not only for area of Nikitsky Botanical Gardens, for Ukraine in general. Heavy shower occurred on September the 4-6th in 1968 damaged vineyards largely: the soil was extremely rain-washed, deep gullies emerged, the

roads were silted by soil and stones, strong mud flows occurred there, human victims were registered. Within sovkhoz (state farm) Gurzuf damage losses made 197500 roubles. Those days each hectare got 3000 m² of water, what effected on vine yield. Vine berries cracked, began to spoil. That`s why harvest began earlier than usually having low sugar concentration. Output of highly saccharine sorts of wine in the harvest of 1968 wasn`t large.

Shower or intensive rain of ≥30 mm per hour and less, occurred only 5 times for 1930-2009, 4 of them took place last decade (2001-2010). They are: heavy shower in September of 1968 mentioned above and rains occurred on the 14th of September in 2003, the 14th of July in 2004, the 26th of May in 2006 and the 10th of July in 2009. Amount of precipitation in 2003-2009 made 31-43 mm per one rain.

In spite of positive dynamics for good weather with gentle and moderate breezes, sometimes wind velocity reaches considerable values. Information about strong winds is of interest for different branches of national economy (construction and municipal engineering etc.). Especially these data gain importance for health-resort zone, gardening, park construction and etc.

As a rule the strongest and prolonged storm winds occur in cold season on South Coast of the Crimea. Taking into consideration separate storm cases maximum wind velocity reaches 40-45 m/s in the region of Aj-Petri mountain, 28-30 m/s in the region of Yalta city [2,3], 35-40 m/s in Nikita region (table 4). In Yalta wind velocity having northeast or western and southwest flows is lower than in the high seas [2].

Table 4

Occurrence (number, n), frequency and probability of storm winds with various directions by wind velocity of 25 m/s and more in the region of Nikitsky Gardens (1930-2014)

Period (year, season)	Parameter	Wind direction, rhumb								Total
		N	NE	E	SE	S	SW	W	NW	
Year	Number	6	17	1	0	0	19	14	75	132
	Frequency, %	4	13	1	0	0	14	11	57	100
	Probability, %	7	20	1	0	0	22	16	88	155
	Velocity value, m/s	28-39	25-40	25	0	0	25-40	25-34	25-40	25-40
	Probability 25-30 m/s	17	71	100	0	0	63	79	69	67
	Probability 35-40 m/s	1	1	0	0	0	1	0	11	9
Winter (XII-II)	Number	6	8	1	0	0	8	7	41	71
	Frequency, %	8	11	1	0	0	11	10	58	54
	Probability, %	7	9	1	0	0	9	8	48	84
Spring (III-V)	Number	0	6	0	0	0	2	4	15	27
	Frequency, %	0	22	0	0	0	7	15	56	20
	Probability, %	0	7	0	0	0	2	5	18	32
Summer (VI-XIII)	Number	0	0	0	0	0	0	1	0	1
	Frequency, %	0	0	0	0	0	0	100	0	1
	Probability, %	0	0	0	0	0	0	1	0	1
Autumn (IX-XI)	Number	0	3	0	0	0	9	2	19	33
	Frequency, %	0	9	0	0	0	27	6	58	25
	Probability, %	0	4	0	0	0	11	2	22	39

Wind of 25 m/s and more occurs due to strengthening continental anticyclone wedge spreading to southwest, and simultaneous deep cyclone from south or southwest to east of the Black Sea [3]. Frequency of storm winds isn`t similar in different regions of South Coast of the Crimea, but all over this phenomenon dominates in cold season. During November-March in Yalta 80% of all storm winds occur [2], Nikita – 82%. According to observation data for 1930-2014 maximum number of storm winds of 25 m/s and more was registered in the region of Nikitsky Botanical Gardens mainly occur in winter months (54%), minimum number is in summer months (table 4, fig.6). During summer period, storm wind of 25 m/s,

was recorded only once in recent 85 years. Seasonal periodicity keeps in decade periods (Fig.6).

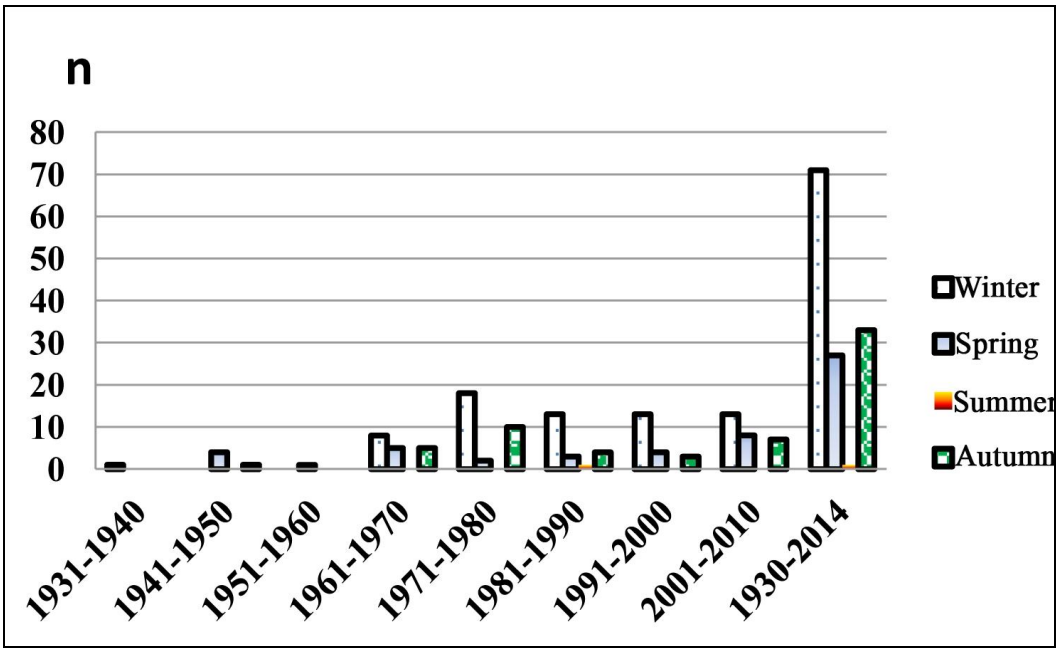


Fig.6 Number (n) of storm winds of 25 m/s and more occurred in the region of Nikitsky Botanical Gardens according to seasons

Within Yalta wind velocity of 25m/s or more is registered annually [2]. According to analysis of wind observations at agrometeorological station "Nikitsky Garden" for period 1930-2014 (table 4) winds of 25 m/s or more occurred in the area of agrometeorological station once or twice a year (132 cases for 85 years). The most frequent wind velocity is 25-30 m/s (up to 67% of total) (see table 4). Winds of ≥30 m/s were registered 49 times for these years, once in two years approximately. Winds of ≥30 m/s mainly occurred in Nikitsky Botanical Gardens during period 1961-1990 (table 5). Hurricane force winds of ≥35 m/s were registered 21 times in recent years, once or twice per 10 years. 9 cases of hurricane force winds of ≥40 m/s occurred for period 1930-2014, approximately ones in 10 years. The highest winds on South Coast of the Crimea within agrometeorological station "Nikitsky garden" have southwest (SW) and northwest (NW) directions. On South Coast of the Crimea on the 15th of November in 1992 strong hurricane was registered. It resembled well-known Balaklava storm which took place on the 14th of November in 1854, it covered the Black Sea and the Crimea during Sevastopol siege by English and French armies and navy. Storm wind on the 15th of November in 1992 started at 03:43 and reached 12 m/s, rushes – 20 m/s. At 06:09 southwest wind reached parameters of NHP (30 m/s). At 8:17 wind velocity decreased till 22 m/s, at 10:09 wind became stronger till 30-34 m/s. At 12:35 wind became weaker - 20 m/s. Atmospheric pressure during this hurricane fell much: from 982 hPa at 21:00 till 965 hPa (724 mm of mercury at 06:00). For 9 hours it became 17 hPa lower. From 00 till 06 – 12 hPa lower. Hurricane was accompanied by heavy rain, a number of precipitations made 36 mm. The losses caused by this hurricane were estimated at billions of roubles. In Yalta port some motorboats went down, many vessels, harbor cranes were damaged, quays and seaside were destroyed. In the sea wave height reached 10-12 m. In forest and parks trees were torn up by the roots, roofs were torn off many houses. As a rule strong winds on South Coast of the Crimea occur from November till March, but sometimes, for example in 1999 hurricane wind was registered on the 18th of May, when a lot of unripen fruits were brought down off

trees, grapes shoots were broken off, electricity cables were torn off. Near Arc at the entrance to Nikitsky Botanical Gardens billboards, set up on two metals were tied into a knot. In May of 2008 this phenomena occurred again.

Table 5

A number of incidents and probability of storm winds of 25 m/s and more within Nikitsky Botanical Gardens (1930-2014)

Years	Wind velocity, m/s			
	≥25	≥30	≥35	≥40
1930-1940	1	1		
1941-1950	5	2	1	1
1951-1960	2	1	1	1
1961-1970	18	10	3	3
1971-1980	30	13	3	3
1981-1990	21	9	3	
1991-2000	20	8	1	1
2001-2010	28	2		
2011-2014	7	3		
1930-2014	132	49	12	9
Probability, %	155	58	14	11
Notes Hereinafter : Empty table cells stand for absence of incident				

In the area of Nikitsky Botanical Gardens winds which become natural hydrometeorlogical phenomena have the following directions: north (N), northeast (NE), southwest (SW), west (W) and northwest (NW) (see table 4). In Nikitsky Botanical Gardens during the whole year, except summer period, when storm winds of NHP category are not registered (only 1 incident for 85 years), northwest winds prevail – 56-58% (fig.7) and probability these wind velocity reaches 40 m/s is the highest – 11%. Percentage of other wind directions in order of decreasing makes: SW – 14%, NE – 13%, W – 11%, N – 4% and E – 1% (Fig.7a).

In winter (December-February) northwest winds prevail. Frequency of other winds is not high and almost similar – 8-11% (fig. 7b). In case of Arctic air invasion northeast winds are accompanied by serious fall of temperature. Storm winds of 25 m/s and more are often phenomena within Nikitsky Botanical Gardens in winter period: 8-9 years per 10.

In spring period frequency of northeast winds increases up to 22% (Fig.7c). They are caused by northeast invasions and cold mountain air; in April-May – steppe hot dry winds occur which wither topsoil. In case of cyclone movement from west or northwest on the territory of the Crimea, west (15%) and southwest (7%) winds become natural hydrometeorlogical phenomena.

In autumn cyclone activity is registered, caused by southwest cyclones in the West of the Black Sea or in direction of Carpathians. As a result frequency of southwest (27%) winds increases considerably (Fig.7d). Frequency of other winds is not so high: northeast – 9%, west – 6%.

In spring and autumn probability of the wind to reach 25 m/s and more is less 2-3 times, than in winter – 3-4 years per 10 (see table 4).

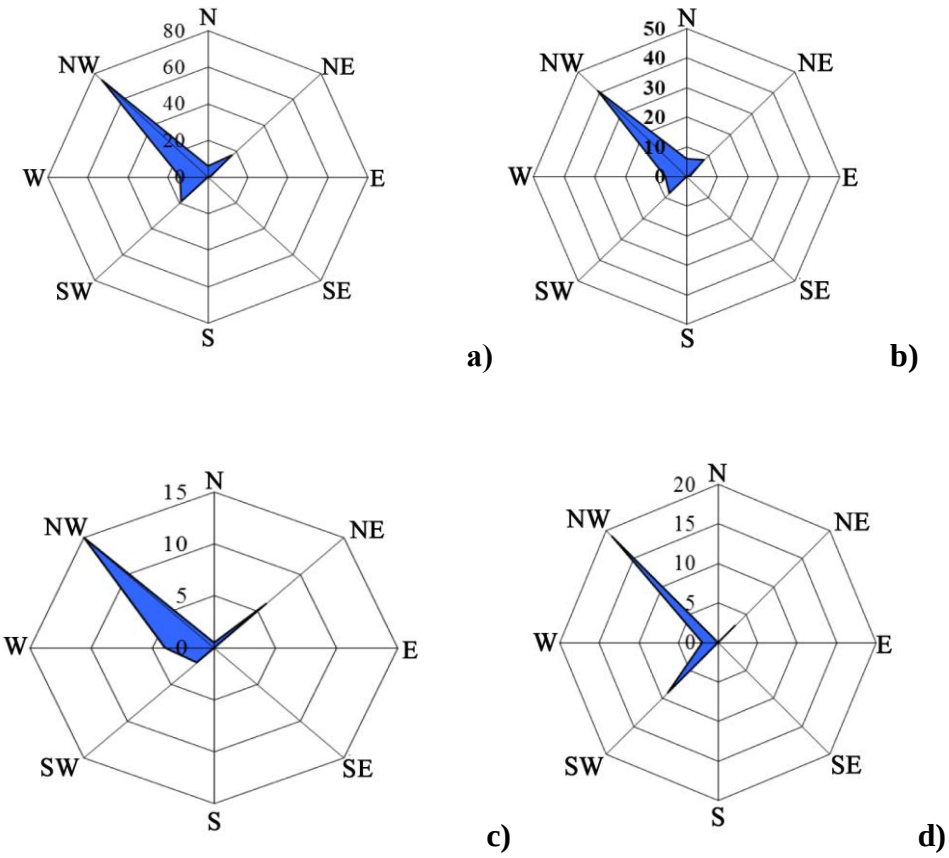


Рис. 7 Annual (a) and seasonal (b-d) roses of storm winds of 25 m/s and more within Nikitsky Botanical Gardens (observations for 1930-2014)
a) I - XII; b) XII – II; c) III – V; d) IX - XI

On South Coast of the Crimea local storms, caused by downdraft from the passes in direction of the sea are rare phenomena. They occur as a result of winter continental monsoon activization. Almost all over storms start like bora, as a strong gusty katabatic current of dry and cold air, which was accumulated on mountain pastures as a result of advection of cold night radiative cooling [3]. Air currents, sliding the mountains and canyons, dropping from the passes, reach considerable velocity and become winds of different directions in certain regions of South Coast of the Crimea, they varies from west to northwest.

Strong bora on the territory of Yalta occurs quite often, approximately once per 4-5 years. But this phenomena isn`t registered either agrometeorological station “Nikitsky Garden” (because of a sudden lowering of mountain level in village Nikita) or maritime hydrometeostation “Yalta” (because of limited observation). During bora wind velocity over mountains reaches 40 m/s (Cape Aj-Todor, canyon of three mountains (Vasiljevka)). Bora continues not so long, less than a day mainly. During bora winds have considerable vertical component, down directed. Bora causes some kind of cloudiness over mountains, spread along the mountains it resembles squall cloud [3].

November-March is a period when frequency of bora phenomena is the highest. During bora sharp variations of meteorological elements are registered: pressure, air temperature and etc. Particularly it concerns air temperature and humidity, their parameters decrease. As a rule wind is gusty.

In winter period atmospheric circulation over Crimean peninsula and the Black Sea is determined by Asian anticyclone. Arctic air invasions from Greenland over Scandinavia or from Taymyr island effected by cyclones which move from West of Europe, are accompanied by wind strengthening, air temperature on SCC is possible to decrease till -12…-17°C (1954)

[3]. Frost phenomenon -10°C and below, causing subfreezing and even destruction of warm-requiring ornamental plants and subtropical cultivars in parks and agricultural areas of South Coast of the Crimea, is registered in the region of agrometeorological station "Nikitsky Garden" (altitude above the sea level 208 m) 35 times in recent 85 years (table 6), on average 4 years per 10. Probability of temperature ≤-11°C is not so high – once or twice per 10 years. More often such low temperature is short-term at dawn, but in some years it kept 3-4 days running. So, in 1950 on 11-14th of January minimal air temperature was -10…-12°C. In 1985 such low temperatures were keeping since 19 till 21 of February. Decreasing of minimal air temperature on South Coast of the Crimea till -13°C and below is a rare phenomenon, during period 1930-2014 it was registered only 6 times. Absolute minimum of air temperature for the period mentioned above was -14,6°C on the 11th of February in 1930. On the 8th of February in 1976 minimal air temperature decreased till -14,5°C.

Heavy snow of ≥20 мм per12 hours and less was registered in the region of Nikitsky Gardens 7 times for 1930-2014 (see table 6). Particular amount of precipitation fell in 1961 on the 24th of December – 31,1 mm, and on the 31$^{st\ of}$ January - the 1st of February in 1988 it made 27 mm. In March of 2003 snowfall of 26 mm was recorded. Taking into consideration all years of meteorological observations in the region of Nikitsky Botanical gardens maximum height of the snow cover reached 56 sm (the third decade of February in 1985). In the end of January 1963 and beginning of March 1987 height of snow cover made 48 sm, the end of January in 1996 and 2012 – 41 sm and 33 sm. Within Nikitsky Botanical Gardens under weight of snow in 2012 large branches of trees snapped, some trees fell. Snow cover caused by snowfall complicates traffic on South Coast of the Crimea. On some road sections characterized by big slope angle traffic is stopped due to snow reel and ice-covered ground. Icy condition of roads, causing considerable troubles for traffic on South Coast of the Crimea, occurs in winter period annually (January-February) not only after snowfall of 20 mm, even of 5-7 mm but accompanied by air temperatures below zero. Probability of heavy rain is once per 10 years.

Table 6

Number (1930-2014) and probability of NHP occurrence within Nikitsky Botanical Gardens

Natural hydrometeorological phenomena	Number	Probability of occurrence (number per years)
Frost ≤-10◦C	35	Once per 4 years
Heavy snow ≥20 mm per ≤12 h	7	Once per 10 years
Hail ≥20 mm	4	Once per 20 years
Fog<100 m for ≥12 h	2	Once per 40 years
Hot dry wind	4	Once per 20 years
Tornado	1	Once per 85 years
Hard frost	1	Once per 85 years
Temperature drop	2	Once per 40 years

Conclusions

This work presents the most comprehensive description of natural hydrometeorological phenomena occurred in the region of Nikitsky Botanical Gardens during period of 1930-2014 according to data of agrometeorological station "Nikitsky Garden". Phenomena dynamics, distribution during a year and probability of occurrence were considered as well.

Analysis of extreme weather phenomena changes which take place within Nikitsky Botanical Gardens area induces to believe that on South Coast of the Crimea they can strengthen and become more frequent against the background of global warming. During period of 1930-2014 in the region of Nikitsky Botanical Gardens 330 cases of NHP were

registered. The most frequent of them are heavy rain, strong wind and decrease of temperature till -10°C.

In-depth researches are necessary to confirming a hypothesis about correlation of changes in amount of precipitation, cycle of solar activity and development of forecasting model.

Rain of ≥30 mm per 12 hours and less are registered 1-2 times a year within Nikitsky Botanical Gardens area. A number of such cases has considerably increased in recent 25 years. In winter months they occur 4 years per 10, in spring period - 1-2 years per 10, in summer – 5- 6 years per 10, in autumn - 6-7 years per 10. Probability of rainfall of ≥50 mm in the region of Nikitsky Botanical Gardens makes 3 years per 10. The most dangerous is rainfall of 100 mm and more (once per 30 years), which causes marked material damages in different sectors; rainfall of more than 150 mm occurs once per 40 years, more than 250 mm – once per 85 years. Heavy shower is registered once per 20 years on average.

Winds of 25m/s and more occur 1-2 per year, ≥30 m/s – once per 2 years. Mainly wind velocity (NHP) reaches 25-30 m/s (67% of total). Probability of hurricane force wind of ≥35 m/s is 1-2 per 10 years. Wind directions capable to become natural hydrometeorological phenomena are north, northeast, east, southwest, west and northwest. The most devastating winds are southwest and northwest. During the whole year, except summer, when storm winds from NHP category don`t usually occur (only once per 85 years), northwest winds prevail – 56-58% of total during the year, at the same time probability these winds reach hurricane velocity of 40 m/s is the highest, up to 11%. Percentage of other wind directions during the year ranges from 1 up to 14%.

In winter period northwest winds prevail. Frequency of other winds is not so high and approximately equal – 8-11%. Storm winds of 25 m/s and more occur quite often in winter within Nikitsky Botanical Gardens area: 8-9 years per 10. Spring period is characterized by often northeast winds – up to 22%, in autumn – southwest (up to 27%). In spring and autumn periods probability that wind velocity reaches 25 m/s and more is 2-3 times less than in winter and makes 3-4 years per 10.

Frost till –10°C and below, causing subfreezing and even destruction of warm-requiring ornamental plants and subtropical cultivars on the territory of parks and agricultural areas on South Coast of the Crimea in the region of agrometeorlogical station “Nikitsky garden” are registered 4 years per 10 on average. Probability that temperature decreases till ≤-11°C is not considerable: 1-2 years per 10. More often such frost phenomenon is short-term, but sometimes it keeps for 3-4 days running.

Probability of heavy snow of ≥20 mm per 12 h and less is once per 10 years, hail with hailstone of ≥20 mm across diameter and dry hot wind is once per 20 years, heavy prolonged fog and temperature drop occurred once per 40 years. Tornado and hard frost were registered only once per 85 years.

These data about natural hydrometeorological phenomena are of interest for different branches of national economy (building, municipal engineering and etc.). They are particularly important for development of recreational field, gardening and landscaping.

Received information should be taken into consideration while planning economic advancement, working out directions on adequate valuation of hazards and managing them, development of protective steps to prevent and reduce negative effect after them.

Results of this research are possible to apply as a reference material to investigate microclimate within Nikitsky Botanical Gardens area and Big Yalta.

Gratitudes

The author appreciates sincerely meteorologists of all generations of agrometeorlogical station “Nikitsky Garden”, who carried out meteorological observations

day and night in spite of shower, hot, freezing or windy weather. She thanks Fursa D.I., a head of agrometeorological station "Nikitsky Garden" (1959-2006) for collection, safety and systematization of archival material, colleagues-meteorologists Fursa V.P., Misyura N.V., Antonnikova L.A., Protsyk V.F., Reshetnichenko V.N., Maistrenko N.A., Korsakova P.B. for primary treatment of meteoelements.

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The detailed analysis of space-time distribution of natural meteorological weather phenomena occurred in the region of Nikitsky Botanical Gardens over a period of 1930-2014 was carried out for the first time. Trends and patterns of the interannual meteorological hazards were identified. Natural hydrometeorological phenomena are the most dangerous result of the climatic instability. Due to considerable climate fluctuations in recent years, their number has increased and in most cases they become catastrophic. Analytical generalization of such phenomena probability is necessary to develop directions in an appropriate assessment of the possible risks and managing them.

Key words: *climate, global climatic changes, meteorological conditions, natural hydrometeorological phenomena, risk.*

UDK 633.8:582.929.4:581.559(477.75)

INVESTIGATIONS OF ESSENTIAL OIL COMPONENT COMPOSITION OF *HYSSOPUS OFFICINALIS* L.

RABOTYAGOV V.D., SHIBKO A.N.

Introduction

Chemical character of essential oils (EO) is quite complex, but in general it presents a mix organic substances such as terpens with acyclic structure (main substances) where this or that component prevails. As a result of long-term essential oils investigations some authors suggested biogenetic schemes which find that essential oil components are formed after consecutive transformations [2, 3, 6, 7, 9]. According to current opinions, each transformation of one terpenic compound into another is under control of a certain gene which codes synthesis of an appropriate ferment. Having a lack of an appropriate ferment sequence of biosynthesis reactions stop and previous ferment is accumulated, that is a key factor which determines essential oil composition of a definite cultivar.

Analyzing open literature sources devoted to essential oil composition of *Hyssopus officinalis* it was revealed that data are quite fragmentary and sometimes even conflicting. Moreoften there is summery data of quantitative content of dominate components sometimes it`s possible to find analysis of component composition of different morphologic forms [1,4, 5, 8, 10, 11]. Data of intraspecific changeability of chemical composition of *Hyssopus officinalis* essential oil, correlations between certain terpenoid compounds, chemical composition of essential oil extracted out of different plant parts are hardly presented. According to mentioned above we carried out investigations aimed at studying the changeability of essential oil compound composition of *Hyssopus officinalis* for further selection.

Objects and research methods

Investigations were being carried out on the South Coast of the Crimea in Nikitsky Botanical Gardens since 2007 till 2012. Plants grown out of seed generation of *Hyssopus officinalis L.* (f.albus, f.cyaneus, f.ruber) were chosen as study material.

Crop inventory was organized during mass blooming. Row material was cut by hand and weighed at ones. Mass concentration of essential oil was determined in field-fresh material by hydrodistillation method applying Clevenger apparatus. Essential oil component composition was investigated using chromatograph Agilent Technology 6890N with mass-spectrometric detector 5973N. Essential oil components were identified according to data of chromatography of mass-spectrum chemical substances included into study mixtures; mass-spectrum database NIST02 (more than 174000 substances) were considered. Indices of component retention were calculated allowing for results of control analysis of essential oils with a set of standard alkanes [17].

Results and discussion

Investigation of EO component composition of *Hyssopus officinalis* seed population revealed intraspecific composition of essential oil isolated out of over-ground material is quite diverse and includes components as follows: pinocamphone, isopinocamphone, α- and β-pinene, sabinene, myrcene, β-phellandrene, linalool, myrtenol, methyleugenol, elemol and etc. EO composition included 60 terpenic compounds, 41 of them were identified. EO analysis (table 1) displayed considerable variations of EO composition. Mainly (70%) plants

synthesized pinocamphone, isopinocamphone, β-pinene, sabinene, myrtenol, elemol and immaterial amount of other components.

The second plant group (20%) of *Hyssopus officinalis* accumulated 5 principal terpenoids: pinocamphone (up to 60%), β-pinene (up to 6,2%), β- phellandrene (up to 6,8%), spathulenol (up to 3,5%), myrtenol (up to 6,3%), *caryophyllene (up to 3,5%).*

And the third plant group (10%) of *Hyssopus officinalis* (special group) synthesized: isopinocamphone (up to 61,1%), β-pinene (up to 10,5%), elemol (up to 19%), eudesmol (up to 7,6%). This plant group is special due to biosynthesis of sesquiterpenes (totally up to 25%). EO composition of all study plants mainly corresponds cultivar of *Hyssopus officinalis,* only proportion of the principal components has some variations.

Concentration of independent hydrocarbons in essential oil didn`t exceed 1,5% only maximum content of β-pinene made 10,5%. Standard way of distribution in the range of low values with single-humped curve and minimal values on the track level was typical for all of them.

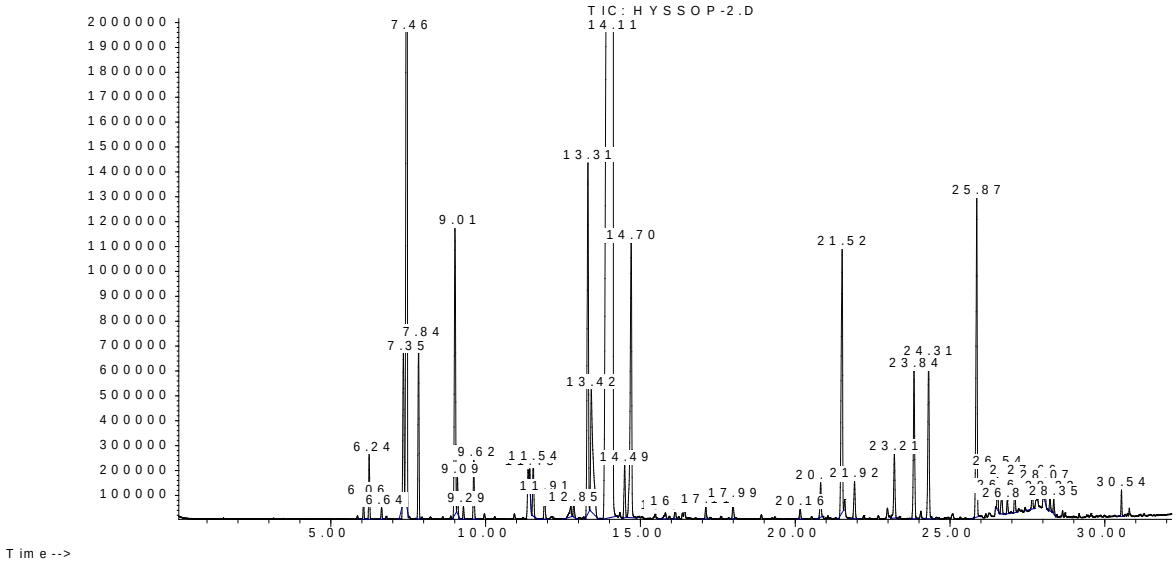
Plant range by mass concentration of pinocamphone and isopinocamphone, as the main dominants in *Hyssopus officinalis* essential oil should be emphasized. It`s a well-known fact that pinocamphone is a bicyclic terpenic ketone which possesses cis- and trans-forms being in the dynamic balance, that causes constant instability of natural pinocamphone isolated out of *Hyssopus officinalis* essential oil [1].

In terms of investigations it was determined that plant distribution by mass concentration of pinocamphone is in range of values from 4,34% up to 60,48% with maximum number of plants (35%) containing pinocamphone (from 10 up to 20%) and the second hump in the range of values is from 30 up to 40%. Other plants are distributed evently with interval up to 10% and from 20 up to 30%. Though, there was revealed a plant with maximum level of biosynthesis of pinocamphone (up to 60,48%) what is a considerable gap in pinocamphone concentration in comparison with other plants. It makes possible to accept two chemotypes of *Hyssopus officinalis:* with middle and high concentration of this terpenoid in the essential oil.

The same situation takes place in plant distribution by isopinocamphone concentration in essential oil. Plant distribution curve by isopinocamphone content in essential oil is bimodal with maximum number of plants (30%) in interval (20-30%) and the second top is in the range of high values of isopinocamphone (from 50-60%) with 35% of plants. Among study plants there is a form with a rather high biosynthesis of isopinocamphone (up to 61,12%) what makes this plant quite appropriate starting material for selection.

Distribution curve by biosynthesis of elemol in the essential oil has a left asymmetry with maximum distribution in interval (2-7%) of average values for this component, only for some plants synthesis of elemol reaches 19%. It should be noted that elemol is monocyclic sesquiterpenic alcohol with a flavor what improves essential oil. Almost all minor components in essential oil of *Hyssopus officinalis* are presented in low concentration with a small interval of variation. In all cases hystograms were unimodal with, there wasn`t the second maximum point in distribution, that is plants belonged to the same chemotype by concentration of terpenoids mentioned above.

Analysis showed the following terpenic compounds were characterized by the highest level of changeability and variation coefficients: β-pinene, sabinene, myrcene, β-phellandrene, caryophyllene.



1	6.05	0.144% α-tuyen	22	16.12	0.094% neral
2	6.24	0.444% α-pinene	23	17.11	0.111% geranial
3	6.64	0.085% camphene	24	17.99	0.162%
4	7.34	1.122% sabinene	25	20.16	0.090% geranylacetat
5	7.46	9.156% β-pinene	26	20.82	0.311% β-burbonene
6	7.83	1.302% myrcene	27	21.51	2.822% methyleugenol
7	9.01	2.680% β-phellandrene	28	21.91	0.382% caryophyllene
8	9.09	0.244% 1,8-cineol	29	23.20	0.670% alloaromadendrene
9	9.28	0.097% trans-ocimene	30	23.84	1.506% germacrene D
10	9.62	0.472% cis-cimene	31	24.31	1.664% bicyclogermacrene
11	11.42	0.132% terpinolene	32	25.86	3.000% elemol
12	11.53	0.456% α-tuyon	33	26.53	0.292% spathulenol
13	11.90	0.244% β-tuyon	34	26.67	0.224%
14	12.75	0.156% pinocarveol	35	26.85	0.108% viridiflorod
15	12.84	0.124% camphora	36	27.09	0.273% ledol
16	13.30	3.491%	37	27.65	0.219% γ-eudesmol
17	13.41	2.247% pinocamphone	38	28.07	0.133% α-eudesmol
18	14.10	61.122% isopinocamphone	39	28.23	0.125%
19	14.49	0.508% α-terpineol	40	28.35	0.118%
20	14.70	3.214% myrtenol	41	30.53	0.169% rosifoliol
21	15.80	0.087%			

Fig. 1 Chemoform of *Hyssopus officinalis* with the highest concentration of isopinocamphone

Researching the EO composition of *Hyssopus officinalis* isolated out of seed population, unique chemotypes were found out. In this way we marked out methyleugenol chemotype which contains 51,32% of methyleugenol, up to 13,1% of isopinocamphone, till 6,77% of elemol. At the same time linalool chemotype was revealed that has the following EO composition: pinocamphone – 2,94%, isopinocamphone – 33,38%, linalool – 34,88%; and uncommon chemotype was found out in terms of this research: pinocamphone – 4,34%, isopinocamphone – 7,77%, methyleugenol – 2,25%, elemol – 10,49%, manool – 21,7%, viridiflorol – 7,51% and others.

Such a pronounced polychemism and a weak connection of some EO components give a great opportunity for an individual plant selection out of *Hyssopus officinalis* seed population. This plant is characterized by high level of plant population heterogeneity. This type of changeability and correlation of biosynthesis processes of some terpenoids is kept in

the seed generation that proves cultivar genetic stability and permits to mark out it as an independent taxonomic unit of *Hissopus* genus.

As a study cultivar *H. officinalis* has three plant forms with white, blue and pink flowers, we carried out investigation of EO component composition of these forms. Comparison study of EO composition revealed there is no a considerable difference by hydrocarbon content between f.albus (9,59%) and f.ruber (10,31) forms. While f.cyaneus form contains hydrocarbon twice less (4,4%). Heighten concentration of alcohols was marked for f.albus (till 8,69%), a bit less than for f.cyaneus (till 5,73%) and f.ruber form had the least value (till 4,61%). As to aldehydes and ketones, basic difference wasn`t noted. F.cyaneus and f.ruber have the same concentration (59,8% each) and f.albus contains a bit more (till 62,17%).

Comparison study of EO composition for some terpenoid compounds revealed basic difference for some specimens. In this way, the largest mass fraction of β-pinene was registered for f.albus form (till 10,50%), a little bit less for f.cyaneus, f.ruber forms (till 9,12%). Though it`s necessary to note that distribution curve by this character for all plant forms has left-side distribution with maximum number of plants containing β-pinene with interval of 3-5%.

Basic differences in EO of f.albus, f.cyaneus, f.ruber forms by mass fraction of β-phellandren, spathulenol, myrtenol, caryophillen, ledol and other terpenic compounds weren`t registered. All of them had a single-humped curve in the range of low values.

Comparison study of plant distribution by biosynthesis of pinocamphone and isopinocamphone in essential oil of different forms presented the followings: maximum level of pinocamphone mass fraction in essential oil was marked for plants of f.ruber form - till 60,48%, f.cyaneus form – till 36,37%, f.albus – till 35,24%. Quite different situation takes place in biosynthesis of isopinocamphone. Maximum mass fraction of isopinocamphone occurred in essential oil of f.albus plants (till 61,12%, see fig.1), a bit less – for f.cyaneus (till 57,93%) and the minimum concentration was registered for f.ruber plants – till 38,14% (table 1).

Table 1

EO component composition of different *H. officinalis* morphological forms

Component	Variations of component mass fraction, %		
	f.albus	f.cyaneus	f.ruber
sabinene	0,19 – 0,42	0,38 – 1,53	0,19 – 0,69
β-pinene	0,41 – 2,89	0,42 – 3,16	0,88 – 4,35
myrcene	0,26 – 0,46	0,25 – 1,35	0,15 – 0,71
β-phellandrene	0,19 – 1,11	0,22 – 2,12	0,32 – 0,82
linalool	0,13 – 34,88	0,22 – 0,93	0,12 – 0,91
α-tuyon	0,17 – 1,09	0,30 – 1,02	0,75 – 1,40
pinocamphone	1,12 – 35,24	12,59 – 36,37	11,54 – 60,48
isopinocamphone	34,65 – 61,12	34,67 – 57,93	4,44 – 38,14
α-terpineol	0,25 – 0,73	0,30 – 0,32	0,29 – 1,43
myrtenol	3,63 – 6,31	1,21 – 3,43	1,68 – 4,17
methyleugenol	0,52 – 37,80	0,23 – 0,73	0,32 – 1,70
caryophillene	1,82 – 3,77	2,12 – 7,70	1,40 – 5,17
elemol	0,33 – 2,81	0,63 – 1,49	0,66 – 3,21
ledol	0,21 – 0,92	0,26 – 0,88	0,11 – 0,52
spathulenol	1,39 – 3,52	0,83 – 3,35	0,48 – 2,39
viridiflorol	0,23 – 2,15	0,10 – 2,16	0,10 – 7,24
manool	0,36 – 5,85	0,10 – 5,76	0,19 – 20,16

Investigation of EO component composition of *H. officinalis* made it possible to mark out of the f.albus plants a valuable chemoform with biosynthesis of a rare terpenoid – methyleugenol (till 37,80%). That`s why we can say with certainly about two chemotypes of *H. officinalis:* with high and low concentration of this terpenoid in essential oil. It`s worth to note this chemotype is characterized by low level of pinocamphone biosynthesis (till 2,2%). Besides among f.albus plants there was found out a chemotype which synthesizes mostly linalool (34,88%). One more peculiarity of this chemotype is a low mass fraction of pinocamphone (till 2,24%). So, talking about revealed chemotypes we have inhibition of pinocamphone biosynthesis while linalool biogenesis takes place.

Essential oil is a complex chemical substance, capable to be effected by a number of factors. Terpenoid composition of essential oil depends upon plant development phase, hydrothermal factors which make a background for plant growth and development, and even the terms of yielding. According to literature data yielding time of flower row material is of great importance as essential oils have considerable changes under influence of daily and seasonal variations [13, 16, 18, 19]. Studies in variation of some EO terpenoids mass fraction of *H. officinalis* during a day aren`t covered in open literature sources in comparison with determination of EO component composition of *H. officinalis* different cultivars which is presented in a lot of scientific works.

That`s why one of the principal tasks of our research was to trace the variations of EO component composition during a day. As a study case we chose *H. officinalis* plants of f.albus form, which EO component composition was determined. Over-ground mass of row material was taken for analysis at 5.00, 8.00, 13.00 and 18.00 o`clock. Data of dynamics of EO component composition of *H. officinalis* during the day are presented in table 5.3.

As to table 2, dominant components of *H. officinalis* essential oil are cys- and trans- forms of pinocamphone, which are in dynamical balance. According to analysis at 5.00 o`clock pinocamphone mass fraction made 38,48%, but isopinocamphone is much lower – 21,03%. Then at 8.00 o`clock pinocamphone biosynthesis reduced till 22,94%, while biosynthesis of isopinocamphone increased till 33,38% that is 1,5 times as much.

Table 2

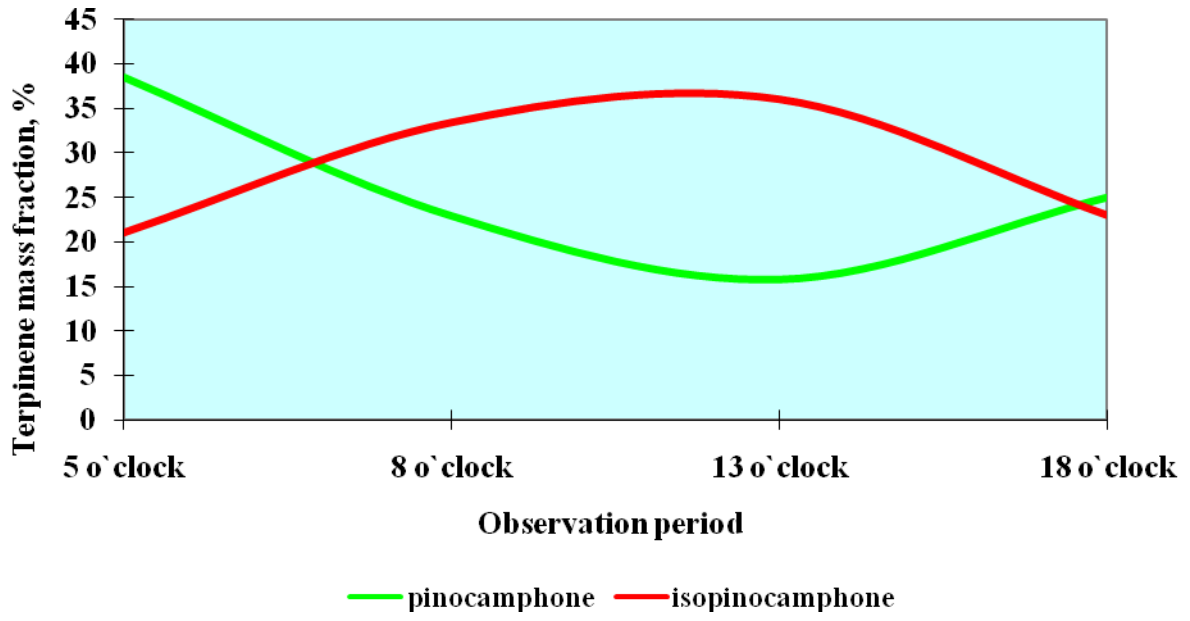
Variations of EO component composition of *H. officinalis* during a day

Component	Time of component occurance, min	Component mass fraction, % for analyzing time			
		5 o`clock	8 o`clock	13 o`clock	18 o`clock
sabinene	7,33-7,37	0,680	0,166	–	–
β-pinene	7,43-7,50	4,769	1,389	3,193	1,372
myrcene	7,83-7,85	0,579	0,333	–	–
β-phellandrene	9,02-9,03	1,231	1,578	0,366	0,225
linalool	11,64-11,81	0,935	4,883	0,708	1,090
pinocamphone	13,45-13,84	38,486	22,940	15,806	25,007
isopinocamphone	14,12-14,37	21,033	33,377	35,968	22,993
myrtenol	14,74-14.93	5,481	3,607	4,895	5,549
methyleugenol	21,50-21,53	0,630	0,351	1,589	2,257
caryophyllene	21,94-21,97	1,207	1,063	1,427	1,654
germacrene-D	23,89-23,93	3,081	4,284	1,806	1,594
elemol	25,85-25,95	4,140	0,309	5,582	8,328
spathylenol	26,56-26,59	–	1,188	2,881	2,110
cayophyllenoxid	26,62-26,66	1,707	0,682	1,748	2,013
viridiflorol	26,86-26,90	0,433	0,236	0,465	0,813
epimanool	33,34	0,319	0,237	3,446	2,366
phytol	33,73	0,657	0,627	1,367	2,148

Reduction of pinocamphone biosynthesis continues by 13 o`clock (till 15,81%), and mass fraction of isopinocamphone slightly increases (till 35,97%). At 18 o`clock pinocamphone biosynthesis intensifies 1,7 times as much till 25,00%, while isopinocamphone biosynthesis decreases 1,6 times less till 22,99%. Thereby, during a day reduction of pinocamphone and increasing of isopinocamphone have the same values. According to figure 2, biosynthesis of pinocamphone and isopinocamphone are in antiphase or dynamical balance. As to dynamics of myrtenol and elemol biosynthesises we have the following data: at 8 o`clock there is a sharp reduction of elemol mass fraction in comparison with its concentration at 5 o`clock, then at 13 o`clock and 18 o`clock its biosynthesis intensifies. The same situation is registered for biosynthesis of myrtenol. Since 5 till 8 o`clock certain reduction of myrtenol mass fraction occurs, but after that we have increasing of myrtenol biosynthesis.

Biosynthesis of β-pinene acts in another way. Its maximum concentration in essential oil is registered at 5 o`clock (4,78%), but then a sharp reduction till 1,39% happens by 8 o`clock. Its biosynthesis increases up to 3,1%, that is 2,3 times as much at 13 o`clock, but by 18 o`clock it sharply reduces till 1,37%, again 2,3 times less.

As to concentration of other components of *H. officinalis* essential oil during a day, their mass fraction variates in terms of mistake and sharp changes are not registered. Anatomico-morphological study of plants found out that *H. officinalis* has essential oil glandulas on all organs that`s why all organs are supposed to contain essential oil. Literature sources [10, 11,1] point only at essential oil availability in over-ground row material. We attempted to isolate essential oil out of stems, leaves, inflorescences, over-ground mass of flower material, seeds and determine its component composition.



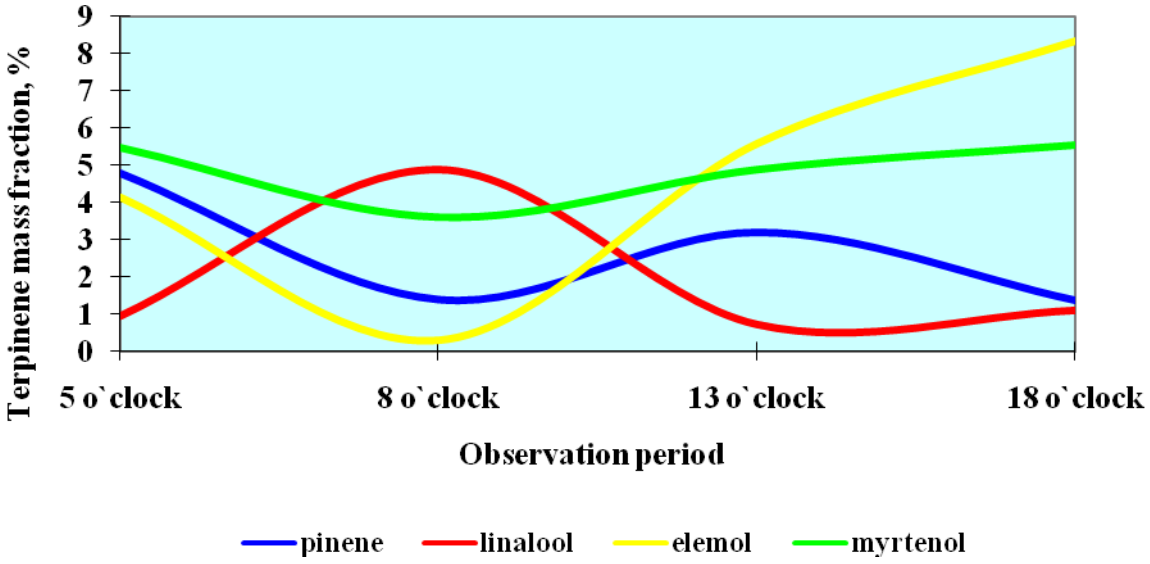


Fig.2 Peculiarities of biosynthesis variations of the principal terpenoids in *H. officinalis* essential oil during day

In terms of investigations it was found out that all parts of *H. officinalis* contained essential oil. Study of EO component composition permitted to identify 41 compounds. Essential oil includes hydrocarbons, alcohols, aldehydes, ketones and esters a bit. Hydrocarbons are presented by sabinene, myrcene, α- and β-pinene, β-phellandren.

Analyzing EO component composition of the over-ground plant mass it occurred that dominant compounds are pinocamphone and isopinocamphone with mass fraction in total ranges from 48,0% up to 77,0 %, then β-pinene takes place (till 10,50%) while some plants had it till 19,51%. Mass fraction of myrtenol ranges from 3,61-5,55%, elemol – 3,13-14,80% (some chemotypes reach 19,04%), methyleugenol – from 0,63% till 4,1% and linalool till 1,50% (table 3).

EO composition isolated out of leaves have some differences from over-ground EO.

Table 3

EO component composition isolated out of *H. officinalis* over-ground mass

Component	Parameters			
	X±Sx	V,%	min	max
sabinene	0,316±0,1227	78,6	0,17	0,68
β-pinene	2,680±0,8169	61,0	1,37	4,77
myrcene	0,505±0,0627	24,9	0,33	0,61
β-phellandrene	0,850±0,3297	77,6	0,22	1,58
linalool	1,055±0,1617	30,7	0,71	1,48
α-tuyon	0,383±0,0661	34,6	0,21	0,52
pinocamphone	20,563±7,4962	72,9	2,94	38,49
Isopinocamphone	28,343±3,7156	26,2	21,03	35,97
α-terpineol	0,708±0,0312	8,8	0,65	0,79
Myrtenol	4,883±0,4492	18,4	3,61	5,55
Methyleugenol	1,208±0,4399	72,9	0,35	2,26
Caryophyllen	1,338±0,1289	19,3	1,06	1,65
Elemol	5,340±1,1014	41,3	0,31	8,33
Ledol	0,660±0,1047	31,7	0,37	0,87
Spathylenol	1,825±0,4177	45,8	1,12	2,88
Viridiflorol	0,485±0,1187	49,0	0,24	0,81

Manool	1,590±0,7902	99,4	0,23	3,44
caryophyllen oxide	1,538±0,2934	38,2	0,68	2,01

The principal component is isopinocamphone with mass fraction ranging from 34 till 52%. At the same time pinocamphone concentration was much less – 9,36 – 42,97%. Less concentration was registered and for β-pinene in comparison with over-ground mass – 0,42-4,35%. Mass fraction of myrtenol and elemol had the same variations as it was in over-ground mass EO. Distinctive feature of EO component composition isolated out of leaves is a high concentration of sesquiterpenes and especially such terpenic compounds as: viridiflorol (till 7,24%), manool (till 20,16%) and also phytol (till 6,75%) and oktakzan (till 22,61%) which are not typical for essential oil isolated out of over-ground mass (table 4).

EO component composition isolated out of stems was of great interest for science, as nevertheless stems are ballast in row material and contains a minimal EO concentration, it influences on EO quality in general. Analysis of stem component composition revealed that a dominant component of this EO is isopinocamphone with mass fraction of 56,7% for some specimens. At the same time a low level of pinocamphone biosynthesis took place, maximum 6,35%. In comparison with other plant organs stem EO is characterized by high biosynthesis of such components as elemol (till 10,39%), viridiflorol (till 7,51%) and maximum level of manool biosynthesis (till 21,7%). Methyleugenol, revealed in EO of one of specimens in maximum concentration (51,32%) is of great importance (table 4).

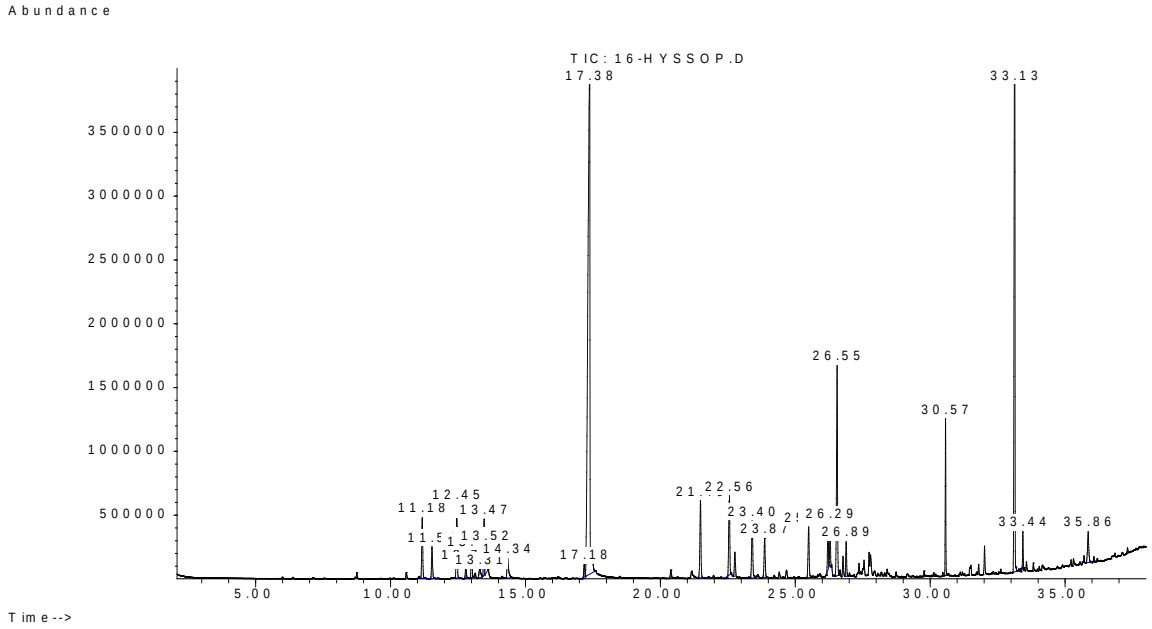
Table 4

Variations of EO component composition isolated out of different *H. officinalis* plant organs

Component	Mass fraction of component in essential oil of the following morphologic forms								
	Leaf			Inflorescence			Stem		
	f.albus	f.cyaneus	f.ruber	f.albus	f.cyaneus	f.ruber	f.albus	f.cyaneus	f.ruber
1	2	3	4	5	6	7	8	9	10
sabinene	0,19-0,42	0,38-0,82	0,24-0,69	0,34-0,39	0,57-1,53	0,,19-0,63	tracks	tracks	tracks
β-pinene	0,41-1,39	0,42-3,16	1,88-4,35	2,30-2,89	0,92-2,31	0,88-3,25	0,34	0,21	1,17
myrcene	0.27-0,36	0,25-0,82	0,20-0,40	0,26-0,46	0,64-1,35	0,15-0,71	tracks	tracks	tracks
β-phellandren	0,19-0,20	0,42-2,12	0,49-0,78	0,47-1,11	0,22-1,42	0,32-0,82	tracks	tracks	Tracks
linalool	0,71-0,81	0,22-0,87	0,69-0,91	0,13-0,89	0,26-0,93	0,72-0,88	0,21	0,28	34,8
α-tuyon	0,17-1,09	0,87-1,02	1,05-1,40	0,25-0,88	0,30-0,90	0,75-0,83	0,30	0,50	0,10
pinocamphone	1,12-9,36	13,81-22,87	11,45-42,97	11,23-13,82	12,59-17,65	40,47-60,48	0,30	6,35	4,34
isopinocamphon	34,65-	34,67-46,73	4,44-24,28	51,52-60,98	57,11-57,93	14,98-38,14	13,10	56,70	7,77
A-terpineol	0,43-0.73	0,31-0,32	0,39-1,43	0,25-0,37	0,30-0,31	0,29-0,33	tracks	tracks	tracks
myrtenol	5,35-5,75	1,42-3,43	1,83-4,17	3,63-6,31	1,21-2,70	1,68-2,70	1,27	4,39	0,56
methyleugenol	0,55-3,60	0,28-0,73	0,49-1,70	0,52-2,46	0,23-0,41	0,32-0,86	51,32	0,47	2,25
elemol	2,64-2,73	4,02-7,79	2,03-5,17	1,82-3,77	2,12-4,39	1,40-3,06	6,77	3,05	10,39
caryophyllene	0,33-2,81	0,63-1,49	1,29-3,21	0,24-0,52	0,70-1,09	0,66-0,80	1,22	1,37	3,67
ledol	0,21-0,92	0,51-0,88	0,20-0,52	0,51-0,55	0,26-0,48	0,11-0,35	0,68	0,54	tracks
spathylenol	1,39-2,89	1,82-3,35	1,05-2,39	2,33-3,52	0,83-2,29	0,48-1,68	0,61	0,91	3,55
Caryophyllene	0,35-1,36	0,94-1,11	0,59-1,91	0,29-0,66	0,30-0,50	0,33-0,46	tracks	0,69	2,02
viridiflorol	1,36-2,15	0,91-2,16	1,19-7,24	0,23-0,82	0,10-1,10	1,10-0,18	tracks	2,56	7,51
manool	5,55-5,85	1,05-5,76	1,41-20,16	0,36-1,82	0,10-1,56	0,19-0,32	2,06	8,29	21,70
Octadecene-1	-	-	8,02	-	-	-	-	-	-
phytol	-	-	6,75	-	-	-	-	-	-
oktakozan	22,61	-	-	-	-	-	-	-	-

Anlysis of the EO component composition of *H. officinalis* isolated out of generative organs (inflorescences) revealed certain differences in component concentration in comparison with EO out of other plant parts. EO isolated out of inflorescences is characterized by the highest level of pinocamphone biosynthesis (till 60,48%) and isopinocamphone (till 61%). Though mass fraction of other components ranges in the same way in comparison with EO out of other plant parts. Biosynthesis of such components as methyleugenol, viridiflorol and especially manool is a little bit lower.

As literature sources don`t give component composition of *H. officinalis* essential oil , isolation of EO out of seeds and study its component composition in comparison with EO out of other plant parts was of great importance for us (Fig.3).



1.	11.17	2.22% α-tuyon	13.	22.55	2.92% humulene
2.	11.53	1.18% β-toyon	14.	23.40	2.36% germacrene D
3.	12.45	2.73% camphora	15.	23.87	1.63% bycyclogermacrene
4.	12.79	0,49% menthone	16.	25.49	2.07% elemol
5.	13.00	0.95% pinocamphone	17.	26.21	1.40% spathylenol
6.	13.30	0.53% borneol	18.	26.29	1.24% caryophylleneoxide
7.	13.46	2.00% isopinocamphone	19.	26.55	7.54% viridiflorol
8.	13.52	1.56% menthol	20.	26.88	1.09% hhumulenoxide
9.	14.33	1.14% methyl chavicol	21.	30.57	3.52% hydrofarnesylacetate
10.	17.18	0,48% α-phenchylacetate	22.	33.13	19.57% manool
11.	17.37	38.00% anethole	24.	35.85	1.39% squalene
12.	21.48	3.08% caryophyllene			

Fig.3 Chromatogram of *H. officinalis* EO isolated out of seeds

In terms of investigations it was revealed that component composition of *H. officinalis* EO isolated out of seeds dramatically differs from EO composition isolated out of other plant parts. 24 components were identified in EO composition. The principal component are

anethol (C_{10} H_{12} O), a component characterized by anise flavor and sweet anise taste, its mass fraction in EO makes 38,0%. EO of *H. officinalis* isolated out of other plant parts doesn`t include this component. Besides anethol there are also α-tuyon (2.22%), β-tuyon (1.18%), camphora (2.73%), menthone (1.56%), methylchavicol (1.14%), squalene (1.39% and a quite high mass fraction of manool (19.57%). At the same time it has to be noted, those components which prevail in EO composition isolated out of over-ground row material, make low concentration in EO out of seeds. So, mass fraction of pinocamphone made 0,95% and isopinocamphone – 2,00%.

Analysis of correlations between certain components of *H. officinalis* essential oil revealed both positive and negative connections between biosynthesis of terpenic compounds. Determined conjugacy in biosynthesis of *H. officinalis* EO components has rather low correlation coefficients in many cases (table 5). Such correlation takes place for pinocamphone and myrtenol (R= - 0,19), pinocamphone and β-pinene (R= - 0,20), pinocamphone and linalool (R= - 0,29). There is only negative correlation between pinocamphone and other components. Though isopinocamphone and other components makes both positive and negative correlations.

So, positive correlation between isopinocamphone and methyleugenol (R= - 0,45) makes it possible to select both components simultaneously, while negative correlation between pinocamphone and methyleugenol (R= - 0,40) permits to select one compound with a high concentration, as they are inversely oriented.

Table 5

Correlations between components of *H. officinalis* essential oil

Components	Correlation coefficient, R	Components	Correlation coefficient, R
myrtenol and elemol	- 0,05	pinocamphone and isopinocamphone	- 0,87
myrtenol and β-pinene	- 0,11	pinocamphone and myrtenol	- 0,19
myrtenol and methyleugenol	- 0,14	pinocamphone and elemol	- 0,42
myrtenol and β-phellandrene	- 0,06	pinocamphone and β-pinene	- 0,20
myrtenol and linalool	0,22	pinocamphone and methyleugenol	- 0,40
elemol and β-pinene	- 0,56	pinocamphone and linalool	- 0,29
elemol and methyleugenol	- 0,13	isopinocamphone and myrtenol	0,12
elemol and linalool	0,37	isopinocamphone and elemol	0,22
elemol and β-phellandrene	0,05	isopinocamphone and β-pinene	- 0,34
β-pinene and methyleugenol	0,36	isopinocamphone and methyleugenol	0,45
β-pinene and β-phellandrrene	- 0,54	isopinocamphone and β-phellandrene	0,18
β-pinene and linalool	- 0,08	isopinocamphone and linalool	0,09
methyleugenol β-phellandrene	0,35	β-phellandrene and linalool	0,29
methyleuhenol and linalool	- 0,09		

In marked out chemotypes with a high fraction of pinocamphone (60,48%) it was registered a low concentration of methyleugenol (0,87%) and vice versa having mass fraction of methyleugenol 51,32% level of pinocamphone concentration reduces till 0,30%.

Such correlations indicate biosynthesis conjugacy of some terpenic compounds of *H. officinalis* and strengthen dependence in concentration of certain EO compounds. High negative correlation coefficient (R= - 0,87) between dominant components of essential oil (pinocamphone and isopinocamphone) limits selection of both terpenic compounds simultaneously among seed generation of *H. officinalis,* increasing of one compound concentration causes reduction of another. Though conjugacy in biosynthesis of minor EO compounds for plants from artificial *H. officinalis* population is not so large, as coefficients of their conjugate correlation mainly have low values what indicates a weak mutuality of

their biosynthesises and dominance of fluctuate changeability, caused by intraspecific heterogeneity. *H. officinalis* possesses biochemical heterogeneity with methyleugenol and linalool chemotypes, as well as low and high level of correlation between EO components concentration. Such a type of changeability, conjugacy degree and mutuality of biosynthesis processes of certain terpenic compounds keeps in seed generation what indicates genetic species resistance and permits to classify it as a separate taxonomic unit from *Hyssopus* L. genus.

Determined regularities present wide opportunities for intraspecific selection of perspective for industry and breeding work *H. officinalis* chemotypes as well as new starting material for breeding of new cultivars.

Conclusions

Thereby in terms of this research it was determined that *H. officinalis* accumulates essential oil in all plant organs (stem, leaf, inflorescence, seeds). Chemical analysis of essential oil isolated out of plants from Piedmont Crimea revealed this essential oil is good quality and contains hydrocarbons, alcohols, ketones, phenols and other compounds. *H. officinalis* EO isolated out of over-ground mass includes 60 compounds, only 41 were identified. Intraspecific changeability of EO component composition, caused by seed propagation, was determined as well.

The following chemotypes were found out, not presented in scientific literature before: methyleugenol chemotype, chemotypes with a high concentration of oktakozan, elemol, manool, viridiflorol. It`s possible to come across information about such cultivar of *H. officinalis* as *H. officinalis* var. *decumbens*, which is characterized by lower concentration of pinocamphone and isopinocamphone. Its principal components are linalool (49,6%), 1,8-cyneole (13,3%) and limonene (5,4%) [18, 19]. Besides there is no any descriptions about their morphological characteristics, which could distinguish *H. officinalis* var. *decumbens* and *H. officinalis*. In seed generation of *H. officinalis* we could find out a chemotype with the following EO component composition: pinocamphone – 2,94%, isopinocamphone – 33,38%, linalool – 34,88%. If *H. officinalis* var. *decumbens* was classified as a separate taxonomic unit on the ground of higher linalool concentration in EO component composition, our researches dispute that fact.

Besides for the first time comparison analysis of *H. officinalis* essential oil isolated out of different plant parts was carried out in terms of this research. It made possible to reveal quantitative differences in composition of terpenic compounds. Qualitative analysis of essential oil from *H. officinalis* seeds was conducted for the first time as well. 24 components with dominance of anethole were identified in its composition.

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Rabotyagov V.D., Shibko A.N. Investigations of essential oil component composition of *Hyssopus officinalis* L. // Works of the State Nikit. Botan. Gard. – 2014. – V. 139 – P. 88 – 100.

The article covers data about content and essential oil component composition of three forms of *Hyssopus officinalis* cultivar growing under conditions of the pied-mont area of the Crimea. This data were presented for the first time. 41 terpenic compounds were identified. The main ones are pinocamphone (up to 60%) and izopinocamphone (up to 61%). *H. officinalis specimen* with content of linalool 34.88%, izopinocamphone 33.38% and pinocamphone 2.94% was marked out for further selection..

Key words: *Hyssopus officinalis, pinocamphone, izopinocamphone, component, changeability, plant top.*

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BIOLOGICALLY ACTIVE SUBSTANCES OF SPICES, AROMATIC AND MEDICINAL PLANTS FROM NIKITSKY BOTANICAL GARDENS

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Introduction

Since long ago spices and aromatic plants are widely used in different spheres of human life and the most often in cookery, alcohol and cool drinks production, preparation of cosmetics and parfume output, medical treatments and medicinal forms [19]. They have great popularity in home and traditional medicine for treatment of various diseases and prophylaxis [17].

Biological value of spices and aromatic plant raw materials is determined by wide range of biologically-active substances: essential oils, glycosides, vitamins, mineral substances and phenolic compounds including flavonoids [18]. These substances improve culinary qualities of products, stimulate taste and digestive organs, cause appetite, increase digestability of foods and have beneficial effect on metabolism, work of cardiovascular system and general condition of the person. Many of spices and aromatic plants have preserve, anticeptic and bactericide qualities [4].

It has been known that qualitative and quantitative composition of biologically active substances in plant is highly depends from its genetic origin and growth conditions, besides, the processes extract them from plant raw material are important. Thus, native extracts, that do not undego therm reaction and processes of separation and purification, as a rule have higher biological activity due to the whole complex of physiologically active plant substances [5].

There is large collection of spices, aromatic and medicinal plants in Nikitsky Botanical Gardens (NBG). For many years, works on their introduction, breeding and selection have been carrying out with the researchers of this institution. As the result of those investigations perspective varieties and forms have been selected [6, 7].

On the base of all above-mentioned it should be noticed that complex investigations of biologically active substances compositions in perspective varieties and forms of spices, aromatic and medicinal plants grown in the conditions of the Southern Coast of the Crimea is of great actuality.

The aim of our work was to make scrining investigations of biologically active substances composition in some spices, aromatic and medicinal plants from NBG collection for giving the ground of their further use.

Objects and methods of the research

Objects of our investigation were: *Achillea millefolium* L. var. *rosea, Achillea collina* Becker ex. Rchb., *Artemisia annua* L. 'Novichok', *Artemisia scoparia* Waldst. et Kit. 'Vetvistyi', *Artemisia absinthium* L., *Artemisia dracunculus* L. 'Travnevyi', *Echinacea angustifolia* DC., *Echinacea purpurea* (L.) Moench., *Helichrysum italicum* (Roth.) Guss. 'VIM', *Hyssopus officinalis* L. 'Nikitsky Belyi', *Levisticum officinale* W.D.J. Koch., *Melissa officinalis* L., *Mentha spicata* L., *Mentha longifolia* L., *Nepeta cataria* L. *f. citriodora* Dum. 'Peremozhets-3', *Ocimum basilicum* L., *Origanum vulgare* L., *Salvia officinalis* L., *Satureja taurica* Velen. 'Krymsky Smaragd', *Satureja hortensis* L. 'Martian', *Scutellaria baicalensis* Georgi., *Thymus vulgaris* L., *Thymus vulgaris* L. 'Yalos'. Studied plants were grown in NBG

on the collection plots by the researchers from Technical and Medical Plants Laboratory. Vegetative material for analyses has been collected in 2011-2013 during the period of mass blossom.

Content of biologically active substances was determined in water-ethanol extracts prepared from the air-dry plant raw material. It has been dried in the aired and dark place up to the constant mass. Extraction was made by 50% ethanol with the proportion of plant raw material to the extragen – 1:10 and 10 days saturation under the room temperature was applied.

Component composition of volatile substances was determined by gas-liquid partition chromatography on Agilent Technology 6890 with mass spectrometer 5973. Column HP-1 height 30 m; inner diameter – 0.25 mm. Thermostat temperature rised from 50 to 250 ^{o}C with the step 4 ^{o}C/min. The temperature of injector was 250 ^{o}C. Carrier-gas – helium, the streem speed 1 sm^3/min. Transfer from gas chromotograph to mass spectrometer was warmed up to 230 ^{o}C. Temperature of the source was kept on 200 ^{o}C. Electron ionization was carried out under 70 eV in the range of masses m/z from 29 to 450. Identification was made on the base of comparison of the obtained mass-specters with the data from combined library NIST05-WILEY2007 (nearly 500000 mass-specters).

Content of phenolic substances was determined according to Folin-Ciocalteu (Gerzhikova, 2002) and flavons – by spectrophotometric method (Chemesova et al., 2000). All meanings were calculated for the air-dry mass.

Component composition of phenolic substances was determined by the method of highly effective liquid chromatography on chromatograph Agilent Technologies 1100. Hromatographycal column 2.1 × 150 mm filled with octadescillic sorbent «ZORBAX-SB C-18» granules 3.5 μm was used. Gradient regime of chromatography, that provides changes of correlation between the components A (0.1% ortophosphore acid, 0.3% tetrahydrofuran, 0.018% threethilamine) and B (methanol), was used for analyses. Speed of movable fraction giving was 0.25 sm^3/min; working eluent pressure – 240-300 kPa; volume of one sample - 2μl; time of scanning – 0.5 sec; measurements` scale was 1.0. Idefication of phenolic substances was made according to the time of standards` keeping and spectral characteristics (parameters for taking spectre – each top 190-600 nm; wavelength - 280, 313, 350, 371 nm (Murrough et al., 1982)).

Results and discussion

Qualitative composition and consentration of volatile compounds and phenolic substances in water-ethanol extracts for 22 species of spices, aromatic and medicinal plants from the families Lamiaceae, Asteraceae и Apiaceae have been determined with chromatogrphical methods.

Concentrations of volatile compounds in vegetative extracts were from 0.01 g per 100 g of the air-dried raw material to 3.37 g/100 g (tab. 1). Maximum concentrations of volatile compounds were noticed in the extracts of *Thymus vulgaris* and *Ocimum basilicum*, minimum – in the extracts of *Echinacea angustifolia*, *Echinacea purpurea* and *Scutellaria baicalensis*.

Component composition of volatile compounds in the extracts of studied plants is characterized with great diversity and mostly represented by terpene and aromatic substances (phenolic compounds and phenilpropanoids). The exeption was extract of *Echinacea angustifolia* mostly composed from aliphatic compounds (monocarboxylic acids).

Thus high concentrations of monoterphens are typical for *Achillea collina* and *Helichrysum italicum* 'VIM'. *Mentha arvensis* is also characterized with high enough consentration of limonene (9.7%). Monoterpene compounds have anticeptic (especially to bacterias presented in the air), bactericidal, stimulated and expectorant influence [2]. Though

some researchers consider that antimicrobial and antioxidant features of spices and medicinal plants are due to the presence of monoterpenes, particularly limonene, in their parts [28].

Maximum concentrations of monoterpene alcohols were noticed for *Thymus vulgaris* 'Yalos', *Ocimum basilicum*, *Nepeta cataria*, *Achillea millefolium* and *Artemisia absinthium*. Such monoterpene alcohols as linalool, terpinene-4-ol, α-terpeneol and borneol have antimicrobial activity [13, 24]. Borneol, terpinene-4-ol, α-terpeneol demonstrate antifungal activity [23]. Besides, for some alcohols, particularly geraniol and terpeneol, high antifungal activity has been determined and anti-inflammatory activity was also noticed for cytronellol and virucidal activity – for linalool [13].

Extract of *Levisticum officinale* is characterized with high concentration of α-terpene-acetate (69.8%). Compound alcohol ethers demonstrate different kinds of biological activity: anti-inflammatory, wounder-healing and antimicrobial influence [13]. α-terpene-acetate has pleasant odour of bergamot and is widely used for aromatization parfume production [3].

High content of monoterpene aldegids is a feature of *Melissa officinalis* and *Nepeta cataria* extracts. Monoterpene aldegids demonstrate antifungal activity and also have anticeptic, analgesic, antihistamine and anti-hypertensive effect, stimulate the gastrointestinal tract [13]. These compounds provide sedation [10]. Monoterpene aldegids have pleasant odor and could be used in parfume industry [3, 11].

Significant concentrations of monoterpene ketones were found in the extracts of *Artemisia annua*, *Hissopus officinalis*, *Salvia officinalis*, *Mentha arvensis* and *Mentha longifolia*. Properties of monoterpene ketones haven`t been fully studied yet. It has been known that some ketones have unfavourable influence on human organism [20]. At the same time ketones demonstrate immunostimulated, antispasmodic and sedative effect [22]. Particularly, camphor affects the central nervous system and soothes the heat in stress [13]. Besides, high content of momoterpene ketones give plants their antibacterial and antifungal features [15, 28, 29]. Some researchers consider that presence of ketones, particularly carvone and menthon guaranty antioxidant features of plants [25, 27]. But it should be taken in attention that thujone in high concentrations could be the reason of spasms, so plant extracts with this substance should be used only after carefully determined tujone concentration [16].

Table 1

Volatile compounds in spices, aromatic and medicinal plants

Species	Concentration, g/100 g	Number of identified components	Main components
Achillea millefolium	0.43	35	borneol (18.0%), β-pinen (5,9%), terpenen-4-ol (6.1%)
A. collina	0.12	20	β-pinen (19.2%), 1.8-cinneol (7,5%), γ-kadinol (9.0%)
Artemisia absinthium	0.04	20	sabinol (23.5 %), β-tujone (13.8 %)
A. annua	1.54	43	artemisia-ketone (40.9%), camphor (19.9%)
A. dracunculus	0.32	21	trans-isoelemicin (62.7%), methileugenol (17,1%), cys-methilisoeugenol (4.2%)
A. scoparia	0.43	33	hexa-5-en-1,3-diinyl-benzene (27.4%), scoparon (18.1%), eugenol (12.9%)
Echinacea angustifolia	0.03	22	linoleic acid (31.6%), palmitic acid (31.0 %)

E. purpurea	0.03	22	p-cymene (27.0%), γ-cadinene (9.4%), spathulenol (8.1%)
Helichrysum italicum	0.33	35	α-pinen (43.3%), limonene (12.2%), rozifoliol (4.5%), penduletin (4.3%)
Hissopus officinalis	0.63	33	isopinocamphon (3.2%), β-pinen (4.2%), pinocamphon (78.2%)
Levisticum officinale	0.38	14	α-terpene-acetate (69.8%), *butilidendigidroftalid* (12.5%)
Melissa officinalis	0.22	38	citronellal 4.0%, neral (6.0%), geranial (8.2%)
Mentha spicata	0.37	43	cys-dihydrocarvone (15.1%), carvone 29.3%, ethil linolenat (11.0%)
M. longifolia	0.97	20	methone (53.2%), isomethone (27.7%), trans-sabinene hydrate (8.2%)
Nepeta cataria	0.23	14	citronellol (33,1%), nepetalactone (33.8 %), geranial (10.5%), geraniol (8.0%)
Ocimum basilicum	2.31	43	linalool (46.2%), methyl chavikol (24.0%), 1,8-cineole (9.1%)
Origanum vulgare	0.62	32	p-cymene (10.0%), thymol (10.3%), γ-terpinen (15.4%), carvacrol (37.7%)
Salvia officinalis	1.80	26	α-tuion (33.3%), camphor (30.5%), 1,8-cineole (6.4%)
Satureja taurica	0,32	18	carvacrol (81.6%), p-cymene (9.7%)
S. hortensis	0,24	15	carvacrol (75.1%), γ-terpenen (7.5%)
Scutellaria baicalensis	0,01	26	phenylacetaldehyde (14.7 %), 4-vinylphenol (9.1%)
Thymus vulgaris	2.07	29	thymol (74.2%), p-cymene (4.1%), carvacrol (2.3%)
Th. vulgaris ‘Yalos’	3.73	41	linalool (84.5%), thymol 3.4%, linalyl acetate (3.4%)

The greatest amount of sesquiterpenes was noticed in the extract of *Echinacea purpurea*. Sesquiterpenes are among the strongest anti-inflammatory components of essential oils. Some of them have anasthetic features and others demonstrate antispasm activity [2].

High enough concentrations of sesquiterpene alcohols were determined for *Achillea collina* and *Echinacea purpurea*. Sesquiterpene alcohols are valuable compounds that have relax and stimulative effect, they stimulate heart work and regenerate liver, have antiallergene and anti-inflammatory effect [2].

In other studied species volatile aromatic compounds of nonterpene origin predominate. High concentrations of simple phenolic compounds were determined in the extracts of *Artemisia scoparia*, *Origanum vulgare*, *Satureja taurica*, *Satureja hortensis*, *Scutellaria baicalensis*, *Thymus vulgaris* and phenilpropanoids – in the extracts of *Artemisia dracunculus* and *Ocimum basilicum*. Both simple phenols (thymol, carvacrol and others) and phenilpropanoids (eugenol, methyl chavikol and others) demonstrate significant anti-inflammatory,expectorate, untifungal, protistotsidшc, antiseptic, antispasmodic and antioxidant activity [13, 14, 17].

Thus extracts of *Thymus vulgaris* ‘Yalos’, *Ocimum basilicum* and *Achillea millefolium* due to their high concentrations of monoterpene alcohols and phenilpropanoids could be used for treatment and prevention. Extracts from *Melissa officinalis* and *H. italicum* ‘VIM’ are rich in aromatic substances that let us to recommend them as aromatizes for parfume-cosmetics and food production. More than 85% of aromatic substances in *Thymus*

vulgaris variety ‘Yalos’ bred in NBG are monoterpene alcohol linalool that has delicate odor of lily of the valley. Unusual ordor of this *Thymu*s variety makes it attractive for parfume industry.

Content of phenolic compounds in vegetative extracts is from 0.91 g per 100 g of the air-dried raw material up to 2.48 g/100g (tab. 2). Their maximum concentrations were found in the extracts of *Scutellaria baicalensis*, *Origanum vulgare* and *Levisticum officinale*, minimum – in the extracts of *Achillea collina* and *Hissopus officinalis*. Component composition of phenolic compounds in studied species is mostly represented with flavonoids and hydroxycinnamic acids. In the species of *Artemisia* L. coumarins were found.

At the present time many sides of flavonoid substances` biological effect became clear and their P-vitamin activity has been known long ago [1]. Flavonoids stimulate heart activity and decrease blood pressure for short period of time due to dilatation of the abdominal cavity. They demonstrate hepartoprotective, anti-tumour and antimicrobial action [8, 21]. High flavonoids` content is typical for the extracts of *Scutellaria baicalensis*, *Origanum vulgare* and *Salvia officinalis*.

Flavonoids in the studied species are represented by flavons` glycosides: luteolin, apigenin, acacetin, and also by quercetin flavonol glycosides. *S. baicslensis* that has unequal set of flavonoids originated from scutellarin and baicalin should be highlighted.

Hydroxycinnamic acids are biogenetic predecessors for most of other phenolic compounds. Their imunostimulative, antivirus and anti-inflammatory effects are well studied. It has been determined that ferulic, caffeic, chlorogenic acids and especially cinarin (1,4-caffeoylquinic acid) have bile-pursue effect. Sum of ferulic, caffeic, chlorogenic, coumaric and other caffeoylquinic acids have hypoazotemic effect enhances the function of the kidneys, stimulates the antitoxic function of the liver, has antimicrobial and antineoplastic action [30].

Table 2

Phenolic compounds in spice-aromatic and medicinal plants

Species	Concentrations, g/ 100 g			Number of identified components	Main components
	Phenolic compounds	Flavonoids	Hydroxycinnamic acids		
Achillea millefolium	2.36±0.12	1.44	0.92	11	luteolin-7-O-glycoside (28.6%), 4- caffeoylquinic acid (18.5%)
A. collina	2.34±0.10	1.32	1.02	11	luteolin-7-O-glycoside (24.2%), caffeoylquinic acid (20.5%)
Artemisia absinthium	0.83±0.03	0.10	0,.0	12	chlorogenic acid (27.0%), kaempferrol (8.7%)
A. annua	3.99±0.13	1.28	2.72	16	rosmarinic acid (18.9%), chlorogenic acid (11.5%)
A. dracunculus	1.49±0.10	0.11±	0.32	14	rosmarinic acid (21.4%), coumarin (15.2%)
A. scoparia	2.75±0.12	0.48±	1.07	16	coumarin (36.8%), chlorogenic acid (16,8%)
Echinacea angustifolia	2.41±0.10	0.05	2.29	17	rosmarinic acid (68.5%), caffeoylquinic acid (5.6%)
E. purpurea	3.27±0.16	0.06	2.70	9	caffeoylquinic acid (71.3 %), p-coumaroylquinic acid (2.3%)
Helichrysum italicum	1.69±0.06	0.93	0.76	6	4- caffeoylquinic acid (40.0%), chlorogenic acid (19.6%)
Hissopus officinalis	0.59±0.01	0.16	0.43	17	rosmarinic acid (28.6%), luteolin-7-O-glucoside (18.9%)
Levisticum officinale	4.18±0.18	3.41	0.77	9	quercetin biglucoside (79.3%), caffeic acid (7.9%)

Melissa officinalis	0.91±0.02	0.20	0.72	8	rosmarinic acid (41.8%), glucoside of apigenin (21.5%)
M. longifolia	3.00±0.14	1.99	1.61	13	rosmarinic acid (50.2%), luteolin-7-O-glucoside (13.2%)
Mentha spicata	2.26±0.12	0.85	1.41	17	rosmarinic acid (57.8%), luteolin-7-O-glucoside (5.7 %)
Nepeta cataria	2.21±0.16	0.84	1.41	12	chlorogenic acid (50.2%), apigenin-7-diglucoside (22.5%)
Ocimum basilicum	1.04±0.03	0.16	0.88	11	rosmarinic acid (20.9%)
Origanum vulgare	5.57±0.18	3.01	2.56	12	rosmarinic acid (40.1%), luteolin-7-O-glucoside (1.5%)
Salvia officinalis	2.49±0.08	2.03	0.46	8	luteolin-7-O-glucoside (42.0%), apegenin-7-O-glucoside (26.8%)
Satureja taurica	1.86±0.15	0.68	0.74	16	rosmarinic acid (32.7%), apigenin (11.3%)
S. hortensis	1.33±0.03	0.59	0.74	16	rosmarinic acid (46.0 %), luteolin-7-O-glucoside (18.2 %),
Scutellaria baicalensis	18.51±0.50	18.51	-----	15	dihydroscutellarin (41.3%), scutellarin-7-O-glucoside (11.7%)
Thymus vulgaris	2.84±	0.92	1.62	15	rosmarinic acid (54.5%), luteolin-7-O-glucoside (20.0%),
Th. vulgaris 'Yalos'	1.02±	0.92	0.10	11	luteolin (22.7%), luteolin-7-O-glucoside (24.1%)

From the group of hydroxycinnamic acids caffeic, chlorogenic acids and some isomers of the last were found in all studied species. In some plants isomers of rosmarinic acid were found.

Maximum concentration of hydroxycinnamic acids is characteristic for *Echinacea angustifolia* and *Origanum vulgare* extracts.

Thus on the base of our investigations such species with high conten of biological active substances have been marked out: *Thymus vulgaris*, *Ocimum basilicum*, *Levisticum officinale*, *Scutellaria baicalensis*, *Origanum vulgare*, *Salvia officinalis*.

Conclusions

Qualitive and quantative composition of biological active substances in some spices, aromatic and medicinal plants from collection of Nikitsky Botanical Gardens has been studied.

It has been determined that amount of volatile substances in vegetative extracts was from 0.01 to 3.37 g/100g. Their maximum concentrations were noticed in the extracts of *Thymus vulgaris* and *Ocimum basilicum*.

Amount of phenolic compounds was 0.59 – 18.51 g/100 g. There maximum concentrations were found in the extracts of *Scutellaria baicalensis*, *Origanum vulgare* and *Levisticum officinale*.

On the base of the investigations such species with high conten of biological active substances have been marked out: *Thymus vulgaris*, *Ocimum basilicum*, *Levisticum officinale*, *Scutellaria baicalensis*, *Origanum vulgare*, *Salvia officinalis*.

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Qualitative and quantitative composition of volatile compounds and phenolic substances from the extracts of some spices, aromatic and medicinal plants of Nikitsky Botanical Gardens collection has been determined. Species with a high content of biologically active substances have been marked out. The concentration of volatile compounds in vegetative extracts was from 0.01 to 3.37 g/100 g. and its composition was mainly represented by terpenic and aromatic substances. The content of phenolic substances in the vegetative extracts mostly presented by flavonoids and hydroxycinnamic acids, is in the range of 0.91 – 2.48 g/100 g.

Key words: *spices, aromatic and medicinal plants, water-ethanol extracts, volatile compounds, phenolic substances.*

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PRINCIPLES OF ESSENTIAL OILS USING FOR MEDICAL PURPOSES. A REVIEW

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Introduction

Empirical experience of essential oils successful application as medicines has been confirmed with the latest research methods.

Range of beneficial effects of essential oils (EO) on living organisms is so wide that it is difficult to name the area of medicine or a disease in which application of EO has not been researched on, so literary references cited in the article, should be considered only as the examples of EO particular effects.

The purpose of the review is to inform practitioners about the rules that should be followed when working with EO and possible side effects.

General information about essential oils

Currently, there are about 3000 names of essential oils. Approximately 300 EO are widely used in medical and veterinary practice, food and cosmetic industry and as a part of household chemicals [46]. Essential oils are oily liquids from light yellow to brown, almost insoluble in water (0.05 %), but well-volatile even at room temperature, as a rule, have a pleasant odor. EO are active against bacteria, mycoplasma, fungi, viruses, protozoa and parasites, have anti-inflammatory, antioxidant, antimutagenic properties. It has been shown that they slow down the aging process, activate regeneriration processes in organs and tissues, activate the immune system, prevent development of malignant tumors [21], reduce the toxic effects of xenobiotics [13], ionizing radiation [3] and optimize the performance of systems and tissues, exhibiting the properties of adaptogens and improving quality of life [11].

However, using the EO, it should be remembered that they are complex chemical mixtures which are highly dependent on climate zone where the raw material was grown, harvest season, weather conditions and other factors [38]. Composition and dose of the oil determine severity and direction of its pharmacological effects [23].

It should be also noted that the effect of using any EO for humans will largely depend on sex, age, type of the human nervous system and its state (agitation, fatigue, a state of rest) at the time of aromatherapy.

Standardization of essential oils

Only natural essential oils that are appropriate to the international standards on the content of the main components are recommended to use for aromatherapy [28].

In the composition of essential oils 50-500 or more components could be found, that depends only on the sensitivity of the apparatus. Information about EO composition could be found at the site by Vinogradov B.A. [2]. As an example Table. 1 demonstrates the results of chromatographic analysis of peppermint EO components from Ukrainian raw materials.

Table 1

The result of chromatographic analysis of peppermint EO from Ukrainian raw materials

№	Component	%		№	Component	
2	α- pinen	0.55		**23**	carvone	.04
3	sabinene	0.23			piperitone	.01
4	β- pinen	0.64		**26**	linalyl acetate	.14
5	myrcene	0.27		**28**	neomenthyl acetate	.09
6	p-cymene	0.07		**29**	menthyl acetate	.35
7	1,8-cineole	0.51		**30**	thymol	.04
8	limonene	3.42		**31**	isomenthyl acetate	.21
9	trans-cymene	0.05		**32**	α-cubebene	.05
10	nonanal	0.07		**33**	β-bourbonen	.25
11	linalool	0.14		**34**	β-elemene	.08
12	amyl isovalerate	0.08		**35**	α-gurjunene	.14
13	3-octyl acetate	0.03		**36**	caryophyllene	.67
14	methone	14.4		**37**	gumulene	.09
15	citronellal	0.05		**38**	β- farnesene	.17
16	isomethone	2.25		**39**	germacrene D	.64
17	menthofuran	0.29		**40**	γ- elemene	.13
18	neomenthol	2.97		**41**	α-farnesene	.05
19	menthol	58.7		**42**	δ-cadenene	.08
20	isomenthol	0.84		**43**	spathulenol	.06
21	α-terpineol	0.15		**44**	aryophyllenox	.21
22	pulegone	0.26		**45**	viridiflorol	.11

The table shows that menthol, its isomers and derivatives are ≈84% in peppermint oil, 3 more substance (piperitone, caryophyllene and limonene) are from 1 to 3.5% (of ≈7%), while the remaining 34 components are represented by tenths and hundredths of a percent.

In other mint varieties component composition may vary considerably, but the main components of peppermint oil will be menthol and its derivatives.

Expencive EO are often faked by diluting them with cheaper ones, vegetable oils or synthetic substitutes, which have a similar smell, but do not have the pharmacological properties of essential oils natural componentы could be sold instead of them.

When buying EO it`s better to obtain quality certificate and address of the manufacturer. It is not excluded, however, that adulterated oil will be accompanied by the relevant documents.

Studies of EO samples from different climatic zones showed significant differences in their chemical composition and pharmacological properties. Studies of EO from plants grown in the field in Finland, where a short period of plant growth (2-3 months) is largely offset by a long daylight in summer - 19 hours and more, a lower content of major phenolic components than in their southern analogues was found. So, carvacrol - one of the main components of

Thymus oil, was not found in Finnish sample [38]. For comparison, in EO from Crimean varieties of *Thymus* carvacrol part was from 16% to 72% in carvacrol varieties [7].

EO samples of sage leaves harvested in different seasons, also differed in composition: oil from the raw material collected in January, contained more camphor (12.3%), thujone (1.9%) and camphene (4.8%) was more toxic while intraperitoneal injection in mice (LD50 = 839 mg / kg) and had higher convulsive action. It has be found high degree correlation between these components content and toxicity: spring feedstock oil contained less camphor (7.7%), thujone (1.3%), camphene (3.1%) and was less toxic (LD50 = 1200 mg / kg). Therefore we recommend using of oil from spring raw material for medical purposes [23].

Thus composition and pharmacological properties of the essential oils from the same plant species, varies within wide limits and depend on the genotype, stage of plant maturity, climate, place of growth and other factors [16, 36, 38, 40, 44].

Standardization of EO is carried out by number of the main components, as therapeutic effect may depend on the interaction of several minor components. Sometimes under the influence of minor components toxicity of the main components reduced.

Thus among all possible options the most reliable way is purchasing oils from the same reliable manufacturer – this is the only way to work with the product with already known effect.

EO storage and experiation date

In the public literature, which is uncritically replicated and further cited including the Internet, one can find, for example, such statements: "Terms of use for essential oils are not limited. Moreover, the "thin" oils such as verbena, rose, neroli, sage, as well as "thin" wines are refined during prolonged storage "[10] – it is a completely wrong statement.

After 3 years of storage at + 8-10 °C in glass jars chemical composition of lavender, mint and *Monarda* EO changed within the permissible standards. Absolue rose and jasmine oxidized so that it could be determined even organoleptically.

All EO oxidize and decompose in air and light: changing color, odor, viscosity, acid number increases, resinates isomers and derivatives of original substances form in oils:

- some components of EOof atraktilodis (*Atractylodis macrocephalae* Pall.) self oxidized during the first hours, even at room temperature [26]. After six months of storage in the oil from atractylodis root amount of the main components - β-celine, hinesol, β-eudesmol and α-bisabolol reduced significantly [20];

- when stored for 3 months at 37˚C in EO of Sichuan lovage root (*Ligusticum chuanxiong* Hort.) the amount of the main component - ligustilide reduced, the amount of 2-propylene-1-hexane increased and isomers that were not present in the fresh oil appeared [15].

In the open air and on contact with oxidants limonene, linalyl acetate and geraniol are oxidized forming peroxides, aldehydes, alcohols and ketones, which already have high allergenic properties (influence of oxidation products is described below).

Therefore, EO should only be stored in a dark glass jars with glass or cork plugs, without air. Long-term contact with EO breaks even polyethylene structure and most rubbers destroy and pollute EO with decay products.

In practice, it is necessary to have the vial with EO for long term storage and mold the oil into the working tank when necessary. One should not buy EO in amount greater than needed for 2-3 years of work.

Terms of use for EO mixtures are less than for single ones. Terms of use for mixtures of EO with fatty oils and diluents progressively reduced and their pharmacological effects of varies unpredictably.

Currently it is published hundreds of the recipes for mixtures of several essential oils that are recommended as aphrodisiacs, compositions for "rejuvenation", "weight loss" and so on. Compositions which include up to 40 types of extracts and essential oils are patented. Most of these compositions are the advertising production without scientific confirmation of their useful properties, safety, and allowable storage time. The authors of most of these compositions are people who speculate on the interests of a particular group of customers.

Precautions when working with essential oils

Essential oils are very active pharmacological substances, so before using them all patients should be olfactory or patch testing: a person is given a sniff test probe with EO or it`s making a smear on the skin of the inner surface of the forearm with diluted EO. Patients were followed for at least 30 minutes (longer if possible) and poll the next day. Complaints of sore throat, sneezing, coughing, difficulty breathing, changes in blood pressure and heart rate, itching and irritation of the skin - the emergence of such symptoms on the test day or later should be considered a contraindication to the use of EO to the patient.

Oils should be applied to the skin in a diluted form - in a cream or fatty oils: hazelnut, almond, jojoba, sesame, apricot. The exceptions are the acupuncture points where 1-2 drops of whole oil are applied.

EO should be reccomended with great care for elderly people, children under one year and pregnant women.

Cases of skin irritation often associated with the use of old, oxidized oils. Due to prolonged contact with EO massage therapists often have hypersensitivity, contact dermatitis, and intolerance to some EO. Recurrence of skin symptoms after drinking lemongrass tea has been described. The authors consider it is necessary to inform workers that have prolonged contact with essential oils about the possibility of increasing sensitivity to these substances and risk of further limiting their ability to continue work in this sphere [17].

Of course, care should be taken that the oils do not fall on the conjunctiva of the eye.

Contra-indications for EO use

In the literature there are warnings about use of EO when certain diseases and syndromes are noticed:

- EO of basil and cypress increase blood clotting. This should be considered when assigning patients with thrombophlebitis, not to assign these oils for people with stroke and heart attack.

- EO of juniper, fir and sandalwood are contraindicated in nephritis.

- EO of bergamot, ylang-ylang, lemon, neroli, grapefruit, petit-Grein and orange have photosensitizing effect (increase skin sensitivity to UV rays). Photosensitization and other

negative effects after application of bergamot oil have been noted by many researchers [25, 30, 55, 56].

Special attention should be given to the review of articles about lavender, prepared by the Research Foundation of National USA Standards in 1994-2006. The authors concluded that there was insufficient level of scientific evidence on the safety of lavender essential oil use in medicine, particularly combined with other drugs:

- So, lavender enhances the effect of barbiturates (phenobarbital), tranquilizers and muscle relaxants, (lorazepam and diazepam), narcotics (codeine), certain antidepressants (imipramine), alcohol and substances that reduce cholesterol.

- When used with anticoagulants (aspirin, heparin, and warfarin) or NSAIDs (ibuprofen, naproxen) lavender EO can enhance bleeding.

- Provides information about the possibility of other side effects - allergies, dermatitis and others.

The authors considered there is no sufficient evidence of safety and feasibility of using lavender oil for children and adolescents younger than 18 years. The article is highly recommended to use lavender drugs only after consultation with experienced professionals [52].

If one of the most studied EO, used in medicine for hundreds of years in different countries, found so many accessory effects, probably, exotic oils should be used with caution, as information about them is often limited only by their use in ethnomedicine.

In early pregnancy it does not recommend to use EO in general, especially EO of basil, verbena, cloves, oregano, hyssop, cedar, cypress, myrrh, juniper, patchouli, rosemary, yarrow, thyme and sage [22].

For patients with hypertension, epilepsy, neurological disorders, and kidney disease treatments with oils of black pepper, rosemary and sage are contraindicated [45].

The official medicine in Russia allows medication of essential oils only in diluted form and only in official medicines. The Register of Medicinal Products includes about 40 products of native production, containing essential oils and their components [13] in combinations that allow to use the lowest effective dose of each of them and to reduce the possibility of accessory effects that occur when using high doses of essential oils.

Most products contain in its composition essential oils of mint and eucalyptus; lavender, clove, Ahearn, fir, pine and turpentine oils are used rarely. Pharmacological effects of the essential oils is enhanced by addition to the preparations of terpenoids such as menthol, thymol and camphor or alcoholic extracts and infusions of chamomile, thyme, yarrow, oregano and hops. Mostly these are medicines of antiseptic, anti-inflammatory and wound-healing activity for treatment of respiratory and gastrointestinal tracts and kidney diseases, infected wounds and burns [6].

The composition of foreign drugs except eucalyptus and peppermint can include EO of rosemary, rose, cinnamon, thyme, lemon balm oil and others [4].

Toxicity of essential oils

Some authors consider plants containing EO as sources of potentially toxic substances [24].

Indeed, the toxic effect of vapor terpene compounds was found for workers of aromatic plants industies: under prolonged exposure of menthol and coriander oil vapors at the concentrations of 50-100 mg / m^3 signs of chronic poisoning in the form of changes in the kidney, liver, lung tissue [33], pathology of gastrointestinal tract and cardiovascular system [8, 9] were observed. When collecting aromatic raw materials, some workers complained of headache, nausea, vomiting, conjunctivitis, itching and rashes on the skin [14].

EO of cloves has long been used in medicine, cosmetics and it is considered safe. However, adding 0.03% clove EO and 0.25% lavender EO *in vitro* cytotoxic effect of linalyl acetate and essential oils over the culture of human skin cells was detected. Toxicity of linalyl acetate was even higher than that of whole lavender oil; obviously its toxic effect was inhibited in the whole oil with other components [39].

For lavender EO cytotoxicity properties were described in relation to skin cells [27, 39, 41], and tea tree, mandarin, benzoin and ylang-ylang EO can cause allergic reactions when applied to skin [17, 19, 34, 35, 43, 47, 54, 55]. Allergic reactions are described in contact with the particular components of essential oils - benzyl and cinnamyl alcohols, eugenol, geraniol, hydroxycitronellal and others.

Linalaol (3,7-dimethyl-1,6-octadiene-3-ol) presented in large quantities in many EO has a fresh floral scent and is therefore often used as a flavoring in the goods of household chemicals and cosmetics. In its pure form linalaol is very weak allergen and does not cause skin irritation. In the air or on contact with oxidants linalaol oxidized and forms substances with allergenic properties.

In this model, simulating linalaol storage conditions, it was subjected to air oxidation. After 45 weeks storage mixture had only 30% of linalaola. Furthermore the mixture contains 19% of linalaol hydroperoxides (including 15% of 7-hydroperoxy-3,7-dimethylocta-1,5-dien-3-ol, 4% 6-hydroperoxy-3,7-dimethylocta-1 , 7-dien-3-ol, 20% of 2- (5-methyl-5-viniltetragidrofuran-2-yl) propan-2-ol, 4% of 2,2,6-trimethyl-6-2-viniltetragidro piren- 3-ol), and others. Sensitizing properties were characteristic mostly for hydroperoxides; esters did not exhibit such properties.

The authors supposed that contact allergy to linalaol is more common than previously thought. Thus, patients suffering from skin dermatitis in 5-7% of cases demostrated positive reaction to linalaol. Such a high frequency of positive reactions places linalaol among the most common contact allergens [18, 31, 37, 42, 48, 49, 50, 51].

EO toxic components are α- and β-thujone, ledol, cymene, palyustrol and arbutin. International Society for Polycyclic Aromatic Compounds prohibited for use in aromatherapy EO of tansy, wild rosemary, Cinnamomum, rue, sassafras and arborvitae [29], for which it has been described the possibility of toxic effects on the central nervous system, liver and excretory system002E.

Effective concentration of essential oils

Indications and recommended doses of essential oils can greatly vary from different authors. Therefore, review of the literature should be critical in the part of the recommendations on EO use.

During the aromatherapy session patients inhale vaporized EO in the concentration 1-3 mg / m^3 of air. At relaxed state, in the sitting position a person inhales 5-8 l / min. of the air. Simple arithmetic calculation shows that during aromatherapy session of 20-30 minutes the patient breathes 0.1-0.2m^3 of the air ie ≈0.1-0.6 mg of EO [13].

Doses in tenth parts of a milligram, has a significant impact on health, heart rate, blood pressure, memory, attention, physical performance, regeneration processes, immune system, etc.

Keep this in mind when assigning other procedures with EO - baths, massage, treatment of wounds and burns.

Some authors recommend using of undiluted EO for the massage, and it is at least 5 ml per procedure. We think that recommendations of aromatherapists who advise to delute EO and use much smaller amounts have better foundation. Thus, Brown [1] recommended 3 drops of EO per 10 ml of neutral oils for massage and 2-3 drops of EO three times a day not

more than three weeks for ingestion. The author believes that in large doses essential oils are harmful and dangerous.

From our point of view, the ingestion of 6-9 drops of oil (50-300mg) per day is too much. Drops dosage itself is a very conditional and imprecise method, since the weight of a drop depends on the viscosity of the oil and application tools. When weighing on analytical scales lavender oil drop from the glass pipette was 30-35mg, and a drop of the same oil from syringe with intradermal needle - 5-7 mg.

EO successfully used for the treatment of infected wounds and superficial skin lesions of different nature. We have observed the acceleration of engraftment after skin plasty, acceleration of epithelialization of the wound edges, the appearance of islands of epithelium on the wound surface and reducing scar formation [14]. In the treatment of wounds, ulcers` abrasions and other skin lesions 5-7 times diluted EO should be used [32].

It is also recommended to add not more than 3-5 drops of EO in the bath; the addition of saponificative substances under this amount of oil is not required.

Dose-dependence of various beneficial effects of EO was noted by most of authors. Schematically, this is reduced to the usual sequence: lack of low concentrations effect, the optimal range of concentrations and toxicity of high doses.

This is particularly well illustrated in numerous experiments: when added to animal food small amounts of oregano and pine EO (or fresh herbs of these plants) an increase in fertility, reduction in mortality among young individuals, accelerated weight gain, stimulation of the immune system and improvement of the epidemiological situation in herds were observed [5].

Adding sage, clove or cinnamon EO in the amount of 0.25% and Origanum oil in 0.1% within 2 weeks in female mice food was accompanied by embryotoxic effect - reduction in number of dividing cells and increase of dead cells in the embryos. Concentration of the oil was so high that the animals refused to eat food.

Most of EO authorized for use forms a group of little and virtually non-toxic substances.

According to our data, LD50 for EO of Monarda and eucalyptus was 360-600mg / kg by inhalation and 600-1200 mg / kg intraperitoneal injection for guinea pigs. In the studies of chronic toxicity dystrophic changes of internal organs, pathological changes in the kidney, spleen, peripheral blood and immunosuppression were observed in the animals.

But the dose of EO could be a matter of choice under extreme conditions: in the animals irradiated with a lethal dose of γ-rays observed protective effect of inhaled course with essential oil vapors at concentrations of 20-100 mg / m^3 of air [3].

However, we recommend not creating concentration of EO in the air more than 5 mg / m^3 during the aromatherapy. From our point of view 1-3 mg / m^3 EO concentration in the air at the session duration for 20-25 minutes are optimal.

But the dose of EM can be a matter of choice under extreme conditions : in the animals irradiated with a lethal dose of γ- rays observed protective effect of inhaled course with essential oil vapors at concentrations of 20-100 mg / m3 of air [3].

Conclusions

Natural EO are widely used as an independent and additional medicaments for the treatment and prevention of various diseases and syndromes.

Aromatherapy procedures requires special training of employees as knowledge of chemistry, toxicology and safety in the use of EO are needed.

Before the appointment of aromatherapy it`s necessarily to conduct tests on individual tolerance. Complaints of cough, shortness of breath, skin redness, itching, rash, etc. are the contraindications for this patient taking EO therapy.

High concentrations of EO are toxic for humans and animals.

EO should only be stored in glass containers away from light and air. If not properly stored essential oils lose their therapeutic properties and get allergens` properties.

Mixtures of 2-3 oils should not be stored and used for over a year. Using of multicomponent mixtures is not recommended due to unpredictable changes in their chemical composition and pharmacological properties.

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Tikhomirov A.A. Principles of essential oils using for medical purposes. A Review // Works of the State Nikit. Botan. Gard. – 2014. – V. 139 – P. 109 – 119.

Essential oils are used as an independent as auxiliary medicines for the treatment and prevention of various diseases and syndromes.

The review of the literature contains data about essential oils contents, therapeutic concentration and rules of use in medical practice. The article also covers different points of views in usefulness, harmlessness and dosages of essential oils. The particular attention is paid to safety making a prescription of the aromatherapy: storage precautions, dosages, toxicity, allergenic capacity, ability to use essential oils with medicines. The information is given for practicing physicians.

Keywords: essential oils, composition, contraindications, dosages, toxicity.

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SOME MORPHOBIOLOGYCAL FEATURES OF *GLAUCIUM FLAVUM* CRANTZ. (PAPAVERACEAE)

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Introduction

One of the most important modern problems is the task of biodiversity, including phytodiversity, preservation. This is especially true of those species, which belong to different categories of conservation status. Thus V.N. Golubev [1], V.V. Novosad and L.I. Kritskaia [9] considered *Glaucium flavum* Crantz. to the category of rare species and E.S. Krainiuk [7] brings it to the category of endanged species. This species is known as decorative and medicinal plant, due to alkaloids in its parts that are used for treatment of respiration organs diseases. In spite of its valuable features it hasn`t been studied well enough. In the Crimea *G. flavum* is represented by single specimens or small populations spreaded through the territory of the Southern coast [1, 3]. That`s why features of its reproductive biology and possibilities for naturall reproduction supposed to be important and they were the aim of our investigations.

Materials and methods

Observations have been carried out on the Southern coast of the Crimea on the coastal pebbles and sea slopes. Studies of flowering rhythms and terms were studied with the methods by A.N. Ponomarev [11], Golubev V.N. and Volokitin Yu.S. [2]. For seed production determination method by Yu.A. Zlobin was used [4]. Material preparation (fixation, dehydration) and sections were made by customary embryological methods [10] and stained according to our own method [12, 13]. For taking photos camera Canon A 550 was used. Seeds and embryos measurements were made under the light microscop Axio Scope A.1 (Carl Zeiss) with analyses system Axio Cam ERc5s by the programme Axio Vision rel. 4.8.2.

Results and discussion

G. flavum is a european-mediterrenial species from genus *Glaucium* that includes nearly 30 species [8]. This is biennial or perennial plant and its native area covers sea coasts of Europe, Southwest Asia and North Africa. In the Crimea it grows on the Southern coast on the stone and sand slopes.

During the first year of life *G. flavum* forms the rosettes of leaves and in the second year leaved generative shoots appear. It could form strong specimens with numerous shoots (fig. 1). Blossom was noticed in May – July with the top in June, seed maturity – in July – September. Flowers are single, apical or in the axils of leaves, diameter is about 5 sm. They are bright yellow with two curled sepals and four petals in two rows, without nectary. Sepals are covered with bristles they gradually open and fall down at the beginning of blossoming so tripped flower is presented by the petals only, without sepals (fig. 2).

Fig. 1 General image of *G. flavum* plant

A B C

Fig. 2 Flower buds (A and B – without sepals) and open flower (C) in *G. flavum*

Androecium is presented by numerous single stamens, outer ones are shorter then inner (look fig. 2 B). Anthers with four pollen-sacs and two thecas open extrorse. Microsporangium wall development is centripetal and when it is formed consists of epidermis, endothecium, two middle layers and tapetum (fig. 3 A). Tapetum forms from the cells both of second parietal layer and parenhimal cells of connectivum. At the stage of microspores` tetrads the wall of microsporangium consists of epidermis, endothecium, one

middle layer and tapetum (fig. 3 B). At this stage stransformations of tapetum cells were noticed – their walls begin to degenerate and gradually tapetum transforms into amoeboid type (fig. 3 B). These observations confirm the data recived by G.M. Ilina [5] and O.P. Kamelina for species from Papaveraceae family.

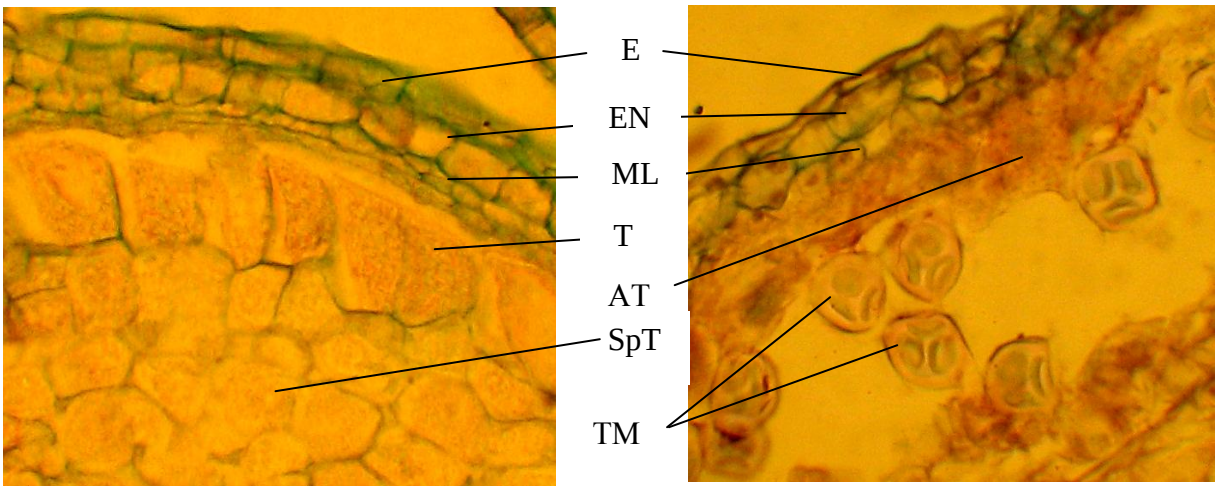


Fig. 3 Parts of the formed microsporangium (A) and at the stage of microspores` tetrads (B): E – epidermis, EN – endothecium, ML – middle layer, T – tapetum, AT – amoeboid tapetum, SpT – sporogenous tissue, TM – microspors` tetrads

Wall of mature microsporangium consists of flattened epidermal sells, fibrous endothecium and parts of the middle layer. Mature pollen grains are two-celled, exine with three furrows and three pores. In microsporangium both normal and anomal pollen grains present. It should be noticed that during meiosis and differentiative mitosis some deviations that further lead to degeneration of some pollen grains were found out.

As in many species from Papaveroideae subfamily [5, 6], ovul in *G. flavum* is funiculose, campylotropous, crassinucellate, bitegmal (fig. 4). Funiculus is short, widen at the base, curved. As the result of its congenital inosculation raphe is formed. Integuments are of epidermal origin, in the apical part both enlarge and form obturators. Obturator of inner integument gradually transforms into an operculum and obturator of outer integument further forms arrilode that is the part of the seed peel.

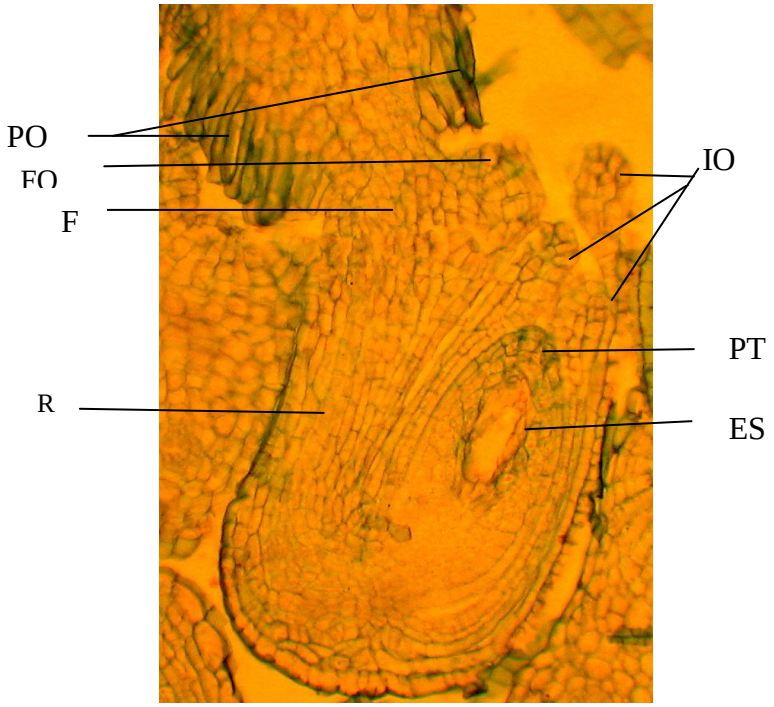


Fig. 4 Ovule of G. flavum (PO – placental obturators, FO – funiculose obturator, F – funiculus, R – raphe, IO – integumentary obturators, PT – parietal tissue bands, ES – embrional sac)

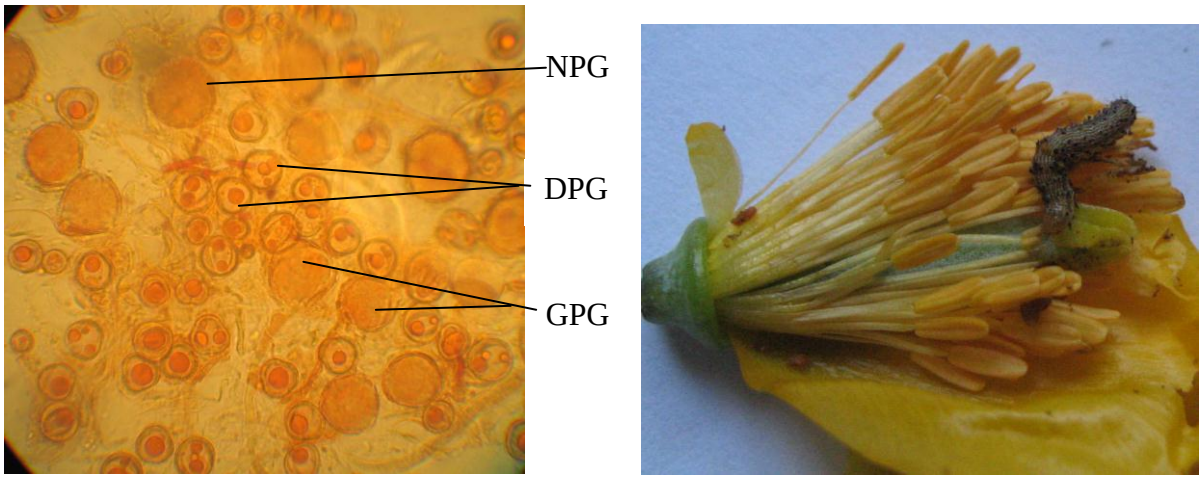
In micropilar zone of nucellus rows of parietal tissue cells are well noticed. They further favour to the pollen tubes growth to the embrional sac. In halasal part of the ovule pedestal, podium and hypostase, placental obturator built of large, radially elongated, nipple-shaped cells (see fig.4). Vascular bundle stretches up to halase. Embrional sac is monosporic, consists of 7 cells, forms by Polygonum type, significantly narrowed in halasal zone. Egg complex includes two pear-shaped sinergids and the egg cell. Polar nuclei are in the central cell of the embrional sac, they are more close to antipods than to the egg cell. Antipods are large; the cells could be binucleate or polyploid and take more than 2/3 of the embrional sac volume, stay long. These cells often grow out and form antipodal complex with haustorial functions (fig. 5).

Fig. 5 Halasal part of *G. flavum* embrional sac with poliploid antipods: ANT – antipods

Blossoming of *G. flavum* flowers starts early in the morning and up to 10-11 a.m. all flowers are open and sepals fall down at once. Anthers` opening is extrorse in the flower just open. Since the staments are moving nearly all the time anthers curve from the pistil to different sides especially while the air blowing (fig. 6). Pollen is fat. It scatters on the petals where the most of it is eaten with insects that are attracted with bright colour of petals and stamens` moving (fig. 7). Insects can eat anthers and tissues inside even before the flowers` blossom (see fig. 7 B). At that time pollen grains attach to the different parts of the small insecst bodies, that comes to the petals or broad stigmas, and they spread pollen grains from one flower to another carrying pollination process (see fig. 6 and 8).

Fig. 6 *Glaucium flavum* flowers with the insects

To the end of the flowering almost all anthers are without pollen grains and they dry rapidly. Some cases of pollen grains` growth just in the anther were noticed (see fig. 7).



A B

Fig. 7 Mature pollen grains of *G.flavum* from the anthers of the open flower (A) and caterpillar inside the closed flower (B): NPG – normal pollen grains; GPG – growing pollen grains; DPG – defective pollen grains with fat drops.

Flower that opens early in the morning seases its blossoming in the evening – its petals lose turgor, become white and droop. At that time pistil curves and it may touches nonfallen petals and take the leaving pollen grains so autogeny occurs in the absence of allogeny (fig. 8).

A B

Fig. 8 *G. flavum* androecium and gynoecium at the time of flowering (A) and at its end (B)

Pollination unit in *G. flavum* is a single flower. This species is characterized with presence of primary attractants (pollen and fatty oils) and secondary ones (visual attraction with bright flower and moving stamens). Neighbouring plants of *Melilotus tauricus* L. (fam. Fabaceae) (fig. 9) often play the role of false attractants.

Fig. 9 Plant of *G. flavum* together with flowering *Melilotus tauricus*

After the pollen grains have landed on the stigma they germinate and spermiogenesis occurs in the pollen tube. The last grows on the surface of the style tissues` cells, comes to the embrional sac through one of the sinergids and discharges its contents. Double fertilization takes place: one spermium fuses with the nucleus of the central cell resulting in the primary endosperm nucleus formation, and the other with the egg cell. Nuclear endosperm develops and its nuclei are located in the parietal strands of cytoplasm. Endosperm cells formation progresses from the periphery of the central cell embryonal sac towards its centre. Embryo development is of Solanad Type, Papaver variation when the derivates of the terminal cell form only the shoot apex with the preliminary differentiation of epiphyseal cell and the main organs of the embryo are formed by the cells derivative from the basal cell.

Fruit in *G. flavum* is dry pod-like capsule, length 18-20 sm, opens with two segments. In each capsule 50-55 seeds are formed. The seed is small, about 1 mm length, with two-layers seed coat and strong endosperm. Endosperm cells are large and loose with droplets of fatty oils. Embryo is poorly differentiated, small (about 200 μm length), takes nearly 1/5 of the seed volume (fig. 10). Seeds germinate in the year of their generation and after a year of storage. For successful germination they need a period of biological rest for their ripening.

It should be noticed that flowering period is long, each plant of *G. flavum* may blossoms more than two months and generally forms great number of flowers so in the time of mass blossom flowers and fruits could be observed on the same plant (fig. 11). Formation of plants with great number of generative shoots with numerous flowers, long flowering period and formation of viable seeds guarantees high reproductive success of this species in the conditions of its native area.

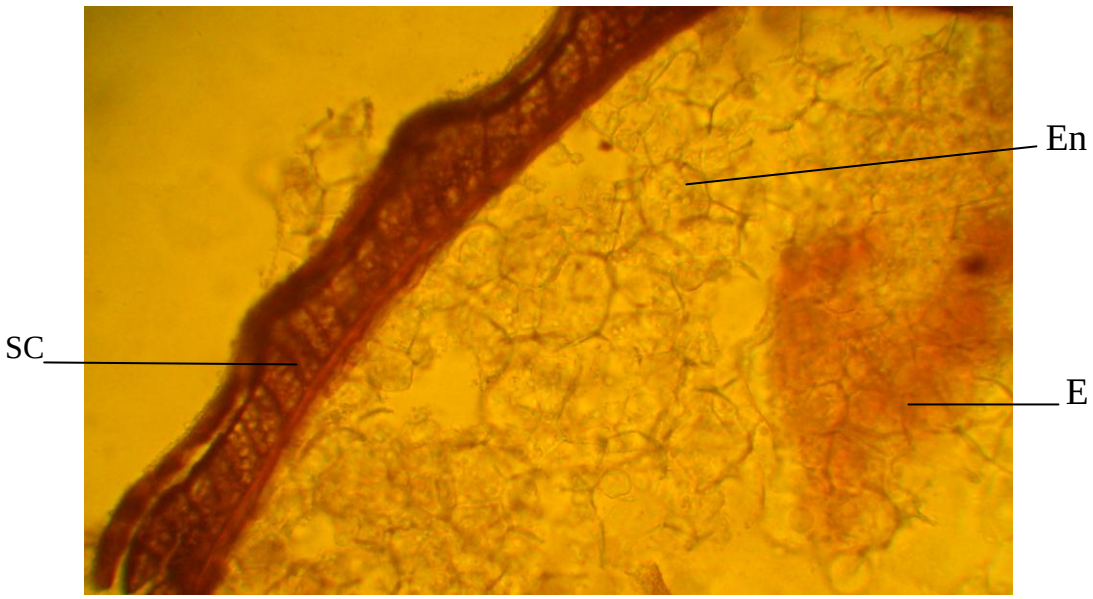


Fig. 10 Part of *G. flavum* seed (SC – seed coat, E – embryo, En – endosperm)

Fig. 11 Plant of G.flavum with fruits (August 8, 2013) (GF – green fruits, DC – dry pod-like capsules)

Three studied *G. flavum* populations differ in number of plants and potential abilities for preservation. Two populations (on the Cape Martjan and on the steep sea slope in Livadja) include 3 plants each. During three years of investigations no changes in their number have been noticed. Population in Yalta cargo port had 48 plants of different age in 2013 and 26 plants in 2014 as the rest plants were destroyed that demonstrates negative antropogenous influence. However, great number of generative shoots, flowers, fruits and seeds per plant let us to suppose potential possibility of this species multiplication in its native conditions but measures for its preservation such as culture cultivation and using as a decorative plant in parks are needed.

Conclusions

Thus *G. flavum* in its biomorphological features doesn`t differ significantly from the other species from Papaveraceae family. Its main embryological features are: centripetal type of microsporangium wall development, amoeboid tapetum and its dual origin; campilotropous, crassinucellate, bitegmal, funicular ovule; formation of placental, funicular and inegumental obturators; long functional haustorial activity of antidods that is supported by nuclei number increasing in the cells or their poliploidisation; Solanad type, Papaver variation for embryo formation; great number of fruits – dry, pod-like capsules, open with two segments, with viable seeds. Formation of numerous generative shoots, long period of each plant flowering and number of fruit and seeds give us possibility to suppose potential abilities of this species for natural reproduction. However, *G. flavum* grow in the coastal area where antropogenous influence is great and it may lead to significant decreasing of this species in nature conditions. That`s why for preservation of *G. flavum* and noted its highly decorative appearance this species could be recommended for culturing and using for creation of ornamental plantings.

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Shevchenko S.V. Some morphobiological characteristics of Glaucium flavum Crantz (Papaveraceae) // Works of the State Nikit. Botan. Gard. – 2014. – V. 139 – P. 120 – 128.

The article presents study results of some aspects of *Glaucium flavum* Crantz reproductive biology. The processes of the flowering, pollination, fruit and seed formation have been described. It covers seeds` characteristic and demonstrates potential possibilities of the species reproduction in the conditions of the Southern Coast of the Crimea as well.

Key words: *Glaucium flavum Crantz., anther, ovule, flowering, pollination, seed formation.*

UDK 582.998.3:581.16

SPECIAL FEATURES OF REPRODUCTIVE BIOLOGY IN SOME SPECIES FROM THE GENUS *CAMPANULA* L.

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Introduction

At the present time one of the most important problems is the one of biodiversity preservation. For further development of rare plants preservation methods knowledges of their reproduction processes are needed. Campanulaceae family is numerous and diverse. According to the data of V.N. Golubev 22 species Campanulaceaeies from Campanulaceae family grow in the Crimea including 16 species from the genus *Campanula* L. Thus, A.V. Ena [9] noticed that in the Crimea Campanulaceae family is represented by 17 species including 12 species from the genus *Campanula* L. this genus is problematic enough in its systematics and some species` position is under discussion [7, 8]. As for species we have investigated - *Campanula sibirica* L., *C. taurica* Juz. и *C. talievii* Juz., there is no consensus in the literature sources. Some authers consider them as three separate species [4, 7, 8] others [2, 9] point that *C. taurica* and *C. talievii* are subspecies for *C. sibirica*. Characteristics of reproductive sphere structural elements of these taxons could be useful for defining and claryfying of their systematic position. Besides, representatives of Campanulaceae family are highly decorative and perspective for using in decorative gardening.

Resulting all reported above the aim of our studies was determination of the special features of reproduction, features of similarity and differences in the reproductive system of three *Campanula* L. species (*Campanula sibirica* L., *C. taurica* Juz. и *C. talievii* Juz.) that grow in the Mountain Crimea.

Objects and methods of he research

Species *C. sibirica*, *C. taurica* and *C. talievii* were investigated in there native growth conditions in the Mountain Crimea on three plots: 1) on the north-eastern slope of the Chatyr-Dag mountain, 2) along the road to Baidarskie Vorota (from Yalta – Sevastopol highway) and 3) on the north-eastern slope of the Chelebi mountain. Flowering rythms and terms were studied with the methods by A.N. Ponomarev [18], V.N. Golubev and Yu.S. Volokitin [5, 6]. Seed productivity was performed by E.A. Hodachek [21]. I.V. Vainagii [1] and Yu. A. Zlobin [11]. Temperature indexes were determined using a laboratory alcohol thermometer. For plant material fixation Karnua solution (ethanol 96% - 6 parts: hlorophorm – 3 parts: acetic acid – 1 part) was used. Customary methods of preparation samples for cytoembriological studies were followed. Staining was by methyl-green and pyronin with alcian blue [24, 25].

Evaluation of ornamental efficiency was made according to the method of State Plant Varieties Testing for ornamental plants [14].

Photoes were taken with the camera Canon A 3100 IS. Seed germination was tested in laboratory conditions on distilled water after a year of storage in paper bags under the room air temperature [17].

Results and discussion

In Campanulaceae family various life-forms are presented including herbaceous plants, climbing plants, treelike forms and treelike climbings. Our studied species are mostly herbs. *C. sibirica* is biennial plant up to 70 sm height. The stem is single and erect. Flowers

are numerous (fig. 1A). *C. taurica* is a perennial plant up to 50 sm height with many stems, the central one is erect (fig. 1 B). *C. talievii* according to N.G. Dremluga and S.N. Ziman [8] and our observations is perennial semishrub up to 25 sm height, sometimes up to 50 sm (fig. 1 C). So as in *C. taurica* its stems are numerous with erect central one, persist after winter. Flowers are numerous. An.A. Fedorov [20] and V.N. Golubev [4] considered that *C. talievii* is endemic Crimean species.

Flowering of studied *Campanula* species is long enough in the Crimea – since May to August. Its initiation in *C. taurica* and *C. talievii* starts 1.5-2 weeks earlier than in *C. sibirica*. Single flower lives for 7-10 days in all three species. Then it gradually wilts, cup and corolla don`t fall down but dry. Flowering of a single plant can last from May to August so in August flower buds, flowers and fruits could be noticed on the same plant. The stage of loose flower bud comes on the 4^{th} day and the flower opens on the 7^{th} day. In the period of flowering 2013, in June, temperature indexes on the Chatyr-Dag Mountain were: on the soil surface +23 ^{o}C, of the air - +21 ^{o}C, in July – +26 ^{o}C and +24 ^{o}C, in August – +28 ^{o}C and +25 ^{o}C, correspondantly. On the mountain Cheleby in 2013 the same temperature indexes in the flowering period were: in June– +22 ^{o}C and +20 ^{o}C, in July – +26 ^{o}C and +25 ^{o}C, in August – +29 ^{o}C and +27 ^{o}C, correspondantly. So in the period of flowering the air temperature varies from +20 ^{o}C to +30 ^{o}C.

A B C

Fig. 1 Plants of *C. sibirica* (A), *C. taurica* (B) and *C. talievii* (C)

Number of flowers per one generative shoot in *C. taurica* is up to 17, *C. sibirica* – up to 13 flowers, *C. talievii* – up to 30 and number of flowers per plant could be up to 45 in *C. taurica*, up to 35 in *C. sibirica* and up to 70 – in *C. talievii*.

Flower is actinimorphic, full and bisexual. Such flowers are characteristic for Campanulaceae family [3]. Flower stem is 8-10 mm. This feature is important for stuied species as in mature fruit it fuctions as an additional tool for dissemination [16]. Flower bed is convex. Sepals are curved out. Cup is toothed, wilted, nonfallen with curved appendages. Petals are toothed. Corolla is bell-shaped, tomentous (fig. 2).

Fig 2 *C. taurica* flower with tomentous corolla and pollinator

Bud becomes coloured when it`s 10-15 mm length. Its colour varies from light lilac to dark violet (see fig. 1). Flowers of *C. talievii* have the most intencive dark violet colour and in *C. taurica* and *C. sibirica* they are significantly lighter.

In all three studied species androecium is represented with 5 stamens that are direct and equal, attached to the base of nectar disc (fig. 3). Bases of filaments are broad; they fuse and form a dome with an aperture on its top.

Gynoecium is syncarpous with one pistil. Nectaries are inner in the form of a disk on the ovary. Style is central, strait, wilted, nonfallen, covered with lots of one cell hairs of epidermal origin (see fig. 3). In studied species stigma is apical, divided, threelobed, turned out, in open flower it`s partly beyond it. Ovary is trilocular, pubescent and low, with great number of ovules.

Anther has 4 microsporangia and 2 thecas. It also has placentoid – radial growth of connectivum tissue that just inside each microsporangium. Microsporangium wall development is centrifugal [13, 23]. Formed microsporangium wall consists of epidermis, endothecium, one middle layer and secretory tapetum. Microsporangium wall in mature anther consists of flattened epidermal cells and endothecium with fibrous bands.

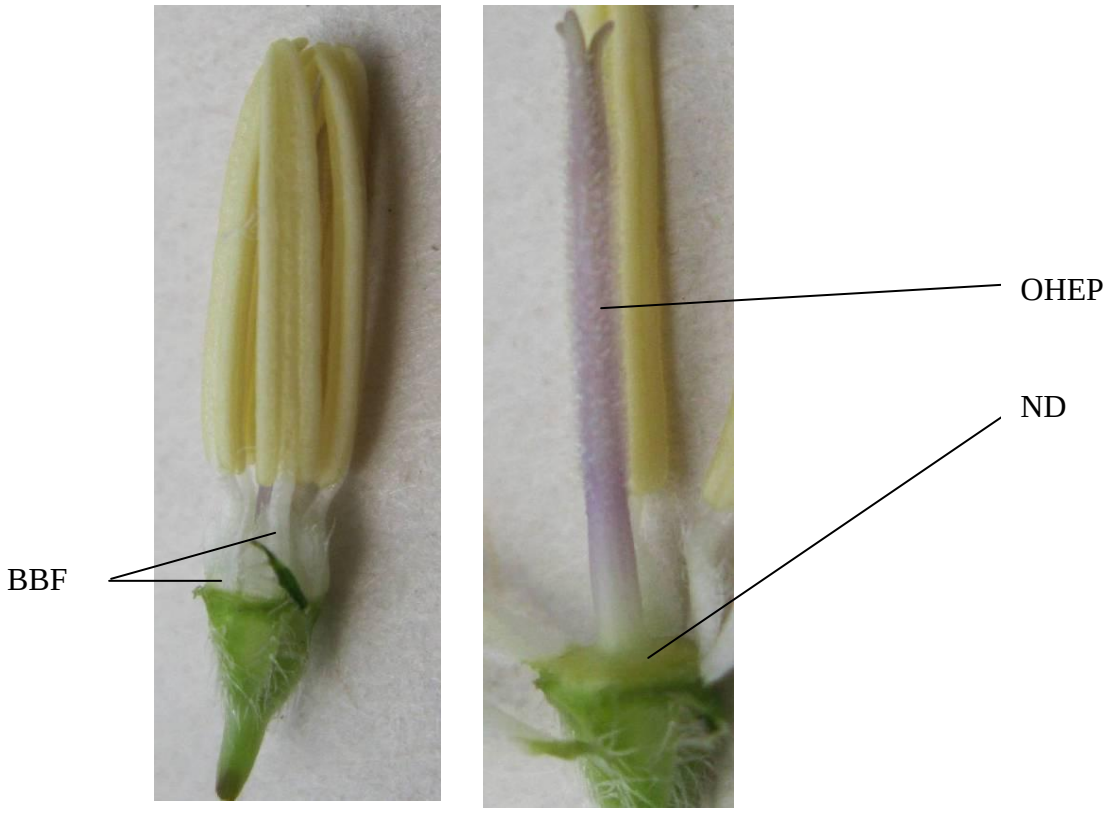

Fig. 3 Parts of C. taurica flower (BBF – broad bases of filaments; ND – nectar disc; OHEP – one cell hairs of epidermal origin on the pistil)

Endothecium could consist of two rows and fibrous bands present both on the cell walls of the outer side of microsporangium and on the cell walls at the side of connectivum. As the result each microsporangium is covered with fibrous layer. Mature pollen grains have three cells, 3 furrows and 3 pores. Together with normal pollen grains defective ones could be noticed in microsporangiums (fig. 4).

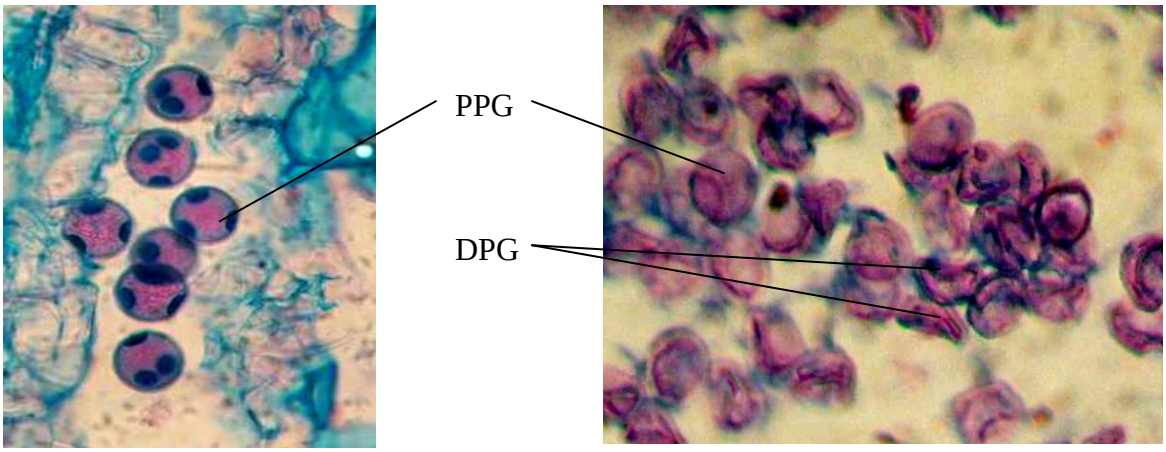

Fig. 4 C. taurica pollen grains (PPG – proper pollen grains, DPG – defective pollen grains

Ovule is anatropous, medianucellate, unitegmal. Integument is of epidermal origin, consists of 6-8 cell rows. Micropyle is simple, narrow, strait. Funiculus is short, funicular obturator presents. Raphe is formed. Integumental tapetum is strongly developed; it reaches the egg apparatus and is presented with flattened round cells with nuclei and nucleoli.

Archesporial cell differentiates in subepidermal layer and after the first division it forms parietal and sporogenous cells, later transforms into megasporocyte. Meiosis results in megaspores tetrad formation and halasal one developes into the embryo sac (fig. 5).

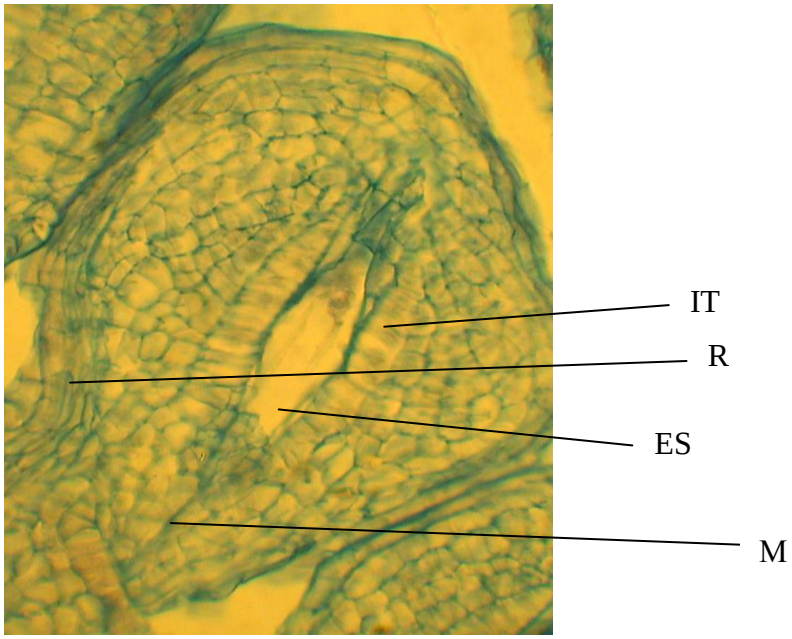

Fig 5 C. taurica ovule (R – raphe, IT – integumental tapetum, M – micropyle, ES – embryo sac)

Embryo sac is 7-celled, monosporic, of Polygonum type. Egg complex is presented by two sinergids with hookshaped outgrowths and paer-shaped egg. Polar nuclei fuse before fertilization. Antipods are placed in T-shape and stay long enough. Between the embryo sac and hypostaze postament-podium forms and its cells have thicker walls than the cells nearby [23].

Pollination process in *Campanula* is very specific. In closed flower bud staments fully cover thestyle – anthers densly surround the style and broad bases of filaments form, as it has been noted above, a kind of dome (see fig 3 A). Anthers open introrse in the closed flower bud and at that time stigma lobes are cerried.

As pistil growth hairs that cover the style hooks the pollen grains and take them out of the anthers and as the result the whole style is covered with pollen grains. In the upper part of the dome there is a whole through which a pollinator reaches a nectar disk with its haustellum. Nectar disk becomes coloured when a flower size is 14-16 mm and it could be from white to bright-lemon.

As an insect moves towards the nectar disk it takes the pollen grains from the style with its legs, abdomen and, sometimes, even wings. When it comes back it touches the style again and takes additional pollen grains (fig. 6)

Fig. 6 Pollinators on *C. Sibirica* flowers

After pollination the staments wilt and curve before the stigma opens so in an open flower we noticed only parts of anthers. When a flower is fully open stigma lobes disclose. For studied species allogamy (geitonogamy and kleistogamy) is typical, sometimes autogamy occurs as it also has been reported by S. Vogel [27] for *C. rotundifolia* L. during the pollination process hairs are pulled in the covers of style so phenomenon of invagination or retraction is observed.

It should be noticed that a typical feature of studied species is protandry, when development of male reproductive structures is much earlier than of female ones and anthers open in closed bud. After pollen grains landed on the stigma they germinate, pollen tube reaches the embryo sac, comes through one of the sinergids, burst and its content pour out. Fertilization takes place. After double fertilization process embryo and endosperm develop. According to Campanulaceae family characteristics [10, 13] its species are characterized with cellular endosperm. Our observations demonstrate that according to O.P. Kamelina classification it`s tubifloral and characterized with transverse division of the first endosperm nuclei and formation of micropillar and halasal haustorium. Exactly endosperm forms from the cells that lie between hausturias. I.I. Shamrov [22] supposed that this type of endosperm formation could be determined as micropilar-halasal with terminal haustorias as a subtype of cellular type.

Almost all flowers in studied species form fruits but they have different number of seeds [15]. Processes of seed maturing and dissemination are gradual and last from Augest to September. Seeds` scattering is also gradual due to ballochory that is one of the most efficient ways for spreading seeds in a short distance. Particularly ballistoanemohorry is typical for these species, when dry stems and capsules move under the wind blows and seeds fall out from the capsules. The species also characterized with ballistozoohorry when parts of the plant move due to the touches of animals. One more type of seed spreading typical for these species is epizoohorry when dry capsules attach to the animals with their hooks and are spreaded for long distances. Besides, after falling from the capsule small and light seeds are carried for a long distance by the wind blowings, that are typical for mountain Crimea, hence anemohorry occurses. All this ways of seed spreading are favourable for species diffusion and colonization of the new territories.

Seeds of all species are small, about 1 mm length, light-brown. Seed coat consists of two layers; endosperm is represented with large cells. Embryo takes nearly 1/3 part of the seed volume and it doesn`t full the entire embryo cavity which is well noticeable. Viability of seed increase correlative to the terms of their storage, just ripened seeds don`t germinate almost, because of undeveloped embryo. Germination of seeds collected in 2011 after year storage under the room temperature was: in *C. sibirica* more than 50%, in *C. taurica* - 65%,

in *C. talievii* – 35%. Germination of seeds collected in 2012 and germinated in December 2013 was: in *C. sibirica* nearly 90%, in *C. taurica* – более 85%, in *C. talievii* – 65%.

Fruit in studied species is wilted, threecelled, hair-covered capsule with numerous seeds. It is formed by dry flower cup and curved appendages (fig. 7)

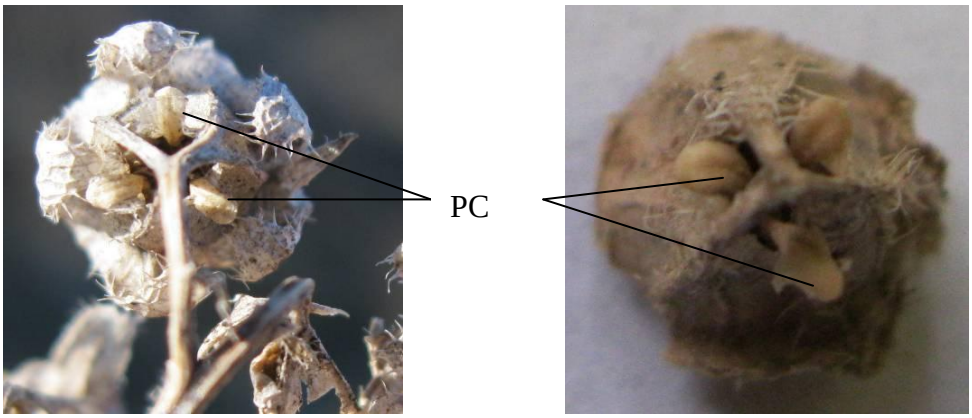

Fig. 7 Dry capsules of *C. talievii* (PC – pore cover)

At the base of the capsule there are three pores formed by axicorns – structure of crescent shape on the centre-axial column of the fruit. Along with the fruit dry axicorn tears a cover between the ridges at the base of the capsule with one of its tips and so pore and its cover are formed (fig. 8).

Fig. 8 C. taurica fruit part with the axicorn (A – axicorn)

Such wilted capsules with pores in their base were noticed in *C. rotundifolia* L. but contrary to *C. carpatica* L. with erect capsule and pores on its top [26, 27]. One more helpful tool for seed spreading is tough hairs that cover the cup and after they dry became hooks and let seeds to attach to animals.

Processes of seed formation and fruit ripening in studied species is long, dissemination starts in the middle of August and lasts to late September. Seeds through pores fall down under the lightest shoots` movement.

Our observations and literature data demonstrates that in *C. talievii* stems wilt but they don`t fall. In the next year, in spring these wilted plants produce new rosettes of leaf so besides seed propagation in *C. taurica* and *C. talievii* vegetative one is possible while in *C. sibirica* we haven`t observe such trait (fig. 9 and 10).

Fig. 9 Parts of the root system in *C. sibirica* (A), *C. taurica* (B) and *C. talievii* (C) (VS – vegetative shoots, NR – new rosette)

Number of new rosettes in studied populations varies from 2 to 4. We observed *C. taurica* plants both with 2 and 4 vegetative shoots. In *C. talievii* population only plants with 2 vegetative shoots were noticed. Studied populations are not full, left-handed with great part of virgin and generative plants while senile plants haven`t been noticed yet. Such structure of populations demonstrates potential possibilities of selfreproduction and propagation in these species.

Fig. 10 *C. talievii* at the stage of wilted plant (A) in November 2013 and with new generative shoot (B) in May 2014

Studied *Campanula* species, as many other representatives of Campanulaceae family, have decorative appearance. Most of signs according to 5-point scale by the method of State Plant Varieties Testing for ornamental plants [14] have marks 4 and flowering duration was evaluated as 5 points (tabl.).

Table

Decorative features evaluation (points)

Name of the sign	Points		
	C. sibirica	C. taurica	C. talievii
Colour of flower	4	4	5
Odour	4	4	4
Stem (length, firmness)	4	4	4
Inflorescence	4	4	4
Flowering abundance	4	4	5
Flowering duration	5	5	5
Flowers` resistance to unfavourable weather conditions	4	4	4
Decorative of plant vegetative part	4	4	4

Due to their ornamental features, long term flowering and number of formed seeds studied species could be used in park expositions.

Conclusions

1. The main embryological features of the studied species could be considered: centrifugal formation of microsporangium wall, presence of placentoid, pollen grains with three cells, 3 furrows and 3 pores, anatropous ovule, integumental tapetum development, 7-celled, monosporic, Polygonum type embryo sac, tubifloral endosperm micropillar and halasal haustorium, Solanad-type of embryo development, protandry.

2. *C. sibirica*, *C. taurica* and *C. talievii* flowering in native growth conditions lasts from May to August under the average day temperature from +20^{o}C to +30^{o}C. C. sibirica flowering starts 1.5-2 weeks later. Great number of flowers is formed both on the single generative shoot and on the whole plant.

3. Allogamy is typical for studied species but autogamy, when pollen grains are taken out of the anthers by one cell hairs of epidermal origin placed on the style, is possible. Phenomenon of invagination or retraction has been noticed.

4. Fruit in studied species is wilted, threecelled, tough hair-covered capsule with three pores at it base and numerous seeds inside. For their ripenning seeds need the period of biological rest.

5. The main type of propagation is seed propagation but in C. taurica and C. talievii vegetative propagation is also possible.

6. Long flowering period of *C. sibirica*, *C. taurica* and *C. talievii*, great number of fruits and seeds formation, data about seed productivity and germination, different tools for successful dissemination demonstrates potential possibilities of reproduction, propagation and spreading of these species.

7. Due to their ornamental features, long term flowering and number of formed seeds studied species could be recommended for using in decorative gardening.

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Myroshnichenko N.N. Special features of reproductive biology in some species from the genus Campanula L. // Works of the State Nikit. Botan. Gard. – 2014. – V. 139 – P. 129 – 139.

The article reveals the research results in formation of male and female generative spheres, flowering, pollination, dissemination, seed formation of three species from the genus Campanula L. (C. sibirica L., C. taurica Juz. and C. talievii Juz.). Also the age structure of their populations, seed and vegetative multiplication as the most important factors of species self-reproduction have been described.

Key words: *Campanula sibirica L., C. taurica Juz. and C. talievii Juz., flowering, pollination, dissemination, seed and vegetative propagation.*

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POTENTIAL FROST-RESISTANCE OF *LONICERA* L. SPECIES AND ANALYSES OF FACTORS WHICH LIMITS THEIR WINTER HARDINESS

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Introduction

Among all the environmental factors that influence on growth, development and ornamental features stability of introduced plants one of the important factors is the climate of the particular territory and weather conditions of exact place of growth and season. It has been known that atmosphere processes in the Crimea are variable [1, 12] and unforable agrometeorological situations which have a conclusive affect for conservation of ornamental qualities of plant introduced from subtropical and tropical regions are often. All these determine necessaty of weather conditions and level of plants` crioresistance carefull analyses for finding both optimal and stress conditions that influence realization of adaptive potential in deciduous and evergreen ornamental introduced plants including species from the genus *Lonicera* L. [2, 5]. Actuality of this question is also connected with the need for Crimean park plantings of assortiment of the plants resistant to winter stress conditions combine ornamental features of flowering and fruiting in those periods of vegetative season when the range of different plants` flowering is limited [3, 5]. Genus *Lonicera* L. includes more than 200 species most of which are decorative [2, 3, 13] and widely used in ornamental plantings and park gardening. Some species are used in medicine and have valuable food qualities [9, 13]. In nature *Lonicera* species are more often in tropical and subtropical regions and only some species grow in the temperate zone [9] and preservation of these plants` viability is needed of all their adaptive mechanisms mobilization.

So we studied potential frost-resistance of some Lonicera species for determination of intraspecies special features of anatomy-morphologycal and physiology-biologycal adaptive mechanisms in their shoots and buds due to the introduction into climatic conditions of the Southern coast of the Crimea.

Material and methods

As the investigation objects we have chosen 9 species and 2 garden varieties from the genus *Lonicera* that grow in the Arboretum of Nikitsky Botanical Gardens: *Lonicera tatarica* L., *L. maackii* (Rupr.) Maxim. (deciduous erect species), *L. pileata* Oliv., *L. pileata* 'Variegata', *L. nitida* Wils., *L. nitida* 'Elegant' (evergreen strait and prostrate), *L. caprifolium* L., *L. etrusca* Santi., *L. henryi* Hemsl., *L. japonica* Thunb. (climbing plants) и *L. fragrantissima* Lindl. et Paxt. (chimochlorous strait plant). Investigations have been carried out in 2012 - 2014. Frost-resistance evaluation was made by method of cutted annual shoots direct freezing under the various temperatures in the climate-control chamber «Gruland» (temperature decrease gradient 2^{o}C/hour) in different wintering periods [11]. This method was used in our modification for adornment shrubs that means optimization of freezing regime and hardening for introduced species. Damages estimation was preceded with slowly melting of the investigated objects (the shoots were kept under the temperature -2…0°C for a day and the same time under 0… +2°C).

Depth and duration of buds biological repose have been obtained in the laboratory conditions with the method by Elmanova, Ahmatova [4]. Stages of intrabud development have been determined by Kouperman [6]. Diagnostics of frost-resistance was also made due

to anatomy-morphological signs of the wood ripening and indexes of carbohydrate cycle. For wood ripening rate determination we used histochemical analyses of annual shoots` tissues with Meule permanganate reaction [8].

Results and discussion

Due to our data in the conditions of the Southern coast of the Crimea frost-resistance of various tissues in *Lonicera* plants is not the same. Lower temperatures, especially after the periods of warming in February damage not only buds but also destructive for vegetative parts of plants. The most sensitive is the apical parts of annual shoots. Shoots` resistance correlates with seasonal changes of the air temperatures so harmful effect of the low temperatures becomes stronger in late winter.

Results of direct freezing in the climate-control chamber under the temperature -12°C in autumn demonstrated some damages in the cells of the vascular system, perimedular zone, sometimes in the pith bands, in February under -20°C damages of cortical parenchyma tissues, not more than 30% of a transvercive stain surface, were noticed. Less damages were observed in the shoots of erect decidouos and evergreen *Lonicera* species compared with climbing ones that could be, possibly, explaned with the rate of wood ripening in shoots. Some authers [11, 14, 15] supposed that winterhardiness in plants and their success in low-temperature stress overcoming depend not only of the winter period but also from their preparation in summer and autumn. One of those processes is shoot lignification that is preceded with wood formation and ripening. Anatomy investigations of annual shoots` tissues were carried out and let to define some features characteristic for erect and climbing *Lonicera* species. Thus, primary cortex consists of epidermis, subepidermal lamellar collenchyme and cortical parenchyma. Epidermis is of one layer, collenchyme depending of the weather conditions and vegetative period has different rate of development and it could consists of 2-3 (*L. tatarica* and *L. maackii* in 2012) up to 4-6 (*L. tatarica* in 2013) cell layers. Cortical parenchyma is geterogenous. It presents as two rows of round or isodiametric cells adjacent to the collenchym. Just near the floem parenchyma cells are some elongated. It`s important to notice that in erect *Lonicera* species round cells of cortical parenchyma form the entire ring of pith rays along the shoot cylinder and in climbing species they are in groups which are connected with the elongated parenchymatous cells. For all studied species presence of calcium oxalate crusts in the bark epidermis cells is typical. Cell walls of the primary cortex in erect *Lonicera* species become wooden and together with bast and sclerenchym fibers could get the functions of mechanical tissue.

L.I. Lotova and A.K. Timohina [7] reported that in *Lonicera* plants secondary ground tissue – periderm is formed deeply in the primary cortex or in the external layers of the centre cylinder. Our researches demonstrate that in some *Lonicera* species in the conditions of the SCC periderm forms deeply in the primary cortex (noticeable in *L. tatarica*, *L. maackii*, *L. pileata* and *L. fragrantissima*) but the rate of its formation differs. In some species felogen just near floem as an etire ring along the whole cylinder (*L. tatarica* and *L. maackii*) but in evergreen and wintergreen erect *Lonicera* this tissue was formed in the basal and middle parts of the shoot from 3-4 floema layers and 1-2 layers of felloderm. In climbing *Lonicera* species, in spite of their decidouos or evergreen state, formation of periderm was poor and in *L. caprifolium* and *L. etrusca* it consists of 1-2 cell layers.

Our investigations demonstrates that in all studied erect *Lonicera* species cells of the secondary cortex are lignificated and in climbing ones the rate of annual shoots` lignifications is significantly lower that could be connected with the growth processes which last through all the period of vegetation. In strait species cambium functions for secondary floem formation (thick bast and sclerenchym fibers) was noticed up to the stage of deep repose (its typical only for *L. tatarica* and *L. maackii*). In *L. henriy* and *L. japonica* wood is poor

differentiated, sclerenchym rings and floem fibers are formed only in the basal parts of annual shoots. In these species stele takes the greates part of the shoot structure.

For successful wintering of trees and shrubs seasonable and full ripening of shoots is very important [7, 10]. Microscope analyses have shown that in annual shoots of *L. tatarica*, *L. maackii*, *L. fragrantissima*, *L. pileata* and *L. nitida* cell walls are lignifyed in the cells of xylem, pith, sclerenchym fibers of floem, isodiametric cells of the bark parenchyma and very seldom in the cells of collenchyme.

On the base of hystochemical analys results we have determined 4 species with high rate of lignifications – these are deciduous and evergreen shrubs: *L. tatarica*, *L. maackii*, *L. fragrantissima* and *L. nitida*. Middle rate of lygnification, when under Meule reaction border between cambium and wood was noticed in 50% of shoots, is characteristic for 3 species - *L. caprifolium*, *L. pileata*, *L. henryi* and two garden forms of the evergreen *Lonicera*. Climbing species *L. etrusca* and *L. japonica* have the lowest rate of lignification.

It is offered to use oligosaccharides` accumulation in the bark of annual shoots as a diagnostic sign [8, 14]. Our researches of seasonal changes in starch amount in the plant tissues have demonstrated that for most of *Lonicera* species two maximums were noticed – in autumn and winter. Storage tissues are xylem rays and perimedulate zone. In floem this polysaccharide is accumulated in 3-4 cell rows at the primary cortex.

In autumn (September – October) starch is more often presented in cortical parenchyma and pith rays and primary hydrolyzed in phloem. In the cold period November – January starch grains were noticed in pith rays, pith and perimedulate zone in *L. fragrantissima*, *L. nitida*, *L. pileata* and *L. caprifolium* only. In the periods of thaws a little amount of starch was noticed in pith rays (February – March). Changes in starch accumulation and resynthesis in *L. maackii* in winter conditions 2012-2013 are presented in fig. 1.

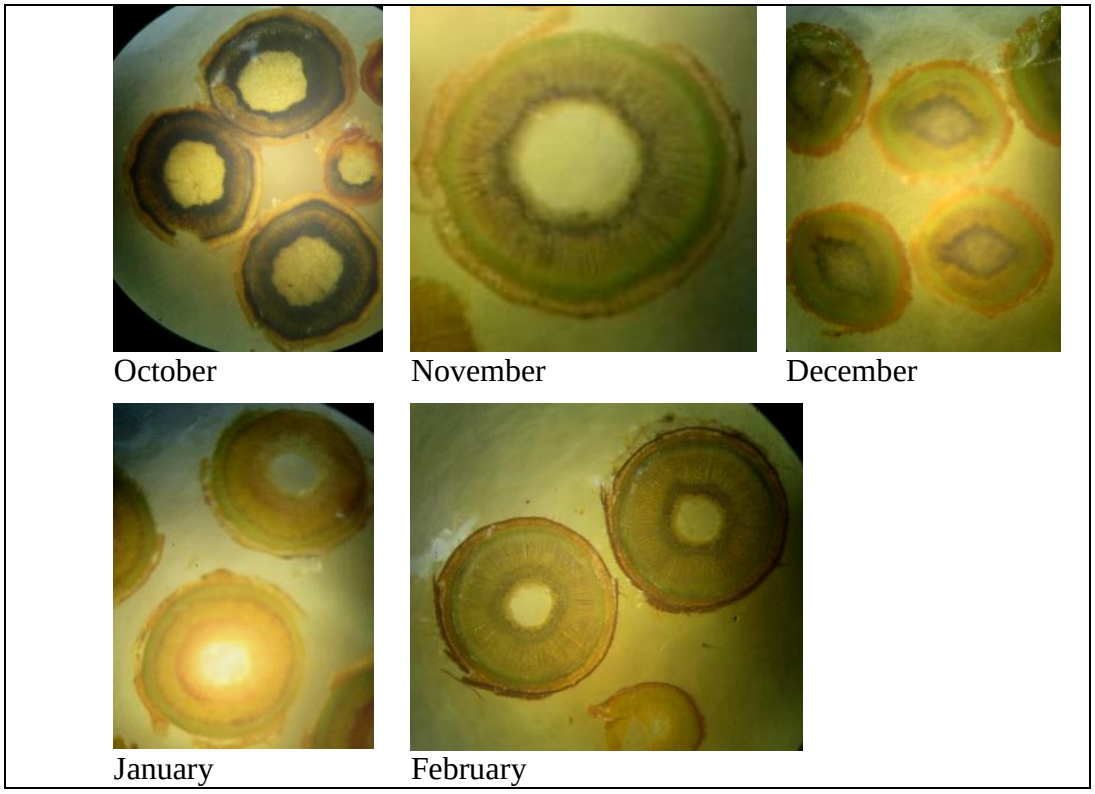

Fig. 1 Starch in *L. maackii* shoot in the autumn – winter period 2012 – 2013.

We have found out that intensive starch hydrolysis started in November when the average day temperature was +11.5…+10.3°C. Probably, in this way carbohydrates in monomeric state could fuction as cryoprotectors for tissues of wintering organs and so to increase their potential frost-resistance.

Deep repose is characteristic only for deciduous erect *Lonicera* shrubs - *L. tatarica* and *L. maackii*. Its initiation suited to the phenological stage of defoliation (second decade of November, 2012 and the third decade of November, 2013). Investigations have demonstrated that deep repose for these species is 68-72 days and it`s followed with forced rest up to the beginning of bud swelling. Duration of forced rest was 20-23 days and strongly depends from the air temperature regime.

In November, 2012 investigations of biological resting depth for *Lonicera* buds have been carried out. Thus the lowest concentration of gibberellic acid (GA) for breaking resting in *L. tatarica* and *L. maackii* was 200 mg/l and after 10-14 days of experiment terminal and low serial buds were fully open proportionally along the shoot.

For climbing and evergreen *Lonicera* species deep resting is not typical. From late October to early November buds of these species are in the forced rest and after they have been placed in favourable growth conditions in most buds active formation processes were noticed. The same phenomenon but more slowly was observed in nature conditions especially during the thaws. For bud swelling and opening keeping shoots in water with GA (50 mg/l) for 5 days is enough. However, in climbing *Lonicera* species all buds were swelling and the first open were buds on the orthogonal side of shoot at its base. In the evergreen species *L. nitida* and *L. pileata* and their garden forms *L. pileata* 'Variegata' and *L. nitida* 'Elegant' terminal buds open first and then the buds on the apical part of the shoot.

The earliest beginning of the resting was noticed for winterflowering species *L. fragrantissima* (late August – September). Bud formation in this species occuers in April – May and it is followed with rapid differentiation of tissues and initial flowers` formation. To September meiosis and microspores formation are finished (it should be noticed that this species has prolonged bud development: in the same plant in September – November buds in various development stages – from the beginning of flower organs formation to sporogenious tissue in microsporangium). It has long blossom in winter (initial blossom is in the second decade of December, full blossom in January – February, the end of blossom – the second decade of April).

Regulated freezing under various temperature regimes gave us possibility to determing temperature parameters for appearance of various types of damages and also to find out the lethal temperatures for each species. It has been found out that for *L. nitida* and *L. pileata* lethal temperature was -12.5°C and for the garden form *L. nitida* 'Elegant' it was -14.0°C. Climbing species and evergreen *L. fragrantissima* demonstrated middle rate of frost-resistance, the lethal temperature for them was -18.0°C. The most frost-resistant species are *L. maackii* and *L. tatarica* (the lethal temperatures for them were -20.0°C and -24.0°C correspondently). In autumn before the first cold in *L. tatarica* and *L. maackii* buds with already formed initial flowers at the stage of flower organs formation had no significant damages under the temperature 10.0°C (frost-resistance of buds was 82.8% – 88.5%) (fig. 2). At this stage tissues at the base of the buds were damaged, probably, because of their high water content. Further (in November) when flower organogenesis processes have been finished and influence of hardening temperatures has been initiated in nature conditions these species demonstrated high frost-resistance (64.1% – 88.1%) under the temperatures -12.0°C…-14.0°C. As the period of deep rest began, frost-resistance increased and its maximum was in December. At that time buds of *L. tatarica* and *L. maackii* stayed alive under the temperatures -18.0°C – -20.0°C; the most often damages were noticed in

generative sphere tissues, apex and leaf primordium (not more than 20 – 30% of the bud stain surface).

During the period of forced rest (late January – February) when it occured intensive growth of flower elements, sporogenous tissue formation, meiosis and microspores development cryoresistence significally decreased. Bud death under controlled freezing with the temperature -20.0°C was 52.8% – 56.9% at this developmentale stage. According to our observations in February – March phenologycal phase of bud swelling and the first leaves opening began. At this stage under the temperature -12.0°C influenced for 48 hours 63.2% – 66.2% of buds were alive and the most often damages were noticed for buds of the basal part (for *Lonicera* species prolonged development is typical and buds of the basal part are more progressive in their growth and development) and young open leaves. At the same time damages of generative sphere, that is usually more sensitive to the frosts, haven`t been noticed. It is shown in fig. 2 that *L. tatarica* demonstrated higher rate of frost-resistance at all stages of the intrabud development compared with *L. maackii*.

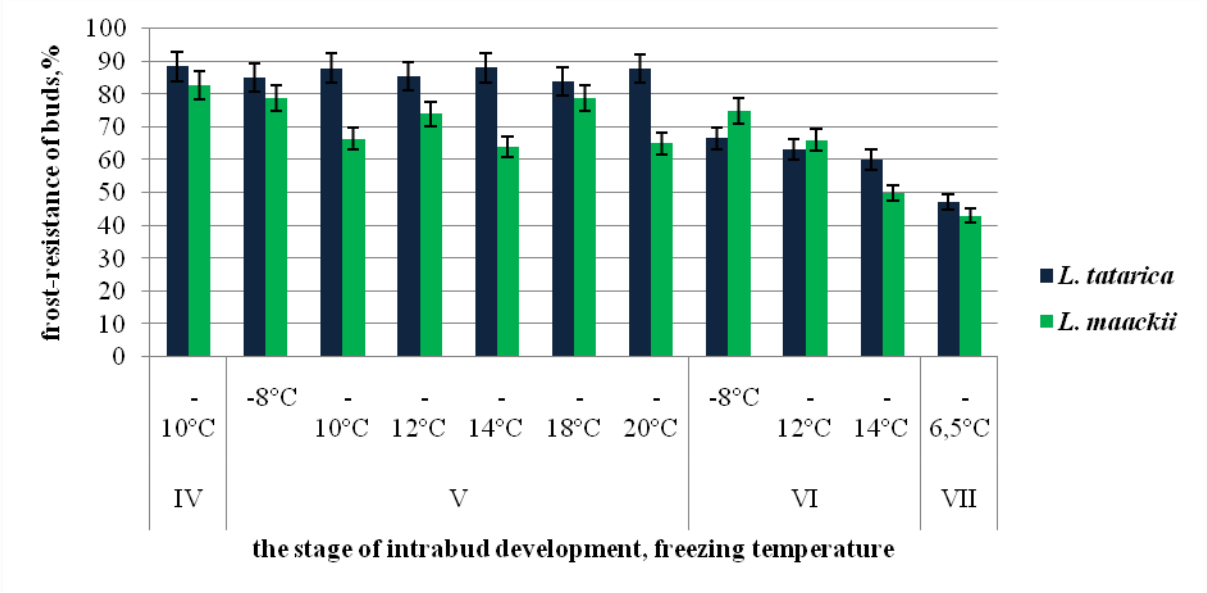

Fig. 2 Changes in bud frost-resistance in erect deciduous *Lonicera* species in the dependence of their development stage: IV – formation of initial flowers, beginning of flower organs formation; V – gradual formation of flower organs, sporogenous tissue formation in microsporangium; VI – intensive growth of flower elements, meiosis and microspores formation in microsporangium; VII – pollen.

Among the evergreen erect and spreading species *L. nitida*, *L. pileata*, their garden forms – *L. nitida* 'Elegant' and – *L. pileata* 'Variegata' were studied. In these species frost-resistance decreased along the row pith – cortical parenhyme of shoots – buds – leaves. Under the freezing with the temperature -7,0°C… -10,0°C typical damages of the leaf tissues were necrotic spots, that appeared mostly on the low (abaxial) side of the leaf blade in the among veins space. Under the temperature -12,5°C…-14,0°C for 12 hours all leaves died in *L. pileata* 'Variegata' and in *L. pileata* base of the leaf blades were damaged, necrosis were more than 40% of the leaf surface. In *L. nitida* and *L. nitida* 'Elegant' under this temperature only point necrosis of among rip parenchyma and rips was noticed.

The most sensitive to the low temperature stress during the cold period were leaves in *L. pileata* 'Variegata' that partly lost their decorative features: necrotic spots were on the chlorophyll free parts of the leaf. In other evergreen species in nature wintering conditions leaf damages were not more than 10% (point necrosis) without loose of turgescence and decorative features and ability to renew after frost influence. In late December – January leaf

frost-resistance is higher than in autumn that is probably connected with osmotic water part increasing. Presence of the snow cover on shrubs during some days led to the appearance of chlorotic spots but in some period of times leaves were able to the reparation.

Initial damage temperature for the buds of the evergreen *Lonicera* species was -8.5°C. To the begining of the cold period generative shoot and flower organs have been already formed in buds and they could endure the frost -10.0°C but under the temperature -12.0°C only 20% of buds were undamaged and for *L. pileata* 'Variegata' this temperature at the stage of flower organogenesis was critical. Typical damages were – tissue necrosis at the base of terminal buds and generative structures. In early January intencive growth of flower parts and microspores formation occurred. In this period increasing of bud frost-resistance in the experiment conditions was noticed – under the temperature 12.0°C frost-resistance was 29.7 – 44.3%. *L. nitida* 'Elegant' was resistant to the temperature -13,5°C its buds` frost-resistance was 48.1%. Lethal temperature for above described evergreen erect and spread *Lonicera* was -14.0°C.

During the whole winter period evergreen *Lonicera* species were at the forced rest but during the long thaws their rest was broken and growth processes began. In 2012-2014 phenophase of bud swelling and primary leaves opening was noticed in January – February (due to warm winter and long period without frosts), in the generative sphere mature pollen grains have been formed and young shoots started their growth. At that time buds of the evergreen *Lonicera* species died under the temperature -6.5°C: shoot pith, vascular system and base of the buds, leaf primordiums and flowers were damaged. Among the studied species the most resistant to the low temperature stress were garden forms of *L. nitida* 'Elegant' and *L. pileata* (tabl. 1).

Table 1

Frost-resistance of buds in the evergreen *Lonicera* species during the wintering period

Phase of intrabud development	Flower organs initiation: sporogenous tissue formation in the anthers		Flower elements intensive growth, meiosis and microspores` formation in the anthers		Mature pollen grains
Species, forms / Temperature of experimental freezing	-10°C	-12°C	-10°C	-12°C	-6.5°C
L. nitida	83.6±5.3	14.4±1.2	68.4±4.4	29.7±2.3	31.5±2.6
L. nitida 'Elegant'	84.7±4.9	20.8±0.8	81.8±3.6	44,3±3.1	15.6±1.2
L. pileata '	71.0±3.1	15.3±1.9	72.2±4.1	39,4±2.0	10.7±0.9
L. pileata 'Variegata'	86.5±9.4	0,0	93.6±5.1	28,4±1.3	12.6±1.5

Probability of the year minimums ≤ −7°C in the SCC is 69% these frosts have been noticed almost in 7 years of 10 [12]. Thus in our conditions *L. pileata* 'Variegata' leaves are always damaged partly. Frosts of -12°C are seldom, their probability 26% and under this temperature wintering aboveground organs in all studied species could be damaged. It has been found that absolute air temperature minimum -14,6°C is lethal for all studied species in the introduction conditions but as it`s known from the literature sourses [3, 13] for these species renewal from the undamaged root and axial stems bud is possible.

Particular attention should be payed to the wintergreen and winterflowering species *L. fragrantissima*. As other *Lonicera* species this one also has prolonged intrabud development. It has fully formed flower couples in most of buds in September. Phase of rest is not long. In winter conditions 2011-2012 (minimal temperature was up to -11.9°C, the air temperatures -9,4…11,9°C noticed for more than 12 hours and temperature decrease was accompanied with stomy wind 21-24 m/s) *L. fragrantissima* fell all its leaves and only in the microclimate conditions of the Low Park in NBG Arboretum its plants kept about 40% of foliage. Winter

periods in 2012-2013 and 2013-2014 were warmer and *L. fragrantissima* was foliaceous. In our experiments we have determined the low temperatures influence on leaf decorative appearance in this species: under -12.0°C leaf damages weren`t noticed, temperature -14.0°C could give the damages of leaf parenchyma along the rips on abaxial side of the leaf blade; under the temperature -20.0°C for 12 hours more than 70% of the leaf surface along the edges were damaged. Young leaves appear after mass blossom and they are very sensitive to frosts.

Bud frost-resistance in *L. fragrantissima* under the temperature -8.0°C, in October, was 98.2%. In late November – December in the most of buds intencive growth of flower elements and microspores formation were noticed. Under the temperature -14.0°C bud frost-resistance was 80.6% and under the temperature -20.0°C only 30.9% of buds was alive. At the period of pollen grains formation and flowering cryoresistance decreased significantly. The temperature -15.0°C is lethal for *L. fragrantissima* buds at this developmental stage. Typical damages were necrosis of vascular bridge in buds, generative sphere and leaf primordiums. Bark cracking between the neighbor buds also increased. Flower damages in nature conditions were noticed under the temperature -2.5^{o}C. The most damaged structure was style and the most resistant – anthers. It was found out that flowers died under the temperature -3.5^{o}C, flower buds were alive under the temperature -4.0 … -4.5^{o}C and died at -5,0^{o}C.

Climbing *Lonicera* species are strongly decorative [2, 9]. We studied low temperature resistance for two deciduous species - *L. caprifolium* and *L. etrusca*, one wintergreen species - *L. henryi* and perennial one - *L. japonica*. As it has been noticed above they differ from erect *Lonicera* species with their long shoot growth, unripened part of which died under the winter conditions in 2011-2012. In *L. japonica* winter drying and cracking of bark were also noticed. That led to the death of terminal parts in some shoots. In the experiment under the temperature −10.0°C…-12.0°C damages in the cortical parenchyma tissues were noticed.

In *L. japonica* and *L. henryi* under the influence of the low temperature stress leaves lost their turgor and curved (-8.0°C); necrotic sports appeared on their surfaces (-10.0°C) and they died at -14.0°C. In wintering conditions 2011-2012 leaves of these species were damaged and more tha 50% fell down. Chlorosis and necrosis of apical leaf part and among rip parenchyma were often noticed.

Buds in climbing Lonicera species are undifferentiated for a long time – vegetative and further generative. At this stage of the intrsbud development the most frost-resistant were *L. henryi* and *L. etrusca*. Temperature -20.0°C in late December – January was not lethal for *L. caprifolium* (bud frost-resistance 65.7%), *L. etrusca* (36.0%) and *L. henryi* (33.9%) (tabl. 2).

At the time of bud swelling differentiation of the generative shoot main axile, flattening and separation of the shoot apex occurred. At that period low temperature resistance decreased significantly due to high water content in the bud tissues (57.7% – 72.0%, 1g of dry substances is able to keep 1.36 – 2.56 g of water). In the experiment under the temperature -12.0°C for 12 hours the best frost-resistance was noticed for *L. henryi* and *L. etrusca* (73.4% and 65.7% correspondantly); the temperature -18.0°C brought damages for more than 60% of buds; -20,0°C was lethal for all climbing *Lonicera* species at this stage of their intrabud development (tabl. 2). Base and apex of buds were mostly damaged. When primary leaves opened, shoots grew rapidly and initial flowers formed frost-resistance increased. It could be connected with high photosynthetic activity of young leaves that is favourable to plastic substances accumulation, increase of cell sap consentration and ability to hold water (1g of dry substances in open buds is able to hold 2.13 – 3.42 g of water).

Table 2

Changes of potential frost-resistance in climbing *Lonicera* species depending on their intrabud developmental phase

Phase of intrabud development	t	Deciduous		Wintergreen	Perennial
		L. caprifolium	L. etrusca	L. henryi	L. japonica
Vegetative or further generative buds	−8.0°C	90.4±5.8	94.4±2.1	86.7±5.9	89.3±5.0
	−12.0°C	48.9±3.3	87.9±5.0	77.5±4.1	63.9±3.4
	−14.0°C	34.2±2.1	75.3±4.8	71.8±4.4	65.9±4.2
	−20.0°C	15.7±1.4	36.0±2.1	33.9±2.8	0.0
differentiation of the main axile, flattening and isolation of the shoot apex	−10.0°C	89.2±5.8	66.7±4.8	81.3±5.0	68.8±3.9
	−18.0°C	42.3±2.8	43.5±2.7	32.1±2.1	38.7±2.3
	−20.0°C	6.8±0.7	1.8±0.6	3.0±0,5	0.0
Initial flowers formation	−6.5°C	88.2±5.2	96.4±2.1	91.9±4.2	85.6±5.9
	−12.0°C	49.2±3.0	72.4±4.2	90.7±5.8	89.5±5.5
Flower organogenesis	−12.0°C	86.4±5.9	70.6±5.3	*	85.7±4.2

* – hasn`t been determined

As the result of these processes climbing *Lonicera* species had no significant damages under the temperature -12.0°C (frost-resistance 49.2%-90.7%). Ability to resist hypotermal stress was kept during flower organogenesis in *L. caprifolium*, *L. etrusca* and *L. japonica* in late March. The most sensitive was vegetative sphere – apex, leaf primordiums and young leaves. Under the temperature -6.5°C lack of turgor was noticed but after 5 days they fully renewed.

Many authers supposed that death of plants in cold season often occurred not due to unfavourable environmental conditions but because of plants haven`t been resistant enough [14, 15]. To protect themselves from harmfull winter effects plants should change their growth condition, when they have low resistance, to the state of hardiness and to get high cryoresistance. Thus presence unrippened wintering organs (shoots and buds) with high water content in climbing Lonicera species explane their low frost-resistance compaired with erect dicidous species.

Conclusions

Comparison of annual shoots potential frost-resistance in different *Lonicera* species introduced on the Southern Coast of the Crimea with the changes of minimum air temperature in autumn-winter-spring period let us to define the most deserving species for the exhibitions in the parks of SCC and also to estimate probability of their damages with spring frosts. The first group of species with minimum resistance to the low temperatures includes *L. nitida*, *L. pileata* and *L. fragrantissima*. Absolute minimum air temperature for the SCC in winter-spring period is the main danger for their successful wintering and further vegetation. The second group includes climbing species that possibly lack their decorative features under the enfluence of the absolute minimum air temperature in February. *L. etrusca* and *L. japonica* are especially sensitive during this period and *L. caprifolium* – at the beginning of wintering. Annual shoots have poor tissue differentiation: fellogen forms below pericycle (it doesn`t separate a part of floem with hard bast). *L. tatarica* and *L. maackii* are the most frost-rsistant as they have well developed cover tissues in their annual shoots. Fellogen forms from parenchymous cell of secondary floem with some bands of hard bast increasing the cover layer. We have found out that deciduous species are resistant to the temperatures up to -20.0°C at the beginning of winter period and have high cryoresistance till late January. The

most endanged stage of their intrabud development is microspores formation after the rest was broken. The results of our investigations give more information about adaptation mechanisms formation in *Lonicera* species and could be used as a base for optimization of exotic plants growth under the influence of exogene stress factors in new climatic conditions.

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Data about anatomy-morphological and physiology-biochemical characteristics of some species Lonicera genus have been presented and some features of their growth under conditions of the South Coast of the Crimea have been discussed. Structure-functional rebuildings in the annual shoots have been suggested as criteria for assessment of Lonicer species winter hardiness, terms of their biological repose and temperatures of initial and lethal damages have been determined. Reliable markers for ornamental Lonicera species winter hardiness prognosis have been suggested on the base of complex physiological analyses of some adaptive reactions in their overground wintering organs under the low temperature stress.

Key words: *Lonicera, potential winter hardiness, biological repose, structure-functional rebuildings, intrabud development, carbohydrate exchange.*

UDK 632.4

AN ANNOTATED LIST OF FUNGI ON TREES AND SHRUBS ON THE BLACK SEA COAST OF THE CAUCASUS

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Introduction

In 117 species of trees and shrubs in 9 localities of the Black Sea coast it was found 98 fungi species of three classes: Deuteromycetes - 37 fungi species on 42 plant species, Ascomycetes - 26 fungi species on 42 plant species, Basidiomycetes - 35 fungi species on 92 plant species. Dominated fungi are those causing powdery mildew - 17 fungi species, necrotic diseases of shoots - 35, root rot - 10 species. Most common species are fungi of the genera Oidium – on 19 plant species, Cytospora – on 4 species, Diaporthe – on 5 species, Phyllactinia – on 3 species, Ganoderma – on 14 species, Stereum – on 9 plant species.

Materials and Methods

Studies of fungi species composition on trees and shrubs were carried out in the 9 settlements on the Black Sea coast of the Caucasus: Anapa, Adler, Sochi, Novy Afon, Sukhumi, Zugdidi, Ochamchire, Pitsunda, Batumi. The studies were made in botanical gardens, city plantings, ornamental nurseries, arboretums, natural stands. It has been totally studied mycoflora of 117 species of introduced and native trees from 81 genera. Dominated families were Fabaceae - 8, Rosaceae - 9, Oleaceae - 4, Pinaceae - 4 genera. According to the number of species the genera Pinus - 8, Quercus - 7, Salix - 4 dominated. Mycological investigations were carried out in autumn. They included the expedition route surveys of plantings, which takes into account the prevalence of the disease on number of hosted plants in the planting and intensity of fungus development on a 5-point scale: 1 point - single fruit bodies on the plant organ; 2 - low intensity, fungus revealed on not more than 25% of leaves and/or shoots; 3 - middle intensity, fungus affected 50% of leaves and/or shoots; 4 – strong intensity, about 75% of organs are affected; 5 - fungus found throughout the tree in its respective ecological niches.

Results and discussion

According to its environmental conditions, many areas of the Black Sea coast of the Caucasus are very similar to the Southern Coast of the Crimea, so these areas are considered potential sources of new exotic species introduction to the Crimea. However, together with the introduction of woody plants symbiotrophic related fungi, including pathogens, that could be of a great danger to the life of plants in new environment, are brought. Therefore, knowledges of plant pathogenic fungi species composition has scientific and practical value for successful introduction. Mycoflora of potentially new to the Crimea exotic plants on the Black Sea Coast of Caucasus has not been studied. There are some literature information about bracket fungi of Caucasus [1, 2, 9], fungi of the genus *Cytospora* [4], diseases of laurel [8] and subtropical plants [3, 5], mycobiota of some introduced and native species in the adjacent regions [6, 7, 10, 11]. With this regards, studies of mycobiota in potentially new for Crimea species was carried out in their natural and culture habitats.

Class DEUTEROMYCETES
Order Moniliales, family Moniliaceae

Oidium sp. - powdery mildew fungi, the development of which was fixed in the conidial stage. It was found on the leaves of *Hydrangea arborescens* L., rarely noticed, only on single plants, the intensity of fungus development is middle (Adler).

Oidium sp. - found on the certain *Eucalyptus* species, only the apical leaves on the stool shoots are affected, it occures sporadically (Adler).

Oidium sp. - noticed on the coppice shoots` leaves on *Laurocerasus officinalis* Roem., intensity of fungus development is low (Adler).

Oidium sp. - 100% coppice shoots` leaves on *Quercus ilex* L. were damaged, intensity of fungus development is strong, it`s often found in shaded areas (Sukhumi).

Oidium sp. – rarely noticed on *Mahonia fortune* (Lindl.) Fedde, powdery coating is formed on the bottom side of leaves, in some plants up to 30% of the leaves were affected (Sukhumi).

Oidium sp. - 100% leaf damages was noticed on *Carpinus betulus* L., fungus is found on different age plants, intensity of fungus development is strong, especially in habitats with high humidity (Sukhumi).

Oidium sp. - fungus found on the leaves of coppice shoots on *Robinia pseudoacacia* L., rare, intensity of fungus development is low (Sukhumi).

Oidium sp. - fungus found on the leaves of the young summer shoots on *Platanus orientalis* L., rare (Sochi).

Oidium sp. - fungus is widespread on *Lagerstroemia indica* L., occurs on the leaves, flowers, fruits and young shoots. Prevalence of the fungus is up to 100% through all the areas of the host plant growth, intensity of fungus development is high (Sochi).

Pseudoidium tuckeri (Berk.) Paul et Kap. - fungus found on the leaves *Euonymus japonica* Thunb., it`s common in all the areas of this plant growth, intensity of fungus development is usually low, but in shaded places it`s up to the middle level (Sochi).

Order **Moniliales**, family **Dematiaceae**

Stigmina platani (Fuck.) Sacc. - fungus causes brown spots on leaves *Platanus acerifolia* (Ait.) Willd., damages are up to 70% of leaves` surface, rare, intensity of fungus development is high (Adler).

Order **Moniliales**, family **Tuberculariaceae**

Tubercularia vulgaris Tode - found on dead annual shoots *Kerria japonica* (L.) DC., noticed on single plants, intensity of fungus development is middle (Sukhumi). The species was also noticed on died old trunks and skeletal branches *Pittosporum eugenioides* A.Cunn. Found on single plants, intensity of fungus development is high (Adler).

Order **Melanconiales**, family **Melanconiaceae**

Gloeosporium platani (Lev.) Oudem. - specialized pathogenic fungus, causing wilting and premature shedding of leaves on different *Platanus* L. species. On *Platanus acerifolia* (Ait.) Willd. intensity of fungus development is low (Sochi); on *Platanus occidentalis* L. fungus affects up to 50% of leaves but it has low developmental intensity (Adler); on *Platanus orientalis* L. fungus was found on the coppice shoots and intensity of fungus development is middle (Sochi).

Pestalotia funerea Desm. - pathogenic fungi identified on *Pinus* sp., strongly affects needles on current-year shoots. Affected needles become light, gray and fall prematurely (Ochamchira).

Pestalotia quepini Desm. - fungus causes brown spots on leaves, found on *Acca selloviana* (Berg.) Burr., rare (Sukhumi). Much more frequently was noticed on *Pterostyrax hispida* Sieb. et Zucc., at the first stage of development causes strong (up to 25% of the leaf surface) brown, then light spotting, more common on the autumn leaves (Ochamchira).

Coryneum depressum Kze et Schum. - phytopathogenic necrotrophic fungus found on different age *Quercus suber* L. trees, affects up to 15% of annual shoots, intensity of fungus development is high (Sochi).

Order **Sphaeropsidales**, family **Sphaeropsidaceae**

Phyllosticta mahoniae Sacc. et Speg. - fungus causes brown spots on leaves *Mahonia bealei* (Fortune) Carr., rare, intensity of fungus development is low (Batumi).

Phyllosticta arbuti-unedonis Pass. - found on *Arbutus unedo* L. leaves, on the single trees 100% of leaf damages was noticed, intensity of fungus development is middle (Adler).

Cytospora leucosperma Fr. - one of the most common necrotrophic fungi on woody plants, found on *Platanus acerifolia* (Ait.) Willd., affects IV-I order shoots, occurs on every plant of this species, intensity of fungus development is high (Adler).

Cytospora leucostoma Sacc. - necrotrophic micromycetes identified on *Chaenomeles japonica* (Thunb.) Lindl., damages 10-year-old shoots, intensity of fungus development is low (Sochi).

Cytospora sacculus (Schw.) Gvrit. - found on IV-III order shoots *Castanea sativa* Mill., noticed on every plant of the species, intensity of fungus development is middle (Batumi). This species is wide-spread on *Punica granatum* L., causes necrosis of 1-2-year-old shoots, intensity of fungus development is high (Sochi).

Cytospora chrysosperma Fr. - necrotrophic fungus is wide-spread on many *Salix* L. species. It was revealed on *Salix alba* L. weakened plants, damages annual shoots, intensity of fungus development is low (Anapa).

Phoma berberidis Sacc. - specialized phytopathogenic fungus that causes shoot necrosis on *Berberis thunbergii* DC. resulting in annual shoots death along the tree. It`s spreaded on plants of this species, intensity of fungus development is low (Adler).

Phomopsis laschi v. Hohn. - found on *Euonymus wilsonii* Sprague, causes necrosis of I-II order shoots, intensity of fungus development is middle (Batumi).

Phomopsis dearnesiana (Sacc.) Arx. - noticed on the flowering shoots *Zanthoxylum piperitum* DC., affects I order shoots, intensity of fungus development is high (Batumi).

Phomopsis magnoliicola f. macrosporophora Dias. ex Camar - noticed on *Magnolia delavayi* Franch., causes necrosis of 2-3-year-old shoots, rare (Batumi).

Cytosporina stellulata Sacc. - necrotrophic fungus found on annual shoots *Berberis thunbergii* DC., intensity of fungus development is low (Adler).

Coniothyrium concentricum (Desm.) Sacc. - fungus causes ringed spots on *Jucca* sp. stems, damages all plants of this species, intensity of fungus development is low (Adler). This species is also noticed on all the specimens of *Hedera helix* L. leaves of which are affected up to 70% (Sukhumi).

Fusicoccum sp. - fungus found on 3-5-year-old shoots *Broussonetia papyrifera* (L.) L. Her., has low spreading in tree crown, intensity of fungus development is middle (Sochi).

Sphaeropsis ellisii Sacc. - specialized necrotrophic fungus with epiphytotic type of development. Causes mass shrinkage of *Pinus pytiusa* Stev. young trees, occurs in all areas of the host species growth. Intensity of fungus development is high (Pitsunda).

Camarosporium laburni Sacc. et Roum. - specialized fungus on *Laburnum anagyroides* Medic., fruit bodies are found abundantly on the 1-3-year-old shoots, available on all the speciements (Sukhumi).

Paradiplodia ribis Zer. – fungus found on dead biennial shoots of *Hedera colchica* K. Koch, rare, intensity of fungus development is middle (Sochi).

Diplodia rutaecola Thum. - necrotrophic fungus found on III order shoots *Phellodendron amurense* Rupr., intensity of fungus development is high (Batumi).

Diplodia sp. - fungus found on III-IV order shoots in *Paulownia tomentosa* Steud., has low intensity of development (Batumi).

Diplodia buxi Fr. - specialized necrotrophic fungus found on *Buxus sempervirens* L., causes death of annual shoots. It occurs in all individuals of this plant species, intensity of fungus development is middle. Ascigerous stage of this fungus Otthia sp. was noticed on the single plants (Adler).

Order **Sphaeropsidales**, family **Leptostromataceae**

Malasmia acerinum Lev. - pathogenic fungus causes black spots on leaves *Acer platanoides* L. The prevalence of the fungus is low (5%), found in damp places, mainly in the spherical form of this species (Sochi).

Order **Excipulales**, family **Excipulaceae**

Dinemasporium decipiens Sacc. – fungus found on all dead shoots *Collecia cruciata* Gill. et Hook., intensity of fungus development is high (Sukhumi).

Class **ASCOMYCETES**

Order **Erysiphales**, family **Erysiphaceae**

Microsphaera alphitoides Griff. et Maubl. - powdery mildew fungus in teleomorphic stage. It was found on the leaves *Quercus iberica* Stev., strongly affects up to 100% of leaves (Zugdidi). Fungus is typical for this plant species in other habitats also, noticed in 150-year-old trees in the lower part of the crown (Adler). On *Quercus castaneifolia* C.A.M. mildew was noticed on the coppice shoots (Adler), and on *Quercus petraea* Liebl. it was found on young trees and strongly – on coppice shoots (Sochi). Intensity of fungus development in all cases is high.

Microsphaera sp. - powdery mildew found in dense plantings and shaded areas on *Viburnum tinus* L., fungus affects up to 80% of leaves, intensity of its development is high (Sochi).

Phyllactinia fraxini (DC.) Fuss - specialized powdery mildew fungus noticed on *Fraxinus excelsior* L., available in all of the speciements of this species, intensity of fungus development is high. It also occurs in the decorative forms of this species - *Fraxinus excelsior "monofila-pendula*" and affects 100% of leaves (Sochi).

Phyllactinia roboris (Gachet) Blum. - powdery mildew fungus found on all individuals of *Fagus orientalis* Lipsky through the area of plants` growth, prevalence in the tree is up to 50%, intensity of fungus development is high (Zugdidi).

Phyllactinia guttata (Wallr.: Fr.) Lev. - one of the most common powdery mildew fungi on *Corylus avellana* L., occurs throughout the area, prevalence along a tree in the shaded areas is 70%, intensity of fungus development is high (Sochi).

Uncinula adunca (Wallr.: Fr.) Lev. - powdery mildew fungus found on *Salix caprea* L., affects 100% of leaves, intensive development occurs in damp places (Sochi).

Sphaerotheca pannosa (Wallr.: Fr.) Lev. - fungus causes powdery mildew on *Rosa canina* L., common, intensity of fungus development is high (Adler).

Order **Helotiales**, family **Dermateaceae**

Cenangium abietis (Pers.) Rehm. - necrotrophic fungus with epiphytotic development. Causes the death of *Pinus sylvestris* L. young plants (up to 15 years age), the prevalence along a tree is 100%, intensity of fungus development is high (Sochi).

Order **Hypocreales**, family **Nectriaceae**

Nectria magnusiana Rehm - obligate saprotroph, found on the dead skeletal branches in *Pteroceltis tatarinovi* Maxim, development of fungus takes place after the destruction of fungi pathogenic species fruit bodies (Batumi). It was also found in *Magnolia grandiflora* L., its development is similar (Zugdidi).

Nectria cucurbitula (Tode) Fr. - obligate saprotroph, found on dead shoots in *Pinus pytiusa* Stev., common, intensity of fungus development is middle (Pitsunda).

Order **Hysteriales**, family **Hysteriaceae**

Hysterographium fraxini (Pers.) De Not. - plant pathogenic necrotrophic fungus, specialized in plants of Oleaceae family, more rarely - in Bignoniaceae. In particular, this fungus was found on *Catalpa steciosa* Ward where it affects skeletal branches; intensity of fungus development is very high. The fungus was also found on *Ligustrum chinensis* Koehne in all the areas of its growth, affects shoots 2-5 mm in diameter, intensity of fungus development is high (Batumi). Has low prevalence on *Osmanthus fragrans* Lour. wintering shoots of 3-years age (Sochi, Batumi). Fungus is mostly widespread on *Olea europaea* L., occurs in all the growth areas. In plantings it affects up to 100% of the trees and up to 30% of shoots in the crown, intensity of fungus development is high (New Athos).

Hysterographium biforme (Fr.) Rehm. - saprotroph, identified on trunk bear wood on *Hibiscus mutabilis* L., *Xylosoma racemosa* Miq., *Pittosporum tobira* Ait., *Cornus iberica* G.Woron. and skeletal branches *Ulmus parvifolia* Jacq. Rare, intensity of fungus development is middle (Batumi).

Hysterium pulicare Pers. - saprotroph, revealed on the annual shoots on *Genista hispanica* L., common, intensity of fungus development is high (Sochi).

Order **Diaporthales**, family **Diaporthaceae**

Cryptodiaporthe hranicensis (Petr.) Wehm. - phytopathogenic necrotrophic fungus found on annual coppice shoots *Ligustrum lucidum* Ait., rare, intensity of fungus development is high (Sukhumi).

Cryptodiaporthe pyrrocystis (Berk. et Br.) Wehm. - phytopathogenic necrotrophic fungus found on *Osmanthus fortune* Carr., spreaded on young annual shoots, rare (Adler).

Cryptosporella aurea Sacc. - necrotrophic fungus is widespread on the 8-10-year shoots *Sophora japonica* L., occurs in all individuals of the species, prevalence along a tree is up to 25%, intensity of fungus development is very high (Sochi).

Diaporthe leiphaemia (Fr.) Sacc. - necrotrophic fungus found on annual coppice shoots *Quercus myrsinaefolia* Blume. Anamorph of this species is fungi from genus *Phomopsis*. It occurs in all growing areas of the host plant, intensity of fungus development is high (Ochamchira).

Diaporthe eres Nits. - necrotrophic fungus found on all the annual shoots *Staphylea colchica* Stev. Ubiquitous, everywhere has a high developmental intensity (Pitsunda). This species has also been found on IV order shoots *Magnolia delavayi* Franch, intensity of fungus development is middle. Simultaneously with this fungus its anamorph - *Phomopsis magnoliicola f. macrosporophora* was noticed (Adler). Significantly spread on *Magnolia grandiflora* L., occurs on skeletal branches (diameter 16 mm), intensity of fungus development is high (Zugdidi).

Diaporthe medusae Nits. - necrotrophic fungus found on shoots with a diameter of 4-7 mm on *Vitis vinifera* L., presents on every plant of the species, intensity of fungus development is high (Batumi). It was also noticed on *Desmodium tiliaefolium* (D.Don) G.Don shoots, where intensity of fungus development is middle (Sukhumi).

Diaporthe oncostoma (Duby) Fuck. - necrotrophic fungus found on shoots with a diameter of 8-10 mm on *Robinia pseudoacacia* L., is widespread, intensity of fungus development is very high. Its development occurs only in teleomorph stage (Pitsunda).

Order **Diaporthales**, family **Valsaceae**

Valsa ceratosperma (Tode: Fr) Maire – teleomorphic stage of necrotrophic fungus from *Cytospora* genus. This fungus was found on I order shoots *Pretoceltis tatarinovi* Maxim, intensity of fungus development is very high (Batumi).

Order **Diatrypales**, family **Diatrypaceae**

Diatrypella verruciformis (Ehrh.) Nits. - necrotrophic fungus, its anamorpha is fungi from genus *Phoma*. It was found on skeletal branches in *Hibiscus mutabilis* L., intensity of fungus development is middle. This species was also noticed on skeletal branches with a diameter of 15 mm on *Phellodendron amurense* Rupr., intensity of fungus development is high (Batumi).

Order **Rhytismatales**, family **Phacidiaceae**

Rhytisma acerinum (Pers.) Fr. – phytopathogenic fungus causes black spots on leaves *Acer platanoides* L. Occurs in conjunction with the anamorph *Malasmia acerinum*, fungus prevalence in plantings is 100%, intensity of fungus development is middle (Sochi).

Order **Xylariales**, family **Xilariaceae**

Daldinia concentrica (Bolt.) Ces. et de Not. - xylotrophic micromycete found on bare wood on *Machilus thunbergii* Sieb. et Zucc., rare. It was also noticed on skeletal branches *Phellodendron amurense* Rupr., intensity of fungus development is very high (Batumi). Besides, the fungus occurs on II-III order shoots *Desmodium tiliaefolium* (D.Don) G.Don, where it has a strong degree development (Sukhumi).

Hypoxylon fuscum Fr. - xylotrophic micromycetes identified on thick skeletal branches *Photinia serrulata* Lindl., rare (Sochi).

Class **BASIDIOMYCETES**

Order **Uredinales**, family **Pucciniaceae**

Phragmidium tuberculatum Mull. - rust fungus, found on different varieties of *Rosa canina* L., affects leaves, found everywhere, intensity of fungus development is high (Adler).

Tranzschelia pruni-spinosae (Pers.) Diet. - rust fungus found on *Prunus divaricata* Ldb., in all places of the host plant growth it has a very poor developmental level. In areas with high humidity affects 100% of leaves, intensity of fungus development is high (Sochi).

Order **Aphyllophorales**, family **Popyporaceae**

Abortiporus biennis (Bull. Ex Fr.) Sing. - bracket fungus causing root rot. Found on *Trachycarpus fortune* (Hook.) H.Wendl. stumps in wet conditions, rare (Adler). This fungus has also been found on the stumps of *Padus racemosa* (Lam.) Gilib., single finding (Sukhumi).

Antrodia albida (Fr.) Donk. - xylotrophic macromycetes found on the dieing trunks *Laurocerasus officinalis* Roem., rare (Adler).

Bjercandera fumosa (Pers. Ex Fr.) Karst. – xylotrophic macromycetes noticed on the broken trunks *Chamaecyparis lawsoniana* (Murr.) Parl., rare (Adler).

Coriolus versicolor (L. ex Fr.) Quel. - one of the most common xylotrophic macromycetes in some deciduous plants and conifers. Found on trunks and drying skeletal branches *Laurus nobilis* L., in the points of mechanical damage on *Pittosporum tobira* Ait. trunks (Sukhumi), on stumps and bare wood on *Castanea sativa* Mill., masse on *Sapindus mucorossii* Gaerth. stumps, everywhere on the felled trunks *Fagus orientalis* Lipsky, rarely on *Cryptomeria japonica* D.Don. (Batumi) stumps, often on large deadfallen wood on *Carpinus betulus* L. (Adler).

Daedalea guercina L. ex Fr. – specialized to *Quercus* genus plants, xylotrophic macromycetes found on stumps of old trees, the species of which could not be established, rare (Sochi).

Fomitopsis cytisina (Berk) Bond. et Sing. - xylotrophic macromycetes causes root rot fruit bodies appear at the base of dead old trees *Populus pyramidalis* Roz. and *Platanus orientalis* L. (Zugdidi), at the base of alive trunks with a diameter of 60 cm on *Sapindus drummondii* Hook. et Arn. (Sukhumi) and it has been also found on trunks diameter of 130 cm *Platanus acerifolia* (Ait.) Willd. (New Athos).

Fomitopsis ulmarius (Sow. Ex Fr.) Bond. et Sing. - xylotrophic macromycetes causes stem rot in dead trees *Cinnamomum camphora* (L.) Nees. et Eberm. (Sukhumi) and *Platanus orientalis* L. (Zugdidi).

Fomitopsis pinicola (Sw. Ex Fr.) Karst. - optional macromycetes causes stem and root brown rot, identified on the stumps of dead trunks *Pinus* sp. (Adler, Sukhumi).

Gloeophyllum abietinum (Bull. Ex Fr.) Karst. - xylotrophic macromycetes specialized to coniferous trees found on the dead trunks *Pinus* sp. (Adler).

Hirschioporus pergamenus (Fr.) Bond. et Sing. - xylotrophic macromycetes causes intense peripheral white rot in dead trunks, found on stumps and fallen trunks of deciduous species, common (Sukhumi).

Lenzites betulina (L. ex Fr.) Fr. - xylotrophic macromycetes found on fallen trunks of deciduous trees, rare (Batumi).

Polyporus squamosus Huds. ex Fr. – saddleback fungus, a parasitizes on alive trunks of hardwood, causes a central stem rot, found on *Salix purpurea* L. trunks and at the places of thick side trunks cuts on *Eucalyptus viminalis* Labill. (Sukhumi).

Fomes fomentarius (L. ex Fr.) Gill. - a real tinder, causes central stem rot, found on dead old trees (diameter 100 cm) *Populus pyramidalis* Roz. (New Athos), on the drying trees *Salix matsudana* Koidz. (Sukhumi), everywhere on *Fagus sylvatica* L. (Adler), rarely on *Platanus orientalis* L. (Sochi).

Laetiporus sulphureus (Bull) Bond. et Sing. - sulfur-shelf mushroom, causes intense stem rot, found in urban plantings on injured trunks *Salix matsudana* Koidz., and also on alive trees with mechanically damaged trunks *Eucalyptus viminalis* Labill. (Sukhumi).

Tyromyces sp. - xylotrophic macromycetes found on stumps after *Pinus pytiusa* Stev. young trees cut, rare (Pitsunda).

Order **Aphyllophorales**, family **Stereaceae**

Stereum hirsutum (Willd.) Pers. - xylotrophic macromycetes causes peripheral rot of dead trunks and skeletal branches, found in damp places on dead wood and old trees with mechanically damaged trunks on *Eucalyptus viminalis* Labill. (Adler, Batumi), on dead trunks *Callistemon salignus* DC. (Batumi), massively on stumps *Cerasus vulgaris* Mill. (Batumi), rarely on stumps *Phyllostachys edulis* (Carr.) A.et C.Riviere (Sukhumi), in places of skeletal branches cut on *Quercus bicolor* Willd. (Sukhumi), on stumps *Osmanthus fortunei*

Carr. (Adler), abundant on *Acacia dealbata* Link (Sochi) stumps, rarely on dead skeletal branches *Carpinus betulus* L. (Adler), on trunks *Jubaea spectabilis* HB et K. (Sochi).

Order Aphyllophorales, family Corticiaceae

Schizopora paradoxa Vel. - xylotrophic macromycetes causes weak peripheral wood rot found on dead trunks *Carpinus betulus* L. (Adler).

Order **Aphyllophorales**, family **Hymenochaetaceae**

Phellinus punctatus (Fr.) Pil. - causes peripheral trunk rot in growing trees, rarely found on the trunks of old trees *Buxus sempervirens* L., rarely on the trunks of old trees *Phillyrea latifolia* L. (Sukhumi), rarely on dead trunks *Ligustrum lucidum* Ait. (New Athos), rarely on old trees *Olea europaea* L. (Sukhumi), on the stumps of old trees *Osmanthus fortunei* Carr. (Adler), rarely on dead trunks of ornamental *Acer* sp. (Adler), at the point of the thick trunks breakings on *Platanus orientalis* L. (Adler).

Phellinus contiguus (Pers.: Fr.) Pat. - xylotrophic macromycetes causes wound rot on living trunks *Eucalyptus* sp., rare (Sukhumi).

Phellinus sp. – bracket fungus, identified on the inside-out butt *Carpinus caucasica* A.Grossh., single finding (Zugdidi).

Phellinus tuberculosus (Baumg.) Niem. - plum tinder, a parasitizes on the species of Rosaceae family, commonly found on the trunks of old trees *Prunus domestica* L. (Sukhumi) and *Persica vulgaris* Mill. (Pitsunda).

Phellinus tremulae (Bond.) Bond. et Borisov in Bond. - xylotrophic macromycetes specialized to *Populus tremula* L., parasitizes on living trees, causes white stem rot, noticed in all host plant places of growth (Adler).

Phellinus torulosus (Pers.) Bourd. et Galz. - lumpy bracket, causes root rot in growing trees, identified on *Laurocerasus officinalis* Roem., rare (Batumi).

Phylloporia ribis (Schum.: Fr) Ryv. - currant sponge fungus causes root rot in shrub species, found on *Euonymus latifolia* Mill. (Sukhumi).

Order Aphyllophorales, family Caloporaceae

Phaeolus schweinitzii (Fr.) Pat. – Schweinitz`s polypore, the fungus causes root rot in conifers, found at the base of the living trunks *Pinus* sp., *Pinus strobus* L., *Pinus radiata* Don (Sukhumi), on stumps *Pinus nigra* Arn. (Adler, Sochi).

Oxyporus sp. - xylotrophic macromycetes found on stumps *Eucalyptus rubida* Deane et Maid., rare (Batumi).

Order **Aphyllophorales**, family **Clavariaceae**

Sparassis crispa (Fr.) Fr. - xylotrophic optional macromycetes found on fallen trunks *Pinus* sp., very rare (Sukhumi).

Order **Aphyllophorales**, family **Ganodermataceae**

Ganoderma lucidum (Fr.) Karst. - causes brown root rot in hardwood, rarely found on stumps *Fagus sylvatica* L. (Adler), sporadically at the points of trunks mechanical damages on *Quercus ilex* L. (Sochi), often on old trees *Quercus iberica* Stev. (Sochi), rarely on dying trees *Salix matsudana* Koidz. (Sukhumi).

Ganoderma applanatum (Pers. Ex Wallr.) Pat. - causes white root rot in many leaf and coniferous tree species, found on old trees *Cinnamomum camphora* (L.) Nees. et Eberm. (Batumi, Sukhumi, Sochi, New Athos), on the damaged trunks in *Populus simonii* Carr. (Sukhumi), on old trees with a diameter of 100 cm *Gleditschia triacanthos* L. (Sukhumi), on the dying plants *Hibiscus syriacus* L. (New Athos), massively in urban plantings of *Salix matsudana* Koidz., on the stumps with a diameter of 50 cm *Cedrus atlantica* Manetti, on 50

years aged trees *Elaeagnus multiflora* Thunb., 30-40-year-old trees *Acacia dealbata* Link, on old trees *Cercis canadensis* L. and *Cercis siliguastrum* L. (Sukhumi), abundant on stumps *Picea smithiana* (Wall.) Boiss. and *Abies nordmanniana* (Stev.) Spach, on old trees *Carpinus betulus* L. (Adler), often in damp places on stumps *Zelkowa carpinifolia* (Pall.) K.Koch (Sochi).

Order **Aphyllophorales**, family **Schizophillaceae**

Schizophyllum commune Fr. - xylotrophic macromycetes causes weak surface rot in dead trunks and branches, rarely found on dead trunks *Cupressus sempervirens* L. (Zugdidi), often in *Hedera helix* L. (Sukhumi), rarely on stumps *Pinus densiflora* Sieb. et Zucc. (Adler), singly on leafstocks *Jubaea spectabilis* HB et K. and often on dead trunks *Acacia dealbata* Link (Sochi).

Order **Auriculariales**, family **Auriculariaceae**

Auricularia mesenterica Pers. - xylotrophic macromycetes found on dead skeletal branches *Davidia vilmoriana* Wangh. (Batumi) and on the stumps *Ligustrum lucidum* Ait. (Sukhumi).

Order **Agaricales**, family **Lepiotaceae**

Armillaria mellea (Fr.) Kumm. – honey fungus, causes root rot, found on stumps *Carpinus caucasica* A.Grossh. (Zugdidi).

Order **Tricholomatales**, family **Pleurotaceae**

Pleurotus ostreatus (Jacq. Ex Fr.) Kumm. - xylotrophic macromycetes found on dead trunks in *Nerium oleander* L., common on weakened trees *Salix matsudana* Koidz. (Sukhumi), sporadically noticed at the cut points of side trunks in *Cordyline australis* Hook. (Batumi).

Conclusions

Thus, on 117 species of woody plants growing in different regions of the Black Sea coast of Caucasus, it has been identified 98 species of fungi from three classes: Deuteromycetes - 37 species in 42 plant species, Ascomycetes - 26 fungi species in 42 plant species and Basidiomycetes - 35 fungi species in 92 plant species. In the class Deuteromycetes fungi belong to 4 orders, 7 families and 20 genera have been identified. Predominated fungi are ones of the order Sphaeropsidales - 20 species, most of them cause necrotic diseases. The most widespread in this class are powdery mildew fungi from the genus *Oidium* - 9 species and necrotrophyc fungi of the genus *Cytospora* - 4 species. Ascomycetes are presented with 4 orders, 8 families and 16 genera. Predominated species belong to the order Diaporthales - 9 species, which are ascigerous stage of main necrotrophic fungi, and to the order Erysiphales - 7 species that are teleomorphs of powdery mildew fungi. Class Basidiomycetes includes fungi from 5 orders, 12 families, 27 generas with dominated order Aphyllophorales - 22 species, dominated family Polyporaceae - 15 species, dominated genera *Phellinus* - 6 species. According to the number of host plants genera *Ganoderma* (14 species) pedominates and due to species number - *Stereum* (9 species). Fungi that cause root rot prevail in this class.

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The article contains data about microflora of 117 tree and bush species from 45 families and 81 genuses, growing along the Black Sea Coast of the Caucasus from Anapa till Batumi. Most of arboroeus exotics are potential introduced species for the Southern Crimea. There were found out 98 species fungi from three classes: Deuteromycetes – 37 species, Ascomycetes – 26, Basidiomycetes – 36. The fungi caused leaf illnesses are prevailed - 24, canker and cancer disease of shoots– 40, root rot – 10. Each of the fungi species has its own characteristics in its dissemination and development rate.

Key words: *arboroeus exotics, introduced species, microflora, diseases, dissemination, special sorts.*

UDK 631.4:632.11:635.6(477.75)

AGROECOLOGICAL ZONING OF STEPPE AND FOOTHILL CRIMEA FOR FRUIT CROPS GROWING AT THE PRESENT STAGE

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Introduction

Crimea is the sourse of gardening and an unique fruit-growing region in Russia. Fruit crops horticulture has been and will remain a priority sector of the Republic, but to make it successful it is necessary and possible to plant about 7000 ha of gardens in the near future.

It`s important to note that in the strategy of fruit horticulture adaptive intensification the center point should be given to agroecologycal (i.e. soil and climate) zoning of Steppe and Foothill Crimea and optimization of fruit crops varieties distribution based on long-term and complex agroecological studies. With this approach, yield amount and stability and also efficient life-terms of fruit plantings increase without additional financial and energy outlays.

Existing methods and trends for zoning territories, including the Crimea, for fruit crops were based on agricultural evaluation of such climate factors as heat, humidity, solar radiation, wind [5, 7, 8, 12, 13] and number of indexes specific for fruit crops and limiting their cultivation (sum of effective temperatures, maximum and minimum air temperature, frost-danger for the territory) [1, 3, 4].

On the principle of similar and differing climatic conditions of the territories V.I. Vazhov [2] made agroclimatic zoning of the Crimea, indicating a probability of frost damage to pome and stone fruit crops in general. Further, V.A. Ryabov, V.V. Antyufeev, N.E. Opanasenko [10] evaluated agroclimatic potential in standard values (points) in all administrative regions of the Crimea on their favour to particular fruit crops due to regulative indexes that affect plant growth.

Allocation of areas according to their agroecological homogeneity on the base of long-term soil-biological complex studies, investigations of physical and chemical climate parameters, physiological and embryological indicators of growth, development and yield of fruit crops varieties in combination with the soil and soil-hydrological features of Steppe and Foothill Crimea areas hasn`t been carried out.

The aim of the researches. Based on broad climate, soil and varietal diversity, productivity of different fruit crops genotypes, great research bank of meteorological and soil data, with points of investigations and particular year data to carry out agroecological zoning of Steppe and Foothill Crimea for apricot, cherry plum, peach, sweet cherry, plum, apple, pear and almond.

Materials and methods

Objects of fixed soil and biological studies were soil and climatic (agroecological) resources, industrial orchards in 46 farms in steppe and foothill areas of the Crimea.

Studies of soil, climate and their effects on fruit trees growth and yield were based on the method of soil-biological researches by P.G. Shitt [14], added by S.F. Negovelov [9] and V.F. Ivanov [6]. Agroclimatic methods by G.T. Selyaninov [11] and N.V. Gulinova [5] were used. Statistic analyses have been carried out in "Windows XP" system with the programme Statistica 6.0.

Agroclimatic indicators taken from decade agroclimatic bulletins of all weather stations in Steppe and Foothill Crimea and daily meteorological observations - according to

meteorological posts in Steppe Department of NBG and the farm-factory "Evpatoria" in Saki region.

For soil surveys comparative profile-genetic, laboratory and analytical, historical and cartographic methods, correlation and regression analysis of the results were used.

Results and discussion

Our investigations resulted in improved soil-climatic zonal and agroclimatic zoning of the Crimea for the purposes of fruit horticulture (Fig. 1).

When assessing the agroclimatic areas, mostly in all administrative districts of Steppe and Foothill Crimea, for 25-35-year periods we took the main agro-climatic indicators, most of which hasn`t been previously quantified:

- absolute minimum air temperatures in December, January, February, March, °C and their frequency;
- the degree of winter strength;
- probability of provocative thaws in January - February, % and their depth;
- the last spring frosts, °C, and the latest date of spring frosts end in March - April;
- number of cloudy, rainy and foggy days during tree flowering ;
- rainfalls and hydrothermal coefficients by Selyaninov for May, June, July, August and for the whole vegetative season.

Based on reliable correlation between the crop yield and physiological and embryological parameters (water-retaining capacity, water content, leaf water deficit, morphogenesis phase of flower buds and others.) the main climatological factors with greatest effect on plants were determined. Their permissible parameters (Table. 1, as an example - absolute minimum temperatures of winter-spring period) were found out. These figures also formed the basis of agroecological zoning of the Crimea for fruit crops planting.

40-years results of soil and biological studies made by soil scientists from Nikitsky Botanical Gardens have been summarized, detailed agronomic description and soil fertility assessment for Steppe and Foothill Crimea have been presented.

In Sivash region, dry steppe zone, dark chestnut soils with various alkalinity, salinity and hydromorphism degrees were studied.

In the South steppe area salty southern chernozem (black soil), southern micellar-carbonate chernozems, southern black soils carbonate, skeletal and poor soils were studied in details.

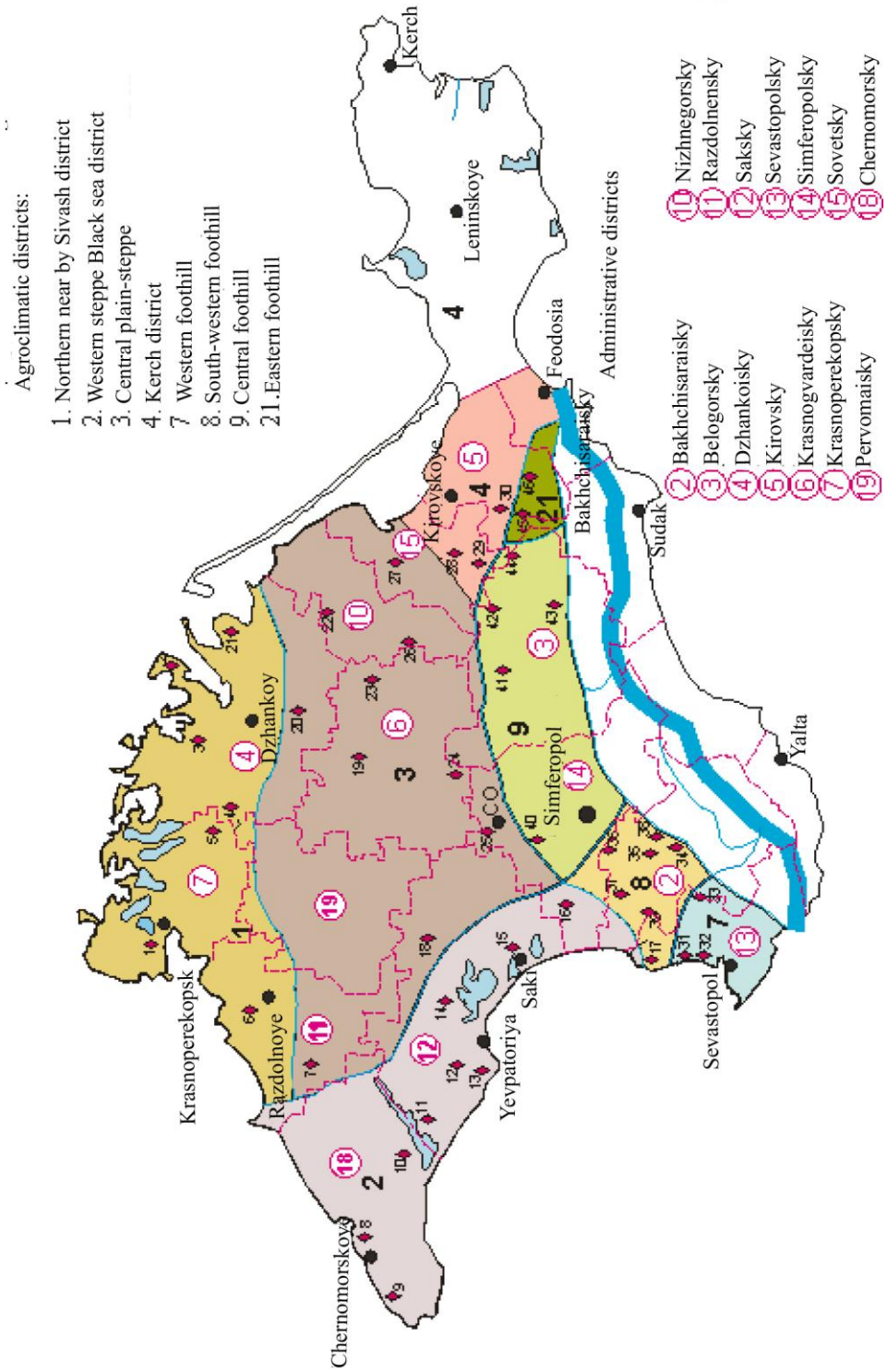

Fig. 1 Agroclimatic and administrative districts and investigated orchards in the Crimea (1 … 46)

Along the river valleys and terraces on the rivers Chernaja (Black), Belbek, Kacha, Alma, Zapadnyi (West) and Vostochnyi (East) Bulganak, Salgir, Bolshaja (Big) and Malaja (Small) Karasevka, Zuja, Burulcha, Indole alluvial-meadow chernozem-meadow and meadow-chernozem soils, including pebble soils within the floodplain and low terraces are described in details.

Table 1

Acceptable absolute minimum air temperatures for fruit crops during winter-spring period in Steppe and Foothill Crimea

Months	Decades	Fruit crops						
		Peach	Apricot	Cherry Plum	Sweet Cherry	Plum	Apple	Pear
January	I	–22º	–22º	–24º	–28º	–28º	–28º	–28º
	II	–22º	–21º	–23º	–27º	–28º	–27º	–27º
	II	–21º…–20º	–20º	–22º	–25º	–26º	–26º	–26º
February	I	–20º…–19º	–19º	–22º	–25º	–25º	–25º	–25º
	II	–18º	–17º	–20º	–21º	–24º	–23º	–23º
	II	–18º…–17º	–16º	–19º	–21º	–23º	–22º	–22º
March	I	–16º	–15º	–17º…–16º	–20º	–21º	–22º	–21º
	II	–15º	–13º	–14º	–17º	–20º	–20º	–20º
	II	–12º	–10º	–12º	–14º	–16º	–16º	–15º
April	I	–9º…–8º	–5º	–7º	–7º	–9º	–10º	–8º
	II	–5º	–1º…–3º	–5º	–3º	–5º	–5º	–5º
	II	–1º…–2º	–1º	–2º	–1º	0º	–3º	–2º

In the foothill and forest steppe ordinary black micellar-carbonate, ordinary black skeletal sod-calcareous and brown soils have been investigated.

In the studied, mainly plantaged orchard soils, constraints limited growth and yield of the fruit crops were determined, acceptable and optimal soil parameters, which were the base for soil fertility and suitability for gardens assessment were found out. They were also used as a regulatory base in the preparation of orchard projects (Table. 2, skeletal soils as an example).

In Nikitsky Botanical Gardens it has been accumulated extensive experience in the development of poor skeletal soils (gravelly and stony-pebble) for planting gardens with trenching method, and also in fruit crops cultivation on highly carbonaceous, saline, alkaline soils with near-surface bedding of fresh and saline groundwater.

Since 1957 to 2005 it has been generally quantified 4300 crop yield years. The average yield of stone fruit in the studied irrigatied gardens was 118 kg / ha and pome fruit - 136 kg / ha. However, the yield of many varieties was significantly higher. Thus, yield of apricot varieties Parnas, Olymp Forum ranged from 185 to 160 kg / ha, peach varieties Ostryakovsky White, Krasnoshcheky, Mayakovsky - from 320 to 225 kg / ha, apple varieties Krymskoe Zimnee, Reinette Simirenko - from 265 to 190 kg / ha, pear varieties Krymskaya Zimnyaya, Bere Ardanpon was more than 205 kg / ha. Nowadays varieties with even higher productivity are cultivated.

All above-presented helped us to implement agroecological zoning of Steppe and Foothill Crimea for apricot, peach (Fig. 2), cherry plum, sweet cherry, plum, apple and pear (Fig. 3), almonds and agroclimatic zoning of skeletal soils for planting gardens in the Crimea has been made (Fig. 4).

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Table 2

Standards of fertility for skeletal heavy clay and light clay soils for commercial orchards of fruit and nut crops in different soil-climatic zones of the Crimea (average for studied soils, cultivars, rootstocks)

Main agronomically valuable soil indexes	Apricot, cherry-plum, almond, peach, walnut for fruit strips,			Pear, walnut for commercial orchards, plum, apple		Pear, apple	
	South steppe	Foothill steppe	Foothill and Mountain forest-steppe	South steppe	Foothill steppe		Foothill and Mountain forest-steppe
	Automorphous soils					Hydromorphous soils	
Skeleton amount, % of soil volume, in the layers: 0-50 sm	<15	<20	<25	<15	<20	<35	<40
50-100 sm and deeper	<30	<35	<45	<30	<35	<50	<60
Depth of dense bedrocks, sm	>127	>117	>117	>150	>145	Fresh groundwater at the depth of 170-180 sm (summer)	
Supplies in the root zone: melkozem, t/ha	>9900	>9000	>9000	>13200	>11900	>9500	>9000
available moisture, mm	>65	>60	>55	>75	>70	>70	>70
Humus supplies in the root zone, t/ha	>155	>140	>140	>175	>160	>120	>125
$CaCO_3$ amount, %, in the layers: 0-50 sm	<30	<25	<25	<25	<20	<15	<15
50-150 sm	<40…45	<35…40	<35…40	<35	<30	<20	<20
Supplies of N, P, K^1 gross forms in the layer 0-100 sm, t/ha	Not less than 7…8; 8…9; 80…100					Not less than 7; 16; 115	

Note. Supplies of mobile forms of main nutrition elements in skeletal soils during the vegetation should be not less than: $N\text{-}NO_3$ – 60, P_2O_5 – 55, K_2O – 2200 kg/ha.

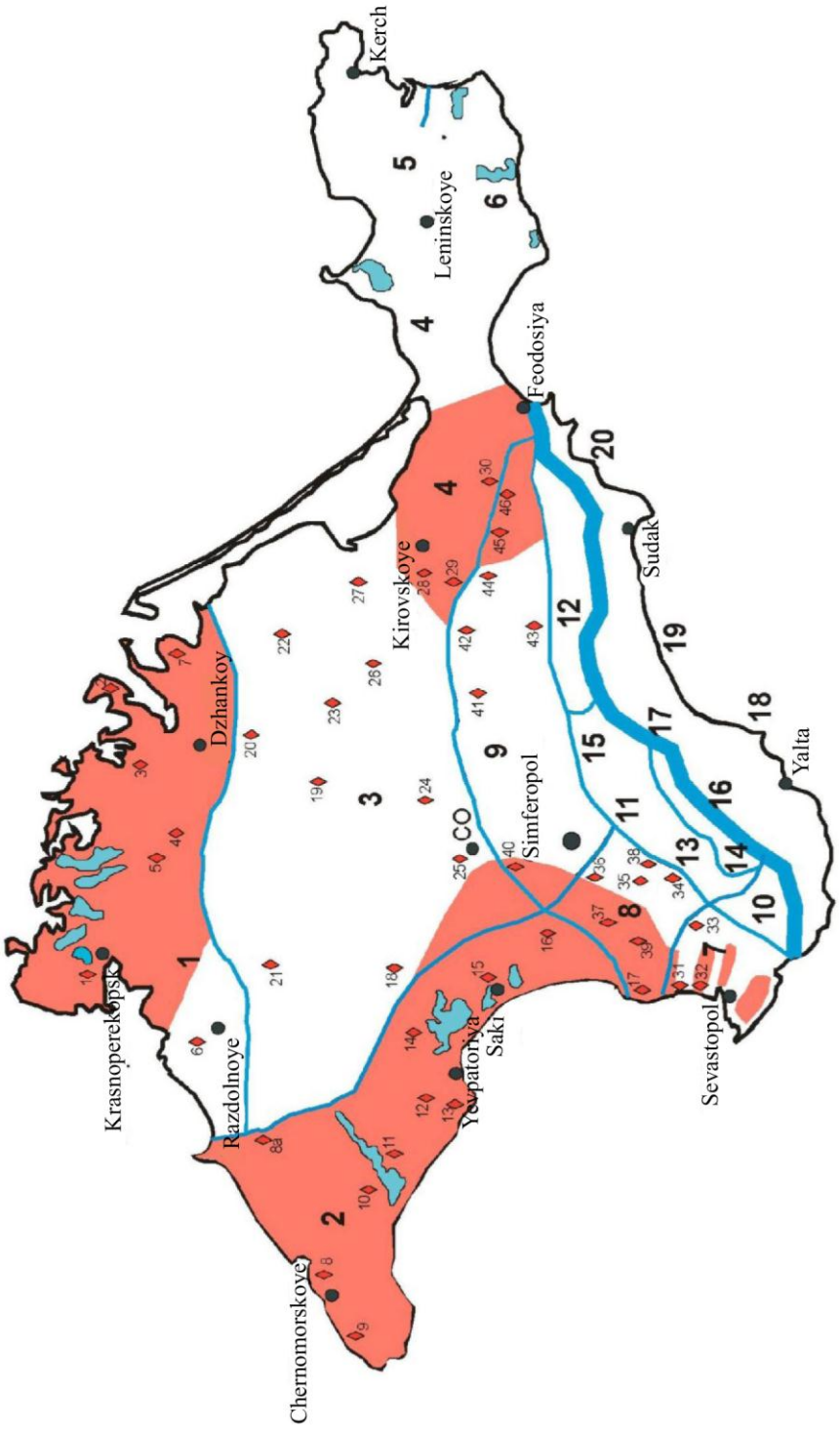

Fig.2 Favorable for apricot and peach territories of agroclimatic districts within steppe and foothill Crimea

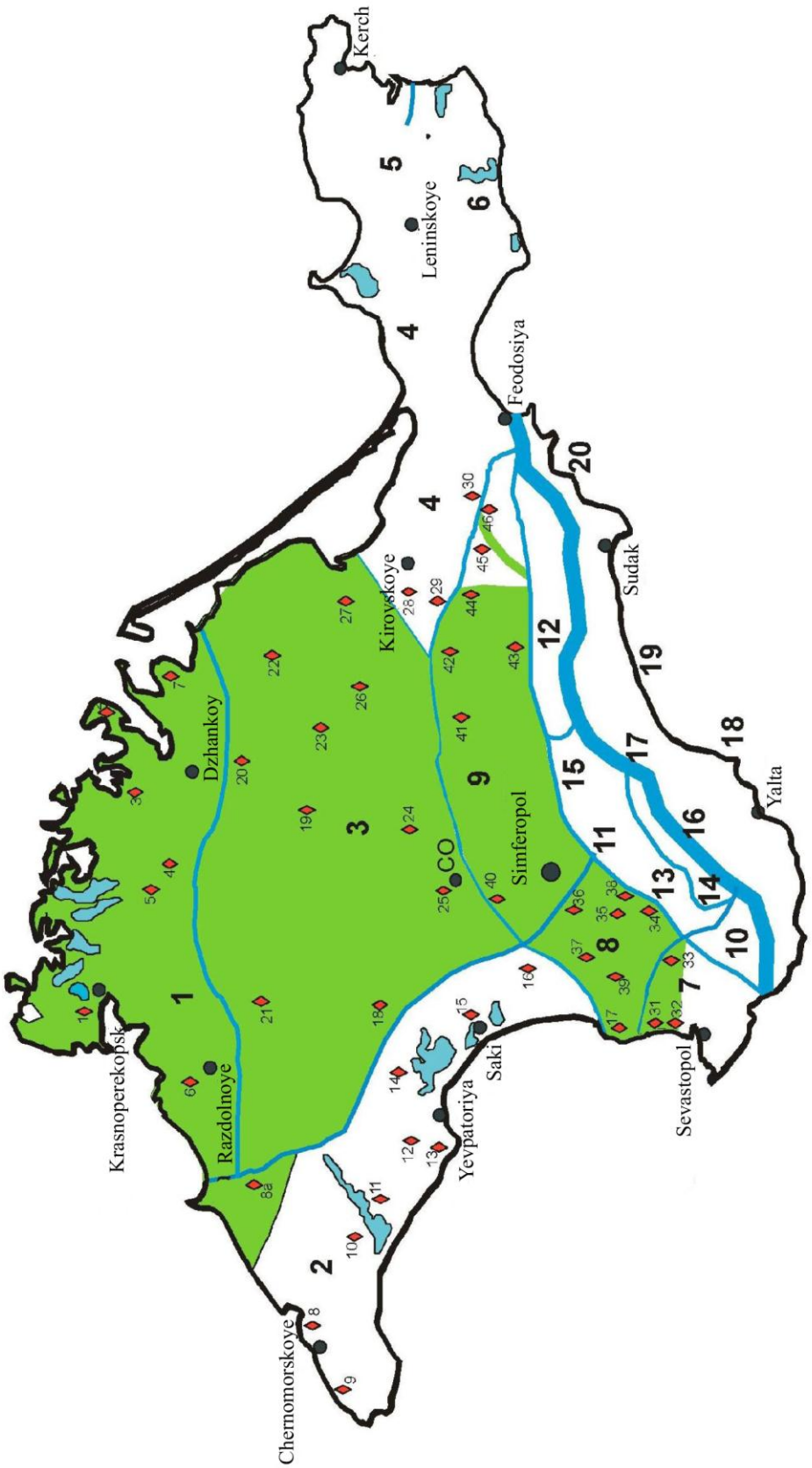

Fig.3 Favorable for apple and pear territories of agroclimatic districts within steppe and foothill Crimea

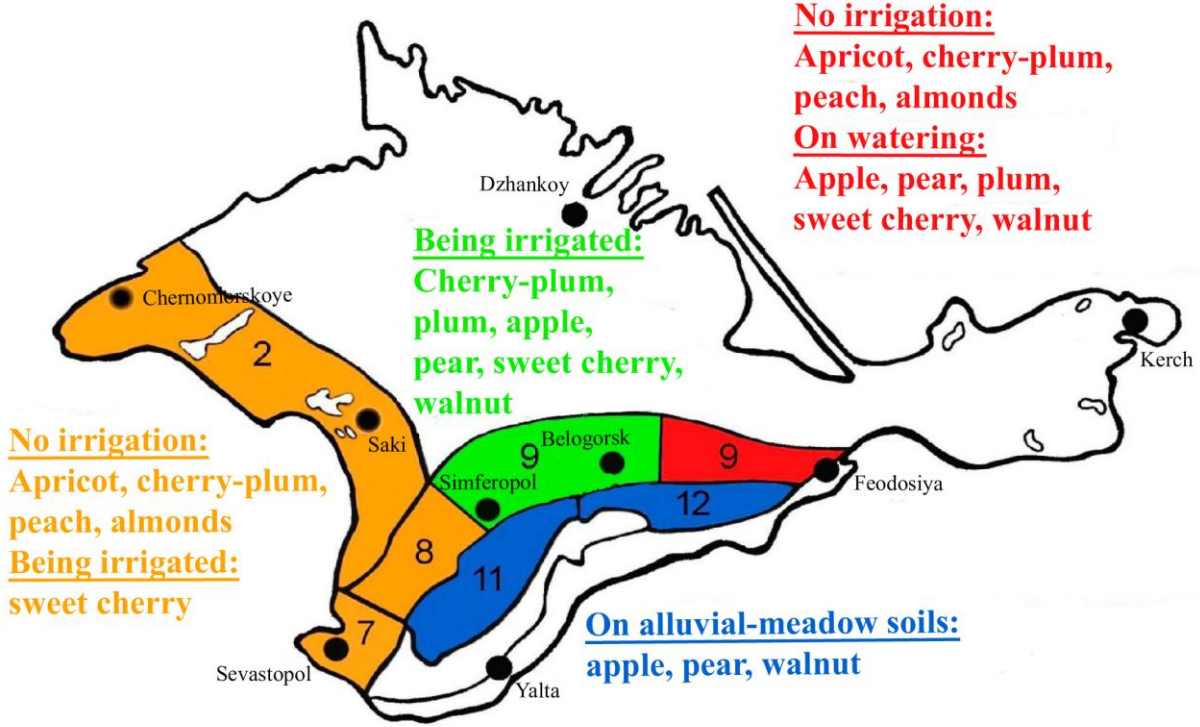

Fig. 4 Agroclimatic districts on the territory with skeletal soils in the Crimea for fruit and nut-bearing crops.

Agroclimatic districts in the Crimea: 2. Western steppe Black sea district); 7. Western foothill (Gerakleiskii); 8. South-western foothill district 9. Central foothill; 11. Kachinsko-Salgirsky low mountain district 12. Eastern low and middle hills; 21. Eastern foothill

In coming years, it is recommended to use for garden plantings undivided lands of the State Concerns - 160 hectares in Krasnoperekopskii region, 520 ha in Dzhankoiskii, 105 hectares in Pervomaiskii region, 1000 hectares in Razdolnenskii, 530 ha in Chernomorskii, 1000 hectares, including 130 hectares in the trenches, in Saksky, 1160 hectares in Simferopolskii region, 100 hectares in Bakhchisaraiskii, 850 hectares in Nizhnegorskii, 640 hectares in Belogorskii and 600 hectares in Kirovskii district of the Crimea. Totally it`s about 7 thousand hectares.

Conclusions

1. Agroclimatic assessment and zoning of the territory of the Crimea, including skeletal soils for gardens, based on the principles of different areas agro-climatic resources and soil fertility conformity to biological characteristics of fruit crops and their varieties has been presented.

2. According to the results of long-term agroecological researches cartograms for eight fruit crops plantings within the agroclimatic regions of Steppe and Foothill Crimea have been grounded and presented.

3. Planting of fruit crops varieties in the determined areas with real ecological optimum makes possible an efficient use of the crimean environment and available gene resources of fruit crops, to increase their productivity.

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The agriclimatic assessment has been given and the Crimean area zoning has been made, including skeletal soil for gardening counting correspondence principle of different territories agroclimatic resources and soil fertility to biological special features of fruit-bearing plants and their species.

Key words: *agroecological zoning, agroclimatic resources, soil fertility, fruit-bearing plants.*

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ELEMENTS OF INNOVATIVE TECHNOLOGY FOR SPIRAEA X VANHOUTTEI (BRIOT) ZAB. SEEDLINGS GROWING IN AN INDUSTRIAL NURSERY

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Introduction

Vegetative propagation is important for plant introduction. In new soil and climatic conditions introduced plants can later come into generative stage, give defective seeds, fruit rarely or poorly, or even not bear fruits at all that makes it impossible to use seed propagation method. Besides, seed propagated plants do not always inherit decorative features that attract introducers and designers. Therefore, vegetative propagation is the most effective way to get planting material in sufficient quantity. It can be obtained from maternal plants, before the generative age. N.F. Dovbish [4] noted that it contributes to the further aromorphosic development of introduced species as the organisms get new opportunities for development of the environmenalt resources.

In soil and plant rhizosphere microorganisms capable to synthesize substances that improve nutrition, stimulate plant growth and development are common. These substances affect various aspects of plant life, regulate particular stages of their growth and development and make possible qualitative changes in the economically valuable plant features [1]. Use of active microorganisms (MO) strains in plant horticulture is based on symbiosis and associations of plant and MO. MO have complex effect on a plant: improve nitrogen nutrition of plants through nitrogen fixation, phosphorus nutrition – through phosphatmobilization [7, 9]; stimulate growth through secretion of growth regulators - indoleacetic acid, gibberellins, auxins et al. [8, 11]; improve the state of plants by allocating other biologically active substances - vitamins, enzymes, antiseptics and others [12].

At present, based on active bacterial strains microbial products (MP) have been created and successfully used for cereals, vegetables and forage crops [2]. MP are used for treating seed at sowing or soil bacterization during the growing season. They are environmentally friendly and help to decrease the use of fertilizers by 20 - 30%.

More rarely MP are used in ornamental plant growing. There is some information about using Fitostimofos preparation based on *Agrobacterium radiobacter* strain 2258 CMF to improve the survival rate of annual and perennial flower crops seedlings [1] and increase seed germination of medicinal and ornamental plants [3]. In fruit crops nursery Fosfoenterin was used to increase the output of peach and sweet cherry planting material [5]. Thus, using of useful microorganisms for increasing of growth intesity, plant nutrition and adaptation to abiotic stresses has great practical importance. Literature sourses have little information about MP use for ornamental shrubs seedling growing. So, investigations of active bacterial strains with stimulating and growth-regulatory effects that let to increase the output of ornamental crops planting material are important.

The aim of our researches was to evaluate the influence of MP on soil agrochemical properties, rooting rate, growth, plant development and output of standard seedlings *Spiraea x vanhouttei* (Briot) Zab. [13] in nursery industry.

Materials and Methods

Objects of the studies were *S. x vanhouttei* plants, MP and soil agrochemical properties. Investigations were carried out in the nursery in NBG (Department of Steppe

Plant Growing) on the small field plots in 2012 - 2014. Experimental varianst were MP: Azotobacterin (*Azotobacter chroococcum* 10702) with nitrogen-fixing properties. Fosfoenterin that is based on *Enterobacter nimipressuralis* 32-3strain and transforms hardly accessible phosphates into plant available state and Complex microbial preparations (CMP) that has plural effect on plants. This Complex is an equal parts mixture of nitrogen fixer - Diazofit (*Agrobacterium radiobacter* 204), Fosfoenterin and Biopolicid created on the basis of *Paenibacillus polymyxa* P strain - antagonist of pathogenic micromycetes.

MP have been developed and provided by the researchers of Microbiology Department at the Institute of Crimea Agriculture. Titer of preparations was 7 - 10 billion. CFU / ml. Working solutions of MP suspensions were prepared in the day of planting through diluting suspensions in 100 times with tap water. For better survival rate and bacteria nutrition on spirea cuttings combined use of MP and organo-mineral fertilizer "Component 2" (0.5% solution), which consisted of macro- and micronutrients set, humates and adhesive were investigated. This drug, according to the developers (NIC "Ecology"), also has immunomodulatory properties. Untreated plants were used as a control.

Lignified cuttings of *S. x vanhouttei* 20 cm length were soaked in tap water for 24 hours before planting, then their basal ends immersed for 1-2 cm in the mixture of MP and Component 2 solutions for 20 minutes. For comparison, Zircon preparation was used as an analogue as it is widely used in ornamental horticulture as an immunomodulator and root stimulater. Before planting cuttings were soaked in 0.01% solution of Zircon for 14 hours.

Cuttings were planted in the first field of nursery in March, 10 - 15 samples per an account plot. Plot area was 0.7 - 1.0 m^2. Experiments had three times replications. Variants` location was rendomized within a row of nursery. Experience was designed for a two-year planting of seedlings. Cultural care of studied plants was carried out in accordance with accepted agricultural practices in the industrial nursery of Steppe Plant Growing Department at NBG.

Account of plants growth and state was carried out according to the procedure [6]. Seedlings quality was evaluated according to GOST 26869-86.

Soil in the plot is southern carbonate plantaged light clayey chernozem on red-brown Pliocene clay. Content of nutrients in the soil before the experiments was low in nitrate nitrogen (5.9 mg / kg) and mobile forms of phosphorus (8.5 mg / kg) and optimal in potassium (325 mg / kg). Humus content ranged from 3.02 to 3.24%, depending on soil and plot.

Soil samples for analysis were collected annually during the period of intensive shoot growth (late July - early August) according to the experiment variants in the layer 0 - 40 cm. Content of phosphorus mobile forms and potassium in soil determined according to Machigin (DSTU 4114-2002), nitrate nitrogen - potentiometrically GOST 26951-86, organic matter - according to Tiurin (DSTU 4289: 2004). Statistical analysis of the results was carried out by the program ANOVA. The level of significance was set at 5% ($p<0.05$).

Results and discussion

Content of nitrate nitrogen and available phosphorus in the soil under *S. x vanhouttei* seedlings was low in control and all other variants and potassium content was high (Table. 1).

Table 1

Content of humus, nitrate nitrogen, mobile forms of phosphorus and potassium in the soil (layer 0 -40 cm) under *S. x vanhouttei* seedlings after cuttings treatment with MP and Component 2, nursery NBG, the average for two years (2012 - 2013).

Variants	N – NO3	P2O5	K2O	Humus, %
	mg/kg soil			
Control	8.1±1.5	8.5±4.2	310±45	2.85±0.20

Azotobacterin	5.6±0.8	10.5±2.9	362±16	2,78±0.29
Azotobacterin + Component 2	5.6±1.0	18.9±7.9	328±94	2.44±0.24
Fosfoenterin	7.6±2.2	12.2±3.4	322±24	2.75±0.14
Fosfoenterin + Component 2	7.0±3.5	21.9±3.5	306±116	2.76±0.28
CMP	8.2±2.3	14.9±4.3	345±50	2.70±0.23
CMP + Component 2	4.7±0.1	15.0±5.0	362±66	2.66±0.24
Optimal [10]	15–20	28–38	211–270	–

On average, two years applied MP and their mixtures with Component 2 helped to reduce nitrate nitrogen in the soil under *S. x vanhouttei* seedlings and only CMP had not changed it, but Fosfoenterin and its mixture with Component 2 - reduced nitrate nitrogen to less extent than other preparations. This is probably due to the increase in the number of established cuttings under the influence of MP and increased consumption of soil nitrogen by rapidly growing seedlings of *S. x vanhouttei*. Therefore, growing ornamental plants using MP and under a low content of soil nitrate nitrogen in the first nursery plot needed low dose of nitrogen dressing to improve nutrition of growing seedlings.

Using of each MP resulted in greater amount of mobile phosphorus in soils. The largest increase of this element content occurred under using a mixture of Fosfoenterin and Component 2 in 13.4 mg / kg that corresponds to 60 kg / ha P_2O_5.

Content of exchangeable potassium in the soil was increased when using most of MP, significantly using Azotobacterin and CMP in combination with Component 2 in 52 mg / kg or 17% of control.

Humus content in the soil was high enough in control. Application of MP and its mixtures with Component 2 gave a tendency to decrease humus content in the soil. It was less when using Azotobacterin, Fosfoenterin and its mixtures with Component 2. However, any significant difference between control and other variants haven`t been found.

The study results in rooting of *S. x vanhouttei* cuttings treated with MP indicated that their survival rate considerably varied in control – from year to year, from 30 to 67% of the planted cuttings and the average for three years was 48% (Table. 2).

Table 2

Survival rate of *S. x vanhouttei* cuttings in NBG nursery (% of planted) under using MP and Component 2, 2011 - 2013.

Variants	2011	2012	2013	Average	% of the control
Control	67±5	30±10	47±12	48.0	100
Zircon	67±7	27±3	–*	47.0	97.9
Azotobacterin	53±2	27±7	60±12	46.7	97.3
Azotobacterin + Component 2	50±3	43±7	60±15	51.0	106.2
Fosfoenterin	43±3	37±9	67±12	49.0	102.1
Fosfoenterin + Component 2	67±7	80±10**	70±2	72.3	150.6
CMP	56±9	33±3	63±13	50.7	105.6
CMP+ Component 2	76±4	85±5**	60±15	73.7	153.5
Notes: * in 2013 Zircon haven`t been used, ** significant difference with the control					

Applied MP changed the survival rate in different ways, and the intensity of their influence depended on the year. So, in 2011, when the survival rate of cuttings was high in control, most preparations either unchanged or somewhat reduced it and only mix of CMP and Component 2 stimulated significant increase of accustomed cuttings. In 2012 and 2013 treatment with most of preparations enhance the survival rate of cuttings, especially in 2012, when it was low in control. This is probably due to increase of plant resistance to adverse environmental factors during the period of rooting (lack of moisture, high temperature, etc.).

In the same year, Component 2, as an immunomodulator, significantly enhanced MP effect. For example, using of Fosfoenterin increased cuttings survival by 7% of planted ones, and adding thereto Component 2 – by 50% compared with the control. Under CMP applying this increase was 3 and 55%, respectively. In 2013 this trend was true for Fosfoenterin. An average of three years studies impact of Zircon and Azotobacterin on the rooting of *S. x vanhouttei* cuttings corresponded to the level of control. Fosfoenterin and CMP slightly increased the rate (by 2 - 6%) respectively to control. The combined use of these preparations and Component 2 stimulatted significant increase in the number of rooted plants by 51 - 54% compared with the control.

The results show that MP affected *S. x vanhouttei* growth and shoot formation in the nursery (Table. 3).

Variants	Number of annual shoots	Total length of annual shoot, cm	The average length of shoot, cm
Control	2.0	15.0	7.5
Azotobacterin	2.7	73.7	27.3
Azotobacterin + Component 2	3.8	70.0	18.4
Fosfoenterin	3.0	60.7	20.2
Fosfoenterin + Component 2	4.3	74.3	17.3
CMP	3.0	27.0	9.0
CMP + Component 2	3.7	73.2	19.8
HCP 05	1.0	35.0	9.2

Thus, using of MP increased number of shoots formed. The highest number of shoots formed under the influence of Fosfoenterin and Component 2 combination which exceeded control in more than half.

Total length of shoots growth was low in control and increased under the influence of MP. Adding of Component 2 to Fosfoenterin and CMP enhanced MP effect on the overall shoot growth; especially significantly Component 2 increased CMP effect - by 2.7 times compared with using CMP only. Total shoot growth increased not only by increasing their number, but also due to increasing a shoot length under MP use. The average shoot length was most significantly increased by Azotobacterin, by 20 cm, compared with the control. Addition of Component 2 to this preparation, as well as to Fosfoenterin had little effect on shoot length, but significantly stimulated shoot growth when used in composition with CMP, by 12.3 cm compared with the control.

In the second year after planting flowering plants were observed (Fig. 1, 2).

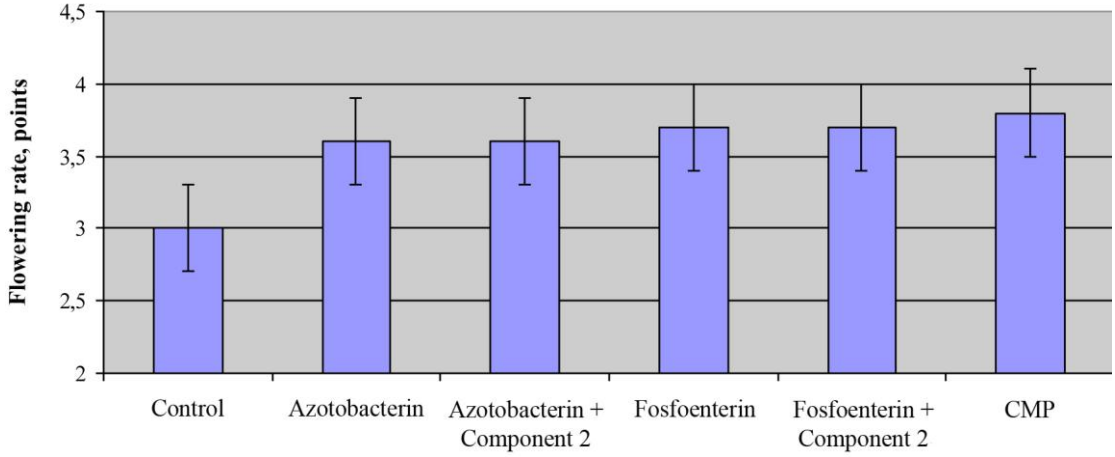

Fig. 1 Effect of MP and Component 2 on flowering rate of *S. x vanhouttei* seedlings in the second year after planting (2013)

Flowering was middle (3 points). All MP increased the intensity of flowering, Fosfoenterin and CMP significantly by 0.7 - 0.8 points or 23 - 27% relatively to control. Therefore, MP affected the rate of flower buds formation in *S. x vanhouttei*.

A B

Fig. 2 Rate of flowering in *S. x vanhouttei*: A – control, B – Fosfoenterin + Component 2, NBG-NSC nursery, 2012.

The main indicator of nursery productivity is the output of standard seedlings. An average index of three year researches for *S. x vanhouttei* was low in control - 35.3% of planted cuttings with changes from year to year from 10 to 51% (Table. 4).

Table 4

***S. x vanhouttei* standard seedlings output in NBG nursery under MP and Component 2 using, 2012 - 2014.**

Variants	2012	2013	2014	An average for three years	
	% of planted cuttings			% of planted cuttings	% of control
Control	51	10	45	35.3	100
Zircon	46	20	not found	33.0	94
Azotobacterin	53	20	60	44.3	126
Azotobacterin + Component 2	50	40	60	50.0	142
Fosfoenterin	42	20	57	39,7	112
Fosfoenterin + Component 2	51	70	70	63.7	180
CMP	53	10	55	39.3	111
CMP + Component 2	57	60	60	59.0	167
NCR 05	Fф < F05	12	15	–	–

Minimum output of seedlings was in control in 2013; however, under MP using maximum increase of seedlings output by 200 - 700% of the control was noticed this year. In the other two, the output of *S. x vanhouttei* seedlings was approximately the same, but MP increased the output of seedlings in 2014 more significantly. This may indicate the influence of particular year environmental conditions on the rate of bacteria development and intensity of their effect. On average for three years the best combination, which obtained maximum additional amount of standard seedlings, was Fosfoenterina combined with Component 2, the

excess relative to the control was 80%. High excess amount compared to seedlings obtained from control was also in the variant with CMP combined with Component 2 - by 67% compared with the control.

All applied MP exceeded Zircon on the effects on *S. x vanhouttei* standard seedlings output in the nursery.

Conclusions

1. It was found that for *S. x vanhouttei* two-year seedlings growing in a nursery treatment of lignified cuttings basal ends by Fosfoenterin and CMP combined with Component 2 in spring before planting was favourable for increasing amount of phosphorus mobile forms and potassium in the soil, and stimulated seedlings growth and shoot formation, increased the rate of flowering. Their influence was more intensive than standard growth stimulator Zircon.

2. It has been demonstrated that using of Fosfoenterina and CMP in combination with Component 2 increased number of rooted *S. x vanhouttei* cuttings by 50% and number of standard seedlings by 67 - 80% compared with standard technology. These combinations can be recommended for wide production testing in nurseries in the southern chernozems.

3. On soils with low content of nitrogen mobile forms under MP using nitrogen fertilizing for plants is required in the first field of nursery. To avoid loss of humus content an introduction of field with green manure (busy couple) or perennial grasses to the crop rotation in a nursery is needed to increase content of organic matter in soil.

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Klymenko O.E., Klymenko N.I. Elements of innovative technology of seedlings Spiraea x vanhouttei (Briot) Zab. growing in industrial nursery // Works of the State Nikit. Botan. Gard. – 2014. – V. 139 – P. 170 – 176.

The data of triennial researches in using of various microbal preparations and organic and mineral fertilizer growing Spiraea x vanhouttei (Briot) Zab. seedlings in industrial nursery have been presented in the article. It was found that the introduction of these elements into the Spiraea x vanhouttei seedlings growing technology increased survival ability of ligneous cuttings, improves their growth, led to the formation of laterals, intensified flowering and increased the standard seedlings output. The best results according to all these parameters were obtained by Fospfoenterin and Complex of microbal preparations in combination with Component 2 treating ligneous cuttings before planting.

Keywords*: microbal preparations, Spiraea x vanhouttei (Briot) Zab., industrial nursery, technology elements.*

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GENETIC DIFFERENTIATION OF HISTORIC CULTIVARS OF HERBACIOUS PAEONIA BASED ON SRAP MARKERS: DOCUMENTATION AND CONSERVATION OF BOTANIC COLLECTIONS

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Introduction

Paeonia L. (family Paeoniaceae) comprises about 35 species of shrubs and perennial herbs distributed widely in the northern hemisphere [20, 26, 29]. The genus possesses great ornamental and medicinal value, which is a reason for its extensive culture, breeding and wide representation in botanical garden collections. Section Paeonia has the most taxa (about 27 herbaceous taxa, including P. lactiflora Pall.) and the most diverse geographic range (from East and Central Asia, the Western Himalayas to the European Mediterranean region). This section has about 1/3 rare to endemic species as well as evidence of complex reticulated evolution that results in incompletely-understood phylogenetic relationships between species [27].

Contemporary cultivated herbaceous peonies mainly belong to P. lactiflora, although there is a great diversity of interspecific and intersectional hybrids. Over 3,000 cultivars have been introduced or bred outside of Eastern Asia since 1820s, half of which are presumed extinct [D. Michener, communication from R. Jakubowoski – ICRA Registrar, unpublished]. Many points of the origin and phylogenetic relationships among P. lactiflora cultivars (and other species) are unclear since their documented history is inconclusive or absent and synonymy is present. To understand the cultivated peony phylogeny, it's domestication history and breeding potential of desirable ornamental characteristics as well as resistance to diseases and adaptability to environmental factors, it is critical to identify, profile, and assess the genetic diversity across the field genebanks of available historical accessions.

Botanical mentioned collections are a national heritage asset and much of it likely now unique. Botanical garden collections are essential for research related to genotypes that will be needed during the pending global climate change [6, 22]. Specifically, botanical gardens collections can function as field genebanks where their rich but selected genetic pool reflects significant artificial selection from complex socio-cultural historical factors as well as acquisition of new genotypes for subsequent educational or research objectives. Effectively understanding this captured diversity and its phylogenetics, evaluating cultivars for their bio-cultural conservation value, and predicting which cultivars carry useful traits for future breeding requires using contemporary molecular genetics approaches [23].

Use of molecular genetics and genomic approaches to resolve fundamental questions on the phylogenetics and origin of cultivated plants from their wild ancestors though domestication has become accepted [3, 5, 13, 17, 18, 28]. Molecular markers for cultivar identification, genetic map construction, genetic diversity assessment, and molecular marker-assisted selection (MAS) have been found useful in many horticultural plants [16], including ornamentals [7]. Within the genus Paeonia, several recent studies document the genetic

diversity of cultivated and wild species of tree and herbaceous peonies and show the high resolution power of different types of molecular markers for phylogenetic and domestication aims [8, 31, 32]. In particular, sequence related amplified polymorphism (SRAP) markers were successfully applied for genetic diversity documentation in various plant species and groups [15, 24], including tree and herbaceous peonies [9, 10, 12]. SRAPs spot coding regions of the genome, for up to 20% are co-dominant, possess capacity to elucidate markers with inherent biological significance, and therefore could facilitate the construction of linkage maps [15, 24].

Molecular certification of the genetic diversity in historical collections (field genebanks) of genus Paeonia is a critical step to resolve the confounded taxonomy and phylogeny of cultivated peonies; this is the first study to survey European, American and Soviet genetic resources of cultivated peonies for analytical depth. These methods provide a unique opportunity to distinguish genotypes/cultivars rigorously, which is intractable when based only on morphological characteristics – especially when historical documentation is lacking. The resultant datasets will help resolve and reconstruct the sequence and geographically dispersed history and process of herbaceous peony domestication in the important regions of its selection – Europe, USA, former USSR.

The aim of this research was to develop SRAP molecular marker systems effective for large-scale fingerprinting of herbaceous Paeonia genetic resources, mainly P. *lactiflora* cultivars, and possessing enough resolution power to discriminate the intraspecific (cultivars), interspecific (hybrids) and species levels, to conduct analysis on the first set of samples and reveal their relationships. Complete molecular profiling of the historically-deep collections of Central Botanical Gardens NAS of Belarus (CBG) and Matthaei Botanical Gardens and Nichols Arboretum of the University of Michigan (MBGNA) and wild parent species should help breeders in their work towards desired characteristics.

Objects and methods of research

Individual research objects were accessions from collections of genus Paeonia of the CBG and MBGNA. The collections of the CBG comprises more than 320 herbaceous genotypes including cultivars from Soviet selection programmes as well as endangered Paeonia species; MBGNA maintains more than 250 herbaceous cultivars of American and European selection as well as Chinese origin. Institutional databases of the peony collections are on-line and contain accession name, morphological description, available information on history and origin [http://mbgna.umich.edu/peony/; http://hbc.bas-net.by/bcb/eng/].

In this study four wild Paeonia species and 50 accessions of cultivated peonies were included: cultivars of P. lactiflora and interspecific hybrids (30 of European, 4 – American and 15 – Soviet selection) and several unresolved accessions for verification. The wild species are P. lactiflora Pall., P. tenuifolia L., P. daurica subsp. mlokosewitschii (Lomakin) D.Y.Hong (further noted as P. mlokosewitschii) and P. anomala L. (Appendix Table).

Material for genotyping was collected at MBGNA and CBG (Table 1) during the growing season (June – August, 2013 and 2014; Table 1). From each analysed peony plant 3 leaves were sampled (bulked), which were dehydrated directly after the harvest using silica gel (Silicagel 60, 0.2-0.5 mm, AppliChem).

Table 1

Locations of the MBGNA and CBG Paeonia collections, sampled for SRAP-genotyping

No	Location	S*	GL, DMS/ DD		GD, km (No)	
			Latitude	Longitude	1	2
1	CBG, Minsk, Belarus	4	53°55'15.1356"N/ 53.920871	027°36'38.8224"E/ 27.610784	4250.85	–
2	MBGNA, Ann Arbor, Michigan, USA	0	42°16'51.7404"N/ 42.281039	083°43'32.3112"E /83.725642	–	4250.85

*Abbreviation: NS – number of samples taken for genotyping, GL – geographic location, GD – geographic distance, noted in km; coordinate format: DMS – degrees minutes seconds, DD – decimal degrees.

Genomic DNA was isolated from silica dehydrated plant leaves by CTAB method [4]. A weighed leaf tissue (100 mg) was ground in a homogenizer (TissueLyser LT, Qiagen), and then 2X CTAB extraction buffer was added, containing 2% w/v of cetyltrimethylammonium bromide (CTAB), 1.41 M NaCl, 0.10 M Tris-HCl, 0.02 M EDTA. RNA degradation in DNA samples was performed as described [11]. Prior the SRAP-analysis the amount of DNA in each sample was equated and its equivalent amount was used for each PCR. DNA samples were stored at -20°C. For the detection of genotypic variability between individuals of the investigated Paeonia genotypes we have tested 4 pairs of SRAP primers (PrimeTech, Belarus): Me05/Em01; Me05/Em10; Me07/Em01; Me07/Em10 (Table 2), described previously for tree and herbaceous peonies of Chinese origin [12]. The primers revealed consistent amplification and polymorphism between species, interspecific hybrids and P. lactiflora cultivars, and were used in our study.

Table 2

Forward and reverse SRAP primers used in this study

Primer	Type	Sequence (5'→3')	T m, °C
Me05	Forward	TGAGTCCAAACCGGAAG	47
Me07	Forward	TGAGTCCAAACCGGACA	47
Em01	Reverse	GACTGCGTACGAATTAAT	43
Em10	Reverse	GACTGCGTACGAATTCAG	48

The PCR reaction mixture (25 µl) contains 60 ng of genomic DNA, 200 µM dNTPs, 2.5 mM MgCl2, 20 pM of each primer, 10x buffer, and 1 U Taq DNA polymerase (Primetech, Belarus). The amplification was carried out in Sure Cycler (Type 8800, Agilent Technologies, USA) using the following program: 3 min denaturing at 94°C, eight cycles of 30 sec denaturing at 94°C, 30 sec annealing at 37°C, and 90 sec elongation at 72°C. In the following 32 cycles the annealing temperature was increased to 50°C, with a final elongation step of 7 min at 72°C. Each PCR product (15 µl) was fractionated into microchips (Bioanalizator 2100, Agilent) or into 1.2% agarose gel and visualized by staining with ethidium bromide. Electrophoresis was carried out at a constant 100 V for 120 min at room temperature. Ladder Markers (100 bp and 1kb, Primetech, Belarus) were loaded each time as the reference for fragments size estimation. Gels were documented using a Molecular Imager VersaDoc MP 4000 image system (BioRad, USA). The molecular sizes of the fragments were calculated using specialized software Bioanalyser Expert 2100 (Agilent) or QuantityOne (BioRad) on the basis of molecular weight standards.

Data analysis. The profiles of amplified DNA fragments obtained by SRAP-PCR analysis were the basis for the creation of binary matrices, where the presence of the amplicon was designated as "1" and the absence - as "0". Only distinct, discrete and reproducible amplicons were scored. A marker was considered as polymorphic if fragment was absent in at least one of the accessions. Reproducibility was estimated by scoring and comparing fragments profiles produced under identical conditions of at least two biological repetitions. Polymorphism information content of each primer (PIC) was calculated according by the following equation: PIC = 1 - ∑pi2, where pi is the frequency of the ith allele for each SRAP marker locus in the set of 54 peony accessions investigated [2].

Genetic similarities between cultivars were measured by the Nei similarity coefficient based on the proportion of shared alleles [19]. The NJ (neighbor-joining), UPGMA (unweighted pair-group method with arithmetic averages) trees were constructed using the Treecon software [30]. The wild species Paeonia daurica subsp. mlokosewitschii (Lomakin) was used as an outgroup in the NJ trees as a most distant species based on known phylogeny [25]. The number of 1,000 replicates was used for all bootstrap tests. Calculation of genetic diversity indices and the number of rare alleles, principal coordinate analysis (PCoA) were performed using GenAlex [21].

Predicting the morphologic characteristics and origin data beyond genotyping data. Predicting the following parameters: type of flower (single, semi-double, double), season of blooming (very early, early, early midseason, midseason, late midseason, late and very late), year of introduction, region (country of introduction) was performed using simple linear regression model and Poisson regression model.

Results and discussion

1. Levels of polymorphism and molecular identification of Paeonia cultivars revealed by SRAP markers. Iteratively selected informative SRAP primers were used to detect polymorphisms at the intra- and interspecific levels, i.e. to show the variability of genomic DNA of different P. lactiflora cultivars and Paeonia species, (see Table 2). The method produced discrete reproducible amplicons; their set were unique to each studied genotype differentiate every genotype. The amplicons' profiles obtained using SRAP primers are shown in Table 3.

Table 3

Characteristics of the amplicons of genotypes of Paeonia obtained with SRAP markers

Primer pair	No markers	Diapason of fragments length, bp	No of fragments per sample (min/max/aver)	No of polymorphic markers/ %	PIC
Me05 / Em01	30	74–1908	3/16/9.5	30/100	0.325
Me05 / Em10	22	84–869	3/13/8	22/100	0.329
Me07 / Em01	36	98–1756	6/16/11	36/100	0.247
Me07 / Em10	25	97–1065	5/13/9	23/92	0.159
Mean	28,3	–	4.25/14.5/9.4	27.8/98	0.265
Total	113				

The selected primers pairs generated amplicons in the size range from 74−1908 bp, the number of received markers varied from 22 to 36. The percentage of polymorphic loci identified with primers Me05/Em01, Me05/Em10 and Me07/Em01 was 100%, with primer Me07/Em10 − 92%. The total number of generated SRAP markers for the studied genotypes of Paeonia was 113, with an average of 9.38 markers per sample. Percentage of polymorphic content of primer pairs varied from 0.159 (for Me07/Em10 combination) to 0.329 (for

Me05/Em10), with an average of 0.265. Primers revealed number of genotype-specific markers. For P. daurica subsp. mlokosewitschii (Lomakin) D.Y.Hong applied SRAP primers detected 4 specific markers; for P. anomala L. – 3; for P. tenuifolia L. – it was revealed 3 unique bands. Among P. lactiflora Pall. cultivars by 1 individual markers possesses 'Albert Crousse' (Crousse, 1893), Augustin D'Hour (Calot, 1867), Suruga (Millet, 1955) and 'Vesennii', 3 individual markers were revealed for 'Novost Altaja' (Lutchnik, 1963).

2. Genetic similarity and cluster analysis of Paeonia species and cultivars. The values of Nei's genetic distance for analysed genotypes (based on the frequency of 113 SRAP alleles) were used to construct a cluster maps using the UPGMA and NJ algorithm. NJ phylogram of pruned genetic distances data (50 accessions) is presented in Fig. 1. (In this analysis accessions with unresolved labels were excluded). We interpret Fig. 1 to represent the genetic relationships among accessions. On NJ dendrogram (see Fig. 1) all accessions are distinctly separated from each other and 2 major groups are evident and assigned as cluster I and II. Cluster I contains species P. mlokosewitschii, P. teniufolia, P. anomala, and interspecific hybrid 'Orlenok'. Cluster II contains wild P. lactiflora itself and its domesticated cultivars. For this analysis P. mlokosewitschii served as an outgroup for the phylogeny data [25, 14]. Its role as an outgroup from the other taxa is also supported by this research (genetic distances; data not presented). Similarly, the clustering of P. lactiflora x tenuifolia hybrid 'Orlenok' between its parent species is consistent with its breeding history (Fomitcheva, 1963) [1] and further confirms the power of developed SRAP markers to resolve hybrid interspecific origin of Paeonia cultivars.

Cluster II has an internal hierarchy: P. lactiflora is relatively separated while clusters A, B and C are suggested. It reflects cultivar-landraces of European (fundamentally French and English), American and Soviet selection. Further subclusterization is observed. Cluster A includes the cultivars 'Albert Crousse' (Crousse, 1893; Double, pink, midseason), 'Arcturus' (Auten, 1933; Single, red, very early) and 'Arlequin' (Dessert & Mechin, 1921; Anemone, pink, midseason). Given the presumed later parentage of the single/anemone forms (if derived from Japanese selections introduced after the mid- to late-1800s), the anomaly is 'Albert Crousse', indicating research with additional related cultivars is needed.

Cluster B1 holds 'Novost Altaya' (CBG accession), 'Arkady Gaidar' (syn. 'Arkadij Gaydar'), 'Pamiati Gagarina', 'Mirnyi', and 'Belyi Parus'. Their breeding history suggests a common derivation. 'Mirnyi' and 'Belyi Parus' (Sosnovets) were selected from open-pollination work involving intervarietal and interspecific parents at the Botanical Garden of the Moscow State University starting in 1951 [1]. Cultivars 'Pamiati Gagarina' and 'Arkady Gaidar' were both bred by Krasnova in 1957 and 1958, respectively; all are historically congruent.

Assessing authenticity of all samples of 'Novost Altaya'(CBG, A, K) – an interspecific hybrid of P. anomala and P. lactiflora, has been requested from this study. When plotted all three 'Novost Altaya' samples from different original sources all placed differently on the dendrogram (data not presented). The accession 'Novost Altaya_A' clusters with the interspecific hybrid 'Orlenok', and is likely authentic. Based on clustering analysis Novost Altaya CBG was likely mislabeled. This shows the method's power to resolve identification queries where only morphological features are suggestive.

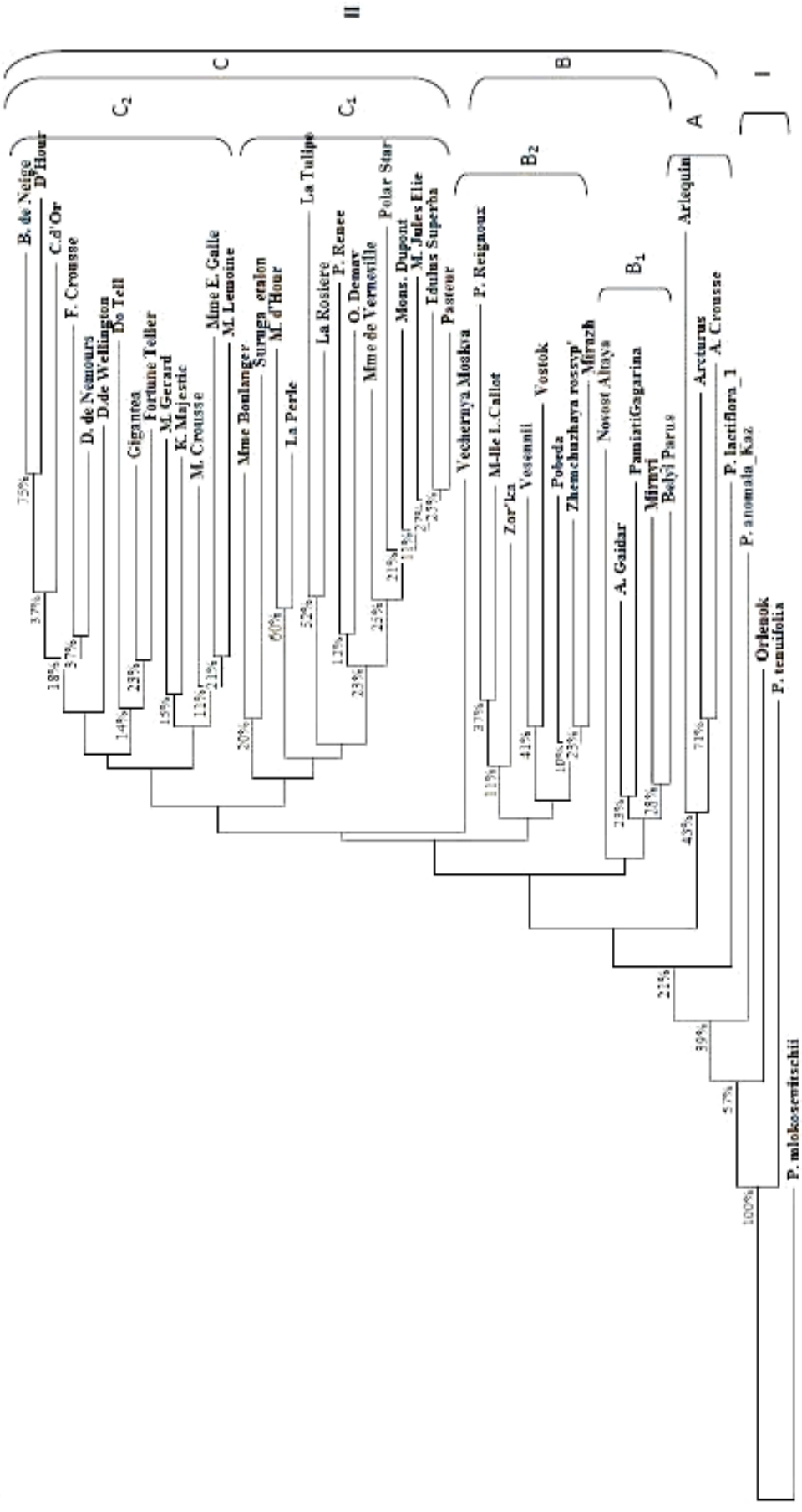

Fig. 2 Consensus Neighbor-joining dendrogram depicting genetic relationships (Nei) between *Paeonia* genotypes generated from 113 SRAP markers. *P. daurica mlokosewitschii* is used as an outgroup. Numbers above branches represent bootstrap values. For genotypes full names see Appendix Table

The rest of Soviet selections and two historical French cultivars (‘M-lle Leonie Callot’ and ‘Pierre Reignoux’) are grouped at cluster B2. A future goal is to much better resolve the historic French phylogeny with additional samples and taxa. One possibility is these cultivars were bred from different parental cultivars than the others, thus grouping them with the Soviet cluster. Lacking breeding records, expanding study to include contemporary sister-cultivars is likely the best approach. Intriguingly in the Soviet group, ‘Vecheriaya Moskva’ was separated yet it; closely distributed ‘Zhemchuzhnaya rossyp’ and ‘Mirazh’ are of Japanese flower type.

The distant position of cluster C from the species (Cluster I) based on developed SRAP polymorphic genomic regions is significant. Although the historically oldest French cultivars are here, these were likely bred from (or were simply renamed) old Chinese landraces-cultivars that were then “new” in Western Europe. The long history of domestication in China, presumably not involving repeated breeding with wild P. lactiflora or any other herbaceous peony species, would account for the genetic distance indicated here. This is counter-intuitive since one would expect the historically “old” cultivars to cluster basally with the species. However, the history of Paeonia domestication in Western Europe and America is doubtlessly based on highly-developed Chinese (and later Japanese) domesticates as ancestral, not the wild species. Thus the unexpected “old” French cultivars are removed from species and opens new research questions.

To examine fine relationships among the peony accessions employed, Principal coordinates analysis (PCoA) was performed using standardized molecular data. It is graphically presented in Fig 3. In general, the relationships between genotypes revealed by PoCA was conceptually consistent with the data obtained in this study by both UPGMA and NJ clustering analysis. The total variance explained by the first, second and third principal coordinates using PoCA was 6.18%, 5.12% and 4.49%, respectively. PC2 differentiates a subset of species and interspecific hybrids from most of the P. lactiflora cultivars; PC1 reflects a clear geographic European-USSR gradient in Paeonia, with spatially dispersed (unclustered) US cultivars.

All analysed wild herbaceous peony species such as P.mlokosewitschii, P. anomala, P. lactiflora and P. tenuifolia were clearly distant from all other peony accessions. The interspecific hybrids ‘Orlenok’ and ‘Novost Altaya’ (A and K accessions) are close to their parent species, so PCoA data are in congruent with previously presented cluster analysis data.

Compact and close enough distribution of Soviet cultivars to species, compared to another groups, may be evidence of breeding that included wild Paeonia species, while the broad range of other cultivars is more of a continuum with the exception of a cultivar cluster centered around the problematic ‘A Crousse’ (as already discussed). The scattered distribution of American cultivars among European may reflect gene flow through additional introductions directly from China and Japan, and may also provide indication for a desire for phenotypically different forms driving novel breeding. The small sample size for the American group (4 accessions) indicates a bigger study is needed.

Further analysis of relationship of Paeonia accessions by their region of origin (wild species, Europe, USSR and USA) by Nei genetic distance/ similarity indices is summarized in Table 4. The closest regions are Europe and USSR (genetic distance – 0.024), and most distant are Wild and USA groups (genetic distance – 0.103). At the same time USA and USSR groups are relatively equally distant to the European group analysed (genetic identity 0.97 and 0.98, respectfully).

This finding is consistent with the suggestion that US breeders by early 1900s were using novel source material rather than re-breeding only from existing European cultivars. What is needed is a better understanding of what was being sought by the breeders and what material was available.

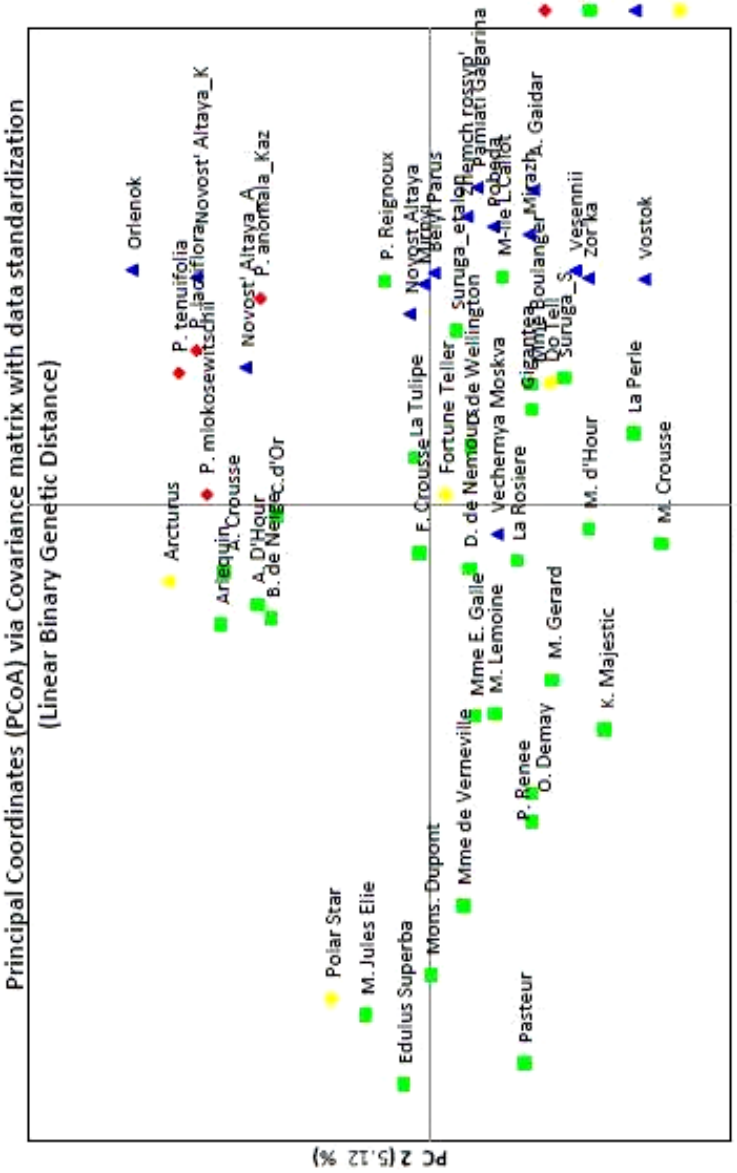

Fig. 2 Diagram showing the relationships among 53 accessions of *Paeonia* based on PCoA using 113 SRAP markers
Visualization of genetic relationships among Paeonia cultivars and species. PC axis 1 and PC2 were calculated from 49 *Paeonia* cultivars samples, and 4 *Paeonia* species were subsequently projected onto these axes. The proportion of the variance explained by each PC is shown in parentheses along each axis. The wild species samples are represented by diamond, European cultivars – as rectangles, USSR – as triangles, USA as circles and the region of their origins are indicated in the legend. Full names, description and geographic origin of the samples are described in the Appendix Table.

In USSR the peony breeding program began in 1949 based on about 200 cultivars, mostly of French selection; wild species hybridization was widely applied [1].

Table 3

Nei's genetic identity (above diagonal) and genetic distance (below diagonal) between groups of Paeonia by region of origin

Group	Wild	Europe	USSR	USA
Wild	****	0.9290	0.9363	0.9018
Europe	0.0736	****	0.9763	0.9683
USSR	0.0658	0.0240	****	0.9544
USA	0.1034	0.0323	0.0467	****

3. Predicting morphologic and non-morphological characteristics for future breeding work. The sets of genetic markers, characteristic to each accession were analyzed for significant interactions with standard morphological descriptors of this genotype that could be logically (including biologically) coded as a numeric value. The ‘year of introduction’ and ‘season of bloom’ (early, middle, late) revealed the significant correlation with several markers at analyzed genotypes (data not shown). The other parameters as type of flower (single, semidouble, double) and ‘region’ (country of introduction) did not reveal significant correlation with genetic data. Since all floral forms have been bred in all regions, this was anticipated. For “year” simple linear regression model revealed significant relationship with SRAP marker 1.6_410bp (p= 0.0171), indicating genetic diversity in the cultivars increases over time, which makes sense if new genotypes were available. Poisson regression analysis of genotypic data and ‘season of bloom’ produced a significant relationship for 1 SRAP marker 1.16_829bp (p= 0.036). Both correlations suggest that a deeper survey of the molecular markers could help finding genetic linkage with favorable traits and will be useful in both applied and theoretical work on herbaceous peony breeding and certification.

Conclusions

Applied SRAP analysis allow to generate 113 markers (in average 9.4 loci per primer), and demonstrated high resolution power for effective discrimination herbaceous Paeonia on the specific level, interspecific (hybrids), and intraspecific (P. lactiflora cultivars). This is well supported by the fact, that species, interspecific hybrids ‘Orlenok’ and ‘Novost Altaya’ and P. lactiflora cultivars were found to be characterized by several unique genotype markers.

Clusterization analysis using UPGMA and NJ algorithm, and also results of principal coordinate analysis allow for the first time to generate relationship between the studied genotypes, which revealed its consistency with the region of origin of genotypes, as well as with available data on the pedigree. Specifically, European landraces and Soviet cultivars of P. lactiflora were clustered distinctly by groups; interspecific hybrids ‘Orlenok’ and ‘Novost Altaya’ were located between their parent species, although it is necessary to study the contribution of each parent more precisely.

Developed markers and genotypic passports of all studied genotypes could be thus used for the delimitation and identification of P. lactiflora cultivars including interspecific hybrids, revision of the unresolved origin issues, and relationship calculation, exchange of the certified material. Regression analysis in combination with SRAP markers is a powerful tool to produce markers important for MAS (such as SCAR, SNP, SSR, QTL), useful to construct linkage map of valuable traits of Paeonia cultivars [15, 24]. When added with markers of chloroplast genome regions, large-scale capability of next-generation techniques, and on a wider set of samples from the studied regions (Europe, USA, USSR, and China as an initial

center of domestication), it could be used for solving phylogeography of cultivated P. lactiflora.

Acknowledgement

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Appendix Table
Characteristics of 54 Paeonia cultivars and species in this study

o*	Name (Transliteration; variants)	Originator	Year	Country	Description**
	Белый Парус (Belyi Parus)	Sosnovets	1961	USSR	Lac. Double, white, semi early
	Памяти Гагарина (Pamiati Gagarina)	Krasnova	1957	USSR	Lac. Double, pink, midseason
	Новость Алтая (Novost' Altaya; Novost Altaja)	Lutchnik	1963	USSR	P.lactiflora x anomala hybrid, Single, lilac-rosy, early
	Мираж (Mirazh; Miraj, Mirage)	Krasnova	1959	USSR	Lac. Japanese, pink, midearly
	Мирный (Mirnyi, Mirnij, Mirnii)	Sosnovets	1952	USSR	Lac. Double, pink white, early
	Аркадий Гайдар (Arkady Gaidar; Arkadij Gaydar)	Krasnova	1958	USSR	Lac. Double, red, very late
	Победа (Pobeda)	Kupoljan	1957	USSR	Lac. Double, red, late
	Suruga_etalon	Cyt.: Millet	before 1955	France	Lac. Japanese, red, late
	Жемчужная россыпь (Zhenchuzhnaya rossyp)	Gorobetz-Tyran	1989	USSR	Lac. Japanese, pink, late
	Вечерняя Москва (Vechernya Moskva)	Sosnovets	1961	USSR	Lac. Double, crimson, magenta, late
	Восток (Vostok)	Krasnova	1957	USSR	Lac. Double, dark violet, midseason
	Зорька (Zor'ka; Zorka)	Sosnowets – Fomitschewa	1965	USSR	Lac. Double, light pink, late
	Весенний (Vesennii; Vesennij, Wesennij)	Krasnova	1959	USSR	Lac. Double, light pink, midseason
	M-lle Leonie Callot (Syn. Mons. Charles Levêque')	Calot	1861	France	Lac. Double, pink, late midseason
	Pierre Reignoux	Dessert	1908	France	Lac. Double, pink, midseason
	Paeonia anomala L.	N/a	N/a	N/a	Pink form
	Paeonia anomala L.	N/a	N/a	N/a	White form
	Suruga	Millet	1955	NL	Lac. Japanese, red, late. R.
	Новость Алтая_K (Novost' Altaya, Novost Altaja)	Lutchnik	1963	USSR	P.lactiflora x anomala hybrid, Single, lilac-rosy, early. R
	Новость Алтая_A (Novost' Altaya, Novost Altaja)	Lutchnik	1963	USSR	P.lactiflora x anomala hybrid, Single, lilac-rosy, early. R
	Орленок (Orlenok; Orlionok)	Fomitcheva	1963	USSR	Lac. Single, red, early
	Paeonia lactiflora Pall.	N/a			
	Paeonia tenuifolia L.	N/a			
	Paeonia daurica subsp. mlokosewitschii (Lomakin) D.Y.Hong	N/a			
	Albert Crousse	Crousse	1893	France	Lac. Double, pink, midseason
	Arcturus	Auten	1933	USA	Lac. Single, red, very early
	Arlequin	Dessert & Mechin	1921	France	Lac. Anemone, pink, midseason

	Augustin D'Hour (Syn. General MacMahon)	Calot	1867	France	Lac. Double, red, midseason
	Boule de Neige	Calot	1862	France	Lac. Double, white, early midseason
	Couronne d'Or	Calot	1873	France	Lac. Double, white, late
	Do Tell	Auten	1946	USA	Lac. Japanese, pink, midseason
	Duc de Wellington	Calot	1859	France	Lac. Double, white, NL, but midseason
	Duchesse de Nemours	Calot	1856	France	Lac. Double, white, early
	Felix Crousse	Crousse	1881	France	Lac. Double, red, late midseason
	Fortune Teller	Auten	1936	France	Lac. Single, red, not listed
	Gigantea	Calot	1860	France	Lac. Double, pink, early midseason
	Kelway's Majestic	Kelway	1929	England	Lac. Japanese, red, early
	La Perle	Crousse	1886	France	Lac. Double, pink, midseason
	La Rosiere	Crousse	1888	France	Lac. Semi-double, white, midseason
	La Tulipe	Calot	1872	France	Lac. Double, pink, early midseason
	Marguerite Gerard	Crousse	1892	France	Lac. Double, pink, midseason
	Marie Crousse	Crousse	1892	France	Lac. Double, pink, midseason
	Marie d'Hour	Calot	1883	France	Lac. Double, pink, midseason
	Marie Lemoine	Calot	1869	France	Lac. Double, white, late
	Madame Emile Galle	Crousse	1881	France	Lac. Double, pink, late
	Madame Boulanger	Crousse	1886	France	Lac. Double, pink, late midseason
	Madame de Verneville	Crousse	1885	France	Lac. Double, white, early
	Monsieur Dupont	Calot	1872	France	Lac. Double, white, late midseason
	Monsieur Jules Elie	Crousse	1888	France	Lac. Double, pink, early
	Octavie Demay	Calot	1867	France	Lac. Double, pink, early
	Pasteur	Crousse	1896	France	Lac. Double, pink, late midseason
	Petite Renee	Dessert & Mechin	1899	France	Lac. Japanese, pink, midseason
	Polar Star	Sass & Interstate	1932	USA	Lac. Japanese, white, midseason
	Edulus Superba	Lemon	1824	France	Lac. Double, pink, early

Note: *Accessions 1-24 – are from the collection of CBG; 25-54 – from MBGNA. ** R – accessions for revision; Lac. – P. lactiflora cultivar; N/a – information not applicable; NL – information not listed in available sources

Vlasava[1] N.B., Michener[2] D.C., Yukhimuk[1] A.N., Gaishun[1] V.V., Bryant[3] R., Agabalaeva[1] E.D., Spiridovich[1] E.V. Genetic differentiation of historic cultivars of Herbacious Paeonia based on srap markers: documentation and conservation of botanic collections // Works of the State Nikit. Botan. Gard. – 2014. – V. 139 – P. 177 – 190.

The article presents data of triennial studies in using of microbial preparations with various spectrum and organo-mineral fertilizer for growing seedlings of *Spiraea x vanhouttei* (Briot) Zab. in an industrial nursery. It was found that integration of these elements in *S. x vanhouttei* seedling growing technology increased a survival rate of the hardwood cuttings, improved their growth, stimulated lateral shoots formation, intesified flowering and increased the output of standard seedlings. The best results on all these parameters were obtained when hardwood cuttings were priviously treated with Fosfoenterin and complex of microbial preparations together with Component 2.

Key words: *microbial preparationss, Spiraea x vanhouttei (Briot) Zab., industrial nursery, technology elements*

UDK 582.912.46: 577.213.3/575.22

ASSESSMENT OF GENETIC DIVERGENCE OF LILAC (*SYRINGA* L.) VARIETIES OF BELARUSIAN SELECTION BASED ON INTEGRATED APPLICATION OF RAPD- AND ISSR-MARKERS

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Introduction

Creation of lilac collection in Central Botanical Garden of NAS of Belarus (CBG) joins the work of several generations of botanists-introducers and breeders: N.V. Smolski, V.F. Bibikova, E.A. Buraya, G.I. Matusevich, N.V. Makedonskaya [13]. For lilac culture employees of Central Botanical Garden in 70-80s widely used a variety of breeding and genetic techniques, such as selection of seedlings obtained after random pollination and hybridization. For hybrid seedlings lilac cultivars were crossed as well as species. By the intercrosses attempts to improve some economical and decorative features in the introduced lilac species were made. On the base of experimental studies Veronica Feodorovna Bibikova obtained lilac cultivars with large simple and double flowers of pure colours, abundant and long blooming 'Lebedushka', 'Nesterka', 'Pavlinka', 'Minchanka', 'Zashchitnikam Bresta', 'Vera Khoruzhaya' 'Pamyati A.T. Smolskoy', 'Uspeh', 'Konstantin Zaslonov', 'Lunnyi Svet', 'Zorka Venera', 'Partisanka', 'Khoroshee Nastroenie', 'Marat Kazei', 'Svityazanka', 'Belaruskie Zori', 'Polesskaya Legenda' [1]. Based on the huge experimental work with the original cultivars of French breeding, with limited contact with breeders of lilac in other countries, minimum access to the main sources of information on lilac collection of Belarusian cultivars was created, and, like the collection of cultivars by Leonid Kolesnikov, it is an unique line of lilac evolution [13].

Along with traditional methods of plant genetic diversity conservation *ex situ*, application of plant cell biotechnology that ensure preservation of valuable breeding samples of previous years, accelerated acquisition and propagation of new forms and lines of ornamental crops with improved traits of stress-resistance and increased productivity is becoming increasingly important in living botanical collections [17]. Regenerative potential of primary meristems most fully realized in micropropagation. This is especially important for propagation of plant genotypes and decorative forms with valuable characteristics that are difficult to maintain during the seed reproduction. In Central Botanical Garden of NAS of Belarus regenerated lilac plants of Belarusian breeding were obtained by methods of biotechnology [15], great assistance was provided by the employees of N.V. Tsitsin`s Botanical Garden RAS [3]. At present, *in vitro* collection of the genus *Syringa* in CBG NASB is presented by 67 varieties; two more species, several cultivars of L.A. Kolesnikov selection and new varieties of American selection are at the stage of sterile culture preparing. An important objective of this work is *in vitro* introduction of all cultivars bred in CBG as the most valuable objects of genetic diversity and national heritage.

A necessary step in creating, preserving and maintaining *in vitro* collections, as well as in the exchange of material between the institutions is harmonization of the rules for keeping collections and development of screening techniques on the base of molecular genetic markers. The last is of great practical importance for collections` certification, assess of their genetic diversity, forming core collections, identification and selection of the most valuable taxa for further breeding. [6]

Systematization and documentation of lilac collection in CBG NASB started in 2002 during the creation of an integrated database of lilac collection, including morphological characteristics of genotypes and the first data on their genetic certification [7, 8]. Nowadays information retrieval database of Belarusian *in vitro* collections is supplemented with molecular genetics passports of the objects providing efficient storage and processing of information, research, enhance cooperation and information exchange in order to preserve biodiversity. [2]

An integrated approach multilocus DNA markers, when some marker systems are used at the same time, is widely used for plant genotyping and it gives the opportunity to differentiate both interspecific and intraspecific genotypes, resolve the problems of cultivars origin and affinity, provide valuable information for further breeding with minimum time and costs [14, 16]. Literature presents a small number of studies on tmolecular marking of *S. vulgaris* cultivars basically involving marker systems and genotypes other than studied in this work [2, 10].

This article presents the results of molecular genotyping of lilac cultivars of Belarusian breeding in the collection of CBG NASB based on polymorphism RAPD- and ISSR-loci identification. This study is aimed for development of effective systems of genetic markers (RAPD and ISSR) for the genus *Syringa*, followed by differentiation and certification of lilac cultivars and creation of a genetic passport for each genotype. Such passports give the possibility not only to distinguish samples from each other, but also to determine the degree of genetic similarity between them, identify the most unique genotypes [5, 11, 12], select the original material for breeding, monitor genetic purity and uniformity of varieties in creating *in vitro* collections and multiplication of plant material.

Materials and methods

The study included 13 lilac varieties of Belarusian selection from the collection of CBG NASB. Data of studied varieties origin are shown below (Table. 1).

Table 1

Lilac varieties of Belarusian selection from the collection of CBG NASB

	Name (Transliteration; variants)	Parent forms	Species identification
	Lebedushka	Mme Abel Chatenay	*Syringa vulgaris* L.
		Reaumur	*Syringa vulgaris* L.
	Pavlinka	Mme Abel Chatenay	*Syringa vulgaris* L.
		Reaumur	*Syringa vulgaris* L.
	Zashchitnikam Bresta (For Defenders of Brest)	Mme Abel Chatenay	*Syringa vulgaris* L.
		Reaumur	*Syringa vulgaris* L.
	Minchanka	Mme Abel Chatenay	*Syringa vulgaris* L.
		Reaumur	*Syringa vulgaris* L.
	Vera Khoruzhaya	Mme Abel Chatenay	*Syringa vulgaris* L.
		Reaumur	*Syringa vulgaris* L.
	Khoroshee Nastroenie (Good Mood)	Mme Abel Chatenay	*Syringa vulgaris* L.
		Reaumur	*Syringa vulgaris* L.
	Lunnyi Svet (Moonlight)	Mme Abel Chatenay	*Syringa vulgaris* L.

		Reaumur	*Syringa vulgaris* L.
	Polesskaya Legenda (Woodland Legend)	Ludwig Shpaeth	*Syringa vulgaris* L.
		Hyacinthiflora	*Syringa* × *hyacinthiflora* Rehder (*Syringa vulgaris* L. × *Syringa oblata* Lindl.)
	Pamyati A.T. Smolskoy	Ludwig Shpaeth	*Syringa vulgaris* L.
		Hyacinthiflora	*Syringa* × *hyacinthiflora* Rehder (*Syringa vulgaris* L. × *Syringa oblata* Lindl.)
0	Partisanka	Ludwig Shpaeth	*Syringa vulgaris* L.
		Hyacinthiflora	*Syringa* × *hyacinthiflora* Rehder (*Syringa vulgaris* L. × *Syringa oblata* Lindl.)
1	Konstantin Zaslonov	Hyacinthiflora	*Syringa* × *hyacinthiflora* Rehder (*Syringa vulgaris* L. × *Syringa oblata* Lindl.)
		Reaumur	*Syringa vulgaris* L.
2	Zorka Venera	Hyacinthiflora	*Syringa* × *hyacinthiflora* Rehder (*Syringa vulgaris* L. × *Syringa oblata* Lindl.)
		Reaumur	*Syringa vulgaris* L.
3	Svityazanka	Hyacinthiflora	*Syringa* × *hyacinthiflora* Rehder (*Syringa vulgaris* L. × *Syringa oblata* Lindl.)
		Reaumur	*Syringa vulgaris* L.

Genomic DNA preparations were prepared from silica dehydrated leaf tissue using CTAB-method with modifications [5, 9]. Quality and concentration of the DNA preparations were measured spectrophotometrically. Multilocus DNA fingerprinting was performed using RAPD- and ISSR-PCR techniques. After a preliminary screening for PCR it was selected five of the most informative primers, i.e. effectively identify genetic variability at intraspecific level among all genotypes: three RAPD (OPA-18, OPE-02, OPP-09) and two ISSR (UBC-808, UBC-862). PCR was performed in 25μl reaction mixture SureCycler device 8800 (Agilent). Fragment analysis and visualization of the amplification products was performed by capillary electrophoresis instrument Bioalalyzer 2100 (Agilent) (Fig. 1). Calculation of genetic distances, UPGMA clustering and construction of phylogenetic trees were performed using the software Treecon ©.

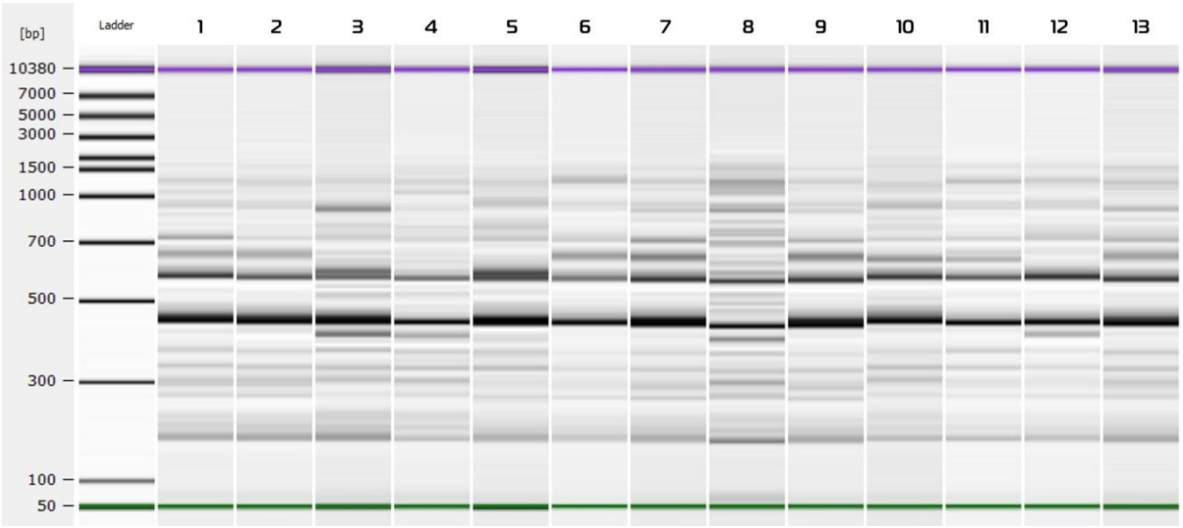


Fig. 1 Representative electrophoretic separation of the amplification products of genomic DNA of 13 lilac varieties with primer UBC-862

1 - 'Lebedushka' 2 - 'Zashchitnikam Bresta' 3 - 'Pavlinka' 4 - 'Minchanka', 5 - 'Zorka Venera', 6 - 'Partisanka' 7 - "Pamyati A.T. Smolskoy" 8 - 'Polesskaya Legenda' 9 -'Vera Khoruzhaya' 10 - 'Lunnyi Svet'11 - 'Konstantin Zaslonov' 12 - 'Svityazanka' 13 -'Khoroshee Nastroenie"; Ladder - molecular weight marker.

Results and discussion

To investigate genetic differentiation of lilac cultivars of Belarusian selection complex method of multilocus DNA fingerprinting based on two PCR techniques: RAPD and ISSR was used. Pre-selected primers (RAPD: OPA-18, OPE-02, OPP-09; and ISSR: UBC-808, UBC-862) generated distinct and reproducible amplicons, which set is unique for each of the investigated cultivars. Table 2 shows the spectra of the amplicons obtained using RAPD and ISSR primers. Informativeness of the primers used varied. Thus, maximum number of loci - 23 (including 15 polymorphic) was identified using primer UBC-808 min - 11 (including four polymorphic) generated with primer OPP-09 (see Table 2). In total, 76 loci markers were identified - 40 RAPD- and 36 ISSR-markers, respectively. In this pool 44 markers were polymorphic. Both PCR techniques revealed high level of polymorphism in the studied lilac cultivars - an average of 57.89%. Maximum polymorphism was detected using primer OPA-18 (66.67%), the lowest - 36.36% with the amplification primer OPP-09.

Table 2

Characteristics of Syringa spp. Amplicons spectra, generated by RAPD- and ISSR-primers

Primer	Nucleotide sequence, 5′→3′	Number of amplicorns		Polymorphism degree, %
		Total	Polymorphic	
RAPD-primers				
OPA-18	AGGTGACCGT	12	8	66.67
OPE-02	GGTGCGGGAA	17	10	58.82
OPP-09	GTGGTCCGCA	11	4	36.36
ISSR-primers				
UBC-808	AGAGAGAGAGAGAGAGC	23	15	65.22
UBC-862	AGCAGCAGCAGCAGCAG C	13	7	53.85
Total number:		76	44	—
An average meaning:		15.2	8.8	57.89

Multilocus DNA fingerprinting for 13 *Syringa* varieties of Belarusian selection using 3 RAPD- and 2 ISSR-primers gave us possibility to differentiate all studied genotypes, to

develop markers, including cultivar-specific ones, create unique profiles for each of them and calculate genetic distances relationship/distance between genotypes. Thus, used RAPD + ISSR approach was adapted for genetic certification of *Syringa* spp. genotypes. Based on RAPD and ISSR-markers for 13 lilac cultivars Multilocus genetic passports were made. Table 3 shows an example of a genetic passport for variety Polesskaya Legenda (Woodland Legend). All data of DNA typing for samples of Belarusian selection lilac were included in a separate section "Molecular genetic passport" of information retrieval system Hortus Botanicus Centralis - Info (№ GR 20053449 from 14.11.2005). This system serves as a source of data for sites "Botanical Collections of Belarus» (http://hbc.bas-net.by/bcb/) and sections of the portal of the Council of Botanical Gardens of Russia, Belarus and Kazakhstan (http://hortusbotanicus.ru), that provides a base for enhanced cooperation and information exchange in order to preserve biodiversity.

Table 3

Representative multilocus genetic passport of Belarusian selection lilac cultivar Polesskaya Legenda (Woodland Legend)

Variety Polesskaya Legenda
Markers
OPA18255, OPA18355, OPA18370, OPA18395, OPA18430, OPA18590, OPA18930, OPA181030, OPA181225
OPE02260, OPE02270, OPE02355, OPE02415, OPE02440, OPE02480, OPE02495, OPE02530, OPE02570, OPE02650, OPE02775, OPE02890, OPE02945, OPE021380
OPP09260, OPP09365, OPP09485, OPP09535, OPP09610, OPP09645, OPP09665, OPP09780, OPP09875, OPP09980
UBC808210, UBC808250, UBC808265, UBC808330, UBC808355, UBC808420, UBC808440, UBC808455, UBC808480, UBC808525, UBC808550, UBC808595, UBC808665, UBC808700, UBC808810, UBC808885, UBC808950
UBC862185, UBC862270, UBC862335, UBC862375, UBC862450, UBC862580, UBC862605, UBC862725, UBC862960

Developed methodical scheme of lilac cultivars genotyping on the basis of complex RAPD- and ISSR-markers was used for molecular genetic documentation of lilac living collections, including for verification samples of *in vitro* collection. It is known that somaclonal variations in plants regenerated *in vitro*, can be very high, so monitoring and maintaining of the genotype stability is important for keeping obtained microshoots. This work was initiated by comparing microclones from *in vitro* collections of CBG NASB and GBS RAS that are supported in the collections of various botanical gardens, in particular varieties Partisanka and Svityazanka [4].

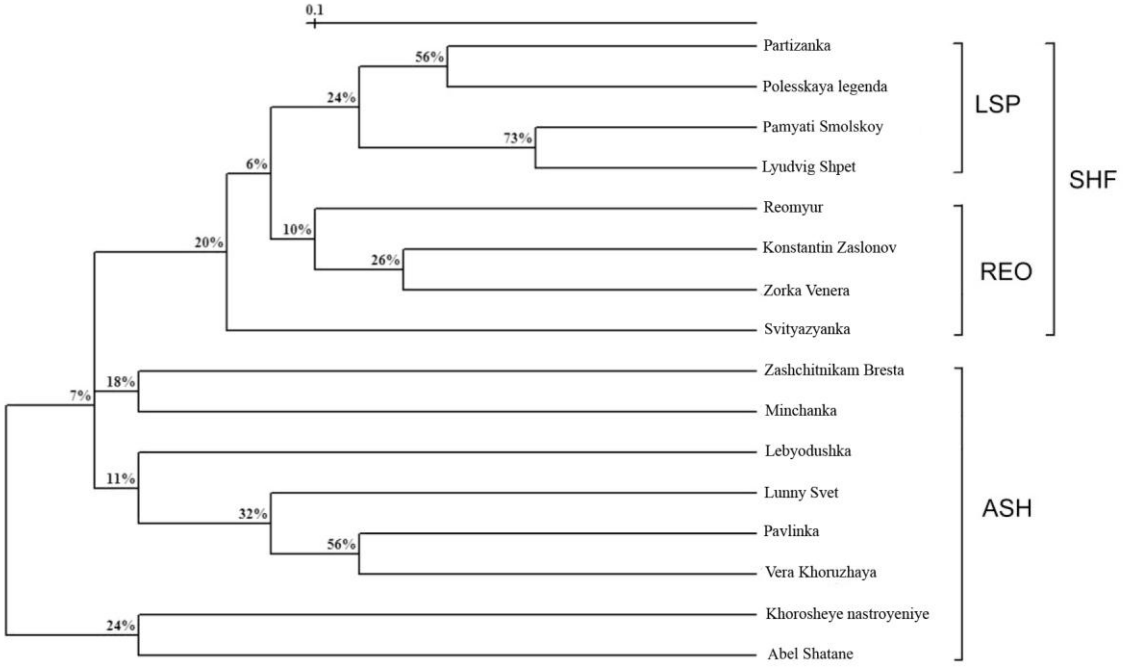
In this work we assessed varietal conformity for genotypes of Belarusian selection 'Lunnyi Svet' (Moonlight), 'Pavlinka', 'Lebedushka', 'Zashchitnikam Bresta' (For Defenders of Brest) under *in vitro* propagation. With the help of developed RAPD- and ISSR-markers conformity of material propagated *in vitro* and collections of the open air was affirmed. In a number of cases variability of genetic homogeneity parameters (profiles of amplicons, genetic distances; data are not shown) was detected. It may be due to the emergence of acceptable changes resulted different responses of genotypes for cultivation under *in vitro* conditions, as well as due to the length of passages.

To study the degree of genetic divergence for Belarusian selection lilac cultivars and to determine their phylogenetic relationships parental lilac cultivars 'Mme Abel Chatenay', 'Ludwig Shpaeth' and 'Reaumur' were included in the study. Based on the identified DNA

markers Nei genetic distances between the studied lilac cultivars were calculated, their clustering by method UPGMA was made. These data were used in the construction of phylogenetic trees for each primer (RAPD and ISSR) (data are not shown), and consensus (RAPD + ISSR) dendrogram shown in Figure 2. These RAPD and ISSR cultivars` genotyping showed similar degree of relationship for studied cultivars. Rod clustering in generated consensus (RAPD + ISSR), as well as RAPD- and ISSR-dendrograms generally preserved; denrograms detect small differences in subclusterization in some varieties.

Fig. 2 Consensus RAPD + ISSR dendrogram, which demonstrates the degree of genetic similarity between lilac cultivars of Belarusian selection based on 40 RAPD and 36 ISSR markers generated with primers OPA-18, OPE-02 and OPP-09, UBC-808 and UBC-862. Magnitude bootstrap (100 replicas) pointed about the corresponding node (%)

Designations clusters: SHF - Hyacinthiflora; REO - Hyacinthiflora × Reaumur; LSP - Hyacinthiflora × Ludwig Shpaeth; ASH - Mme Abel Chatenay × Reaumur



Conclusions

Application of the developed methods of complex RAPD + ISSR-genotyping of *Syringa vulgaris* L. on intraspecific level allowed to differentiate and certify all studied lilac genotypes of Belarusian selection in CBG NASB collection, develop genotypic certificates, verify genealogy of cultivars, clarify phylogenetic relationships between them. Developed method is effective for sampling, monitoring of varietal purity in propagated cultivars, formation and genetic verification of the samples in *in vitro* collections. Developed markers are the basis for further breeding on valuable traits.

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Spiridovich E.V., Vlasova A.B., Yukhimuk A.N., Reshetnikov V.N. Assessment of genetic divergence of lilac (Syringa L.) cultivars of Belarusian selection based on integrated application of RAPD- and ISSR-markers // Works of the State Nikit. Botan. Gard. – 2014. – V. 139 – P. 191– 198.

An integrated approach of RAPD+ISSR molecular certification of lilacs cultivars of Belarusian selection (CBG NASB) was developed and applied aimed at verification of genotypes identity at propagation, collections maintenance and unique genotypes conservation. Generated in total 93 RAPD and 67 ISSR markers (including cultivar-specific) allowed to differentiate 13 studied genotypes, create genetic certificates for each of them, calculate the degree of genetic relationship and clarify the phylogenetic relationships between cultivars. Proposed method of DNA-passportization of Syringa vulgaris cultivars is an effective tool to study the genetic diversity and molecular certification of cultivated lilacs forms, verification of collection banks when depositing in vitro.

Key words: Syringa vulgaris L., lilac cultivars, certification molecular markers, RAPD-, ISSR-loci, genetic distance, in vitro collection, genetic diversity, conservation

UDK 58:069.029:635.92 (477.75)

NEW COLD-RESISTANT VARIETIES OF WATER LILIES IN LANDSCAPING ARTIFICIAL RESERVOIRS IN CONDITIONS OF SIMFEROPOL CITY

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Introduction

Water lilies are widely recognized among the most attractive ornamental plants for decorating water surface of ponds. They are an important part in the life of such complex ecological system as a reservoir. Blades of their floating leaves close significant portion of the water surface do not let it to overheat under the scorching sun in hot summers; protect from the wind during periods of bad weather. They form favourable conditions for normal existence of water biocenosis, enrich it with oxygen, serve as a food source for its inhabitants and place for spawning fish, as well as participate in the process of water purification.

Water lily has long been used in ethnomedicine as a medicinal plant. Rhizome of white lily contains many nutrients: starch (49%), protein (8%) and sugar (20%). Young white water lily rhizomes are eatable when roasted or boiled [7, 11]. Studying valuable features of water lilies in the introduction conditions of Foothill Crimea, we identified some biochemical features of white lilies (*Nymphaea alba* L.) as for accumulation of the compounds causing its scent. Composition of refining oil extracted from the flowers was determined. In its composition high content of saturated hydrocarbons, volatile aromatic compounds of terpene origin and compounds with high biological activity was found [13].

For the first time botanists began to speak about water lilies on a professional level in 1735 - there were scientific works of J.P. de Tournefort, where under the name "water rose" an unusually spectacular plants and their exotic bloom were described. In 1753, Carl Linnaeus named the genus (*Nymphaea*). Systematically family Nymphaeaceae (Nymphaeaca) was identified in 1806 by R.A. Salisbury. Modern genus includes 60 species; most of them are typical in the zones with tropical and subtropical climate. In flora of Russia three species are presented: European white waterlily (*N. alba* L.), snow-white water lily (*N. candida* C. Presl) and pygmy water lily (*N. tetragona* Georgi). All these species have white flowers, although occasionally there are populations with pink ones [7 - 9]. In the Crimea water lily does not occur in wild [6].

The first attempts to grow lilies in Europe associated with the introduction of thermophilic blue water lily (*N. caerulea* Savign), imported from Egypt in 1801 and planted on the territory of National Museum of Natural History in Paris (Musee de «Histore naturelle») [7, 9]. Blooming of tropical and subtropical water lilies differs from the representatives of temperate climate zone so that in addition to morning and afternoon flowering, they demonstrate the night blooming. Besides, flowers in tropical nymphaes are of exotic form and flavor, bright colors, including blue, rare and revered in ornamental horticulture. Due to the wide area and diversity of environmental factors affect different species of water lilies have various morphological features of their root system. There are four ecobiomorphological groups: rhizomatous, tuberous, conditionally-rhizomatous and conditionally-stolonate. At the basis of all the existing cultivars of French and American breeding wintering in our waters, there are five types of rhizomatous lilies: white water lily (*N. alba*), pure-white water lily (*N candida*), fragrant water lily (*N. odorata* Aiton), pygmy

water lily (*N. tetragona*), tuber water lily (*N. tuberosa* Paine) [9]. In the English-language literature rhizomatous lilies are called “hardy winter water lily” [16, 17].

In Europe mass cultivation of species, varieties and cultivars of water lilies began in the mid XVIII - the second half of the XIX century. Creator of the first cold-resistant hybrid lilies was the Frenchman Joseph Bory Latour Marliac. Based on his breeding studies, he received the first cold-resistant cultivars of brightly colored lilies in 1877. According to researches by the International Water Lily Society, for thirty years of his work, until death in 1911, J. Latour Marliac created no less than 60 cultivars of water lilies [16, 17]. For over a hundred years hybrids by J. Latour Marliac dominate the market as a great part of modern assortment of this culture [15], but in recent years in the collections of botanical gardens and florists fans new cultivars, mostly of American and Thai selection began to appear. In water lilies selection originators seek to create new plants with outstanding and/or new decorative features, based on the perception of the whole plant (general habitus) and morphological characteristics of leaf blade and flower, its degree of doubleness, color and form, and so on.

We investigated ornamental and economically valuable biological features of modern breeding cold-resistant cultivars of water lilies in the collection of BG TNU named after V.I. Vernadsky and in the private collection of water lilies in Simferopol to study perspectives for supplement the collection funds in BG TNU with new cultivars.

Materials and Methods

Formation of water lily collection in the Botanical Garden of TNU began in 2009 and it is based on introduction aimed in collecting and demonstrating maximum morphological and biological diversity and giving assess of their efficiency for decorative water gardening in the conditions of Simferopol city. The collection is landscape-integrated - cultivars are exhibited in the garden ponds, now it has two species and 20 varieties of water lilies [12, 14] of different selection periods [15, 16]. To the collection of BG TNU water lilies came from A.V. Fomin Botanical Garden (Kyiv), the Botanical Garden of Ivan Franko National University (Lviv) and the Arboretum of Bolestraszyce (Poland), from private collections [14].

Almost at the same time or some earlier in Simferopol city private collections of water lilies were established by water gardening enthusiasts. One of them - the collection of water lilies by Kashirskaia Yu.K., began to take shape in 1998 and at present time has 68 cultivars of water lilies including more than 40 varieties, created in the late XX - beginning of the XXI centuries - representatives of the new, mostly American and Thai selection. This collection is made up of plants obtained from nurseries in Germany, Poland and private aquatic plants collections of near abroad.

Investigations were carried out in 2009 - 2014 in the ponds of TNU Botanical Garden and private water collection of the open air plants in Simferopol. These collections are in the similar edaphic-climatic conditions and located in the southeastern part of Simferopol city, on the left bank of the river Salgir. Climate in Simferopol is temperate continental, arid with hot summers and cool winters. The average annual temperature 9.2 – 10.3 °C; the average year t°Cmin = -30.0 °C (January); the average year t°C max = + 39,0 °C (July); annual precipitation 450-500 mm. Number of days per year with t ° C> 5,0 °C is 220 - 230, that coincides with the length of vegetative season in this region. The mean annual sum t°C> 10 °C are 3175 °C. The frost-free period is 160 - 200 days [2].

During the observation period the average daily temperature in winter was between -1.9 °C to 2.8 °C, in summer - from 21.6 °C to 26.3 °C. The average air temperature in January - from -3.1 °C to 1.7 °.C, in July - from 22.6 °C to 26.7 °C, that corresponds to the average long-term indexes [1, 2]. In winter 2012 the lowest average daily air temperature (-1.91 °C) and minimum daily temperature (-23.1 °C) were noted in February. Maximum summer daily temperature (33.6 °C) was recorded in August 2010. Minimum precepitation were in 2011, 2012 and 2013 - 304.3 mm, 300.3 mm and 303.7 mm, respectively; maximum

(544.0 mm) - in 2010. During the study, the mean maximum water temperature in the reservoir in summer + 19,4 °C was registered in July 2014, absolute maximum + 24,6 °C was recorded in July 2012 and 2014; the average minimum water temperature + 17,1 °C and absolute minimum + 10,1 °C - in August 2010.

The objects of the study were cold-resistant modern cultivars of water lilies registered by the International Water Lily Society since 1990 to the present time: three lilies in TNU BG collection: N. 'Inner Light' (Kirk Strawn, 1997), N. 'Georgia Peach' (Strawn, 1998), N. 'Lemon Mist' (Strawn, 1997) and forty-three cultivars in the private collection of water lilies: N. 'Perry's Double White' (Slocum, 1990), N. 'White Sultan' (Kirk Strawn, 1991), N. 'White Sensation' (Slocum, 1991), N. 'Rattana Ubol' (Pairat Songpanich, 2003), N. 'Franz Berthold' (Franz Berthold jun., 2001), N. 'Bernice Ikins' (Kirk Strawn, 1996), N. 'Mayla' (Strawn, 1993), N. 'Celebrechion' (Strawn, 1994), N. 'Lily Pons' (Slocum, 1992), N.' Starburst '(Strawn , 1997), N. 'Nigel' (Kirk Strawn, 1993), N. 'Yuh Ling' (Kirk Strawn, 1992), N. 'Red Spaider' (Kirk Strawn, 1993), N. 'Fireball' (Slocum, 1999), N. 'Red Paradise' (Slocum, 1999), N. 'Burgundy Princess' (Strawn, 1993), N. 'Hidden Violet' (McDonald, 2007), N. 'Liou' (Strawn, 1993) N. 'Perry's Double Yellow' (Slocum Water Gardens, 1996), N. 'Yellow Sensation' (Slocum, 1991), N. 'Yellow Queen' (Slocum, 1991), N. 'Gold Medal' (Slocom, 1991) , N. 'Lemon Mist' (Strawn, 1997), N. 'Innerlight' (Kirk Strawn, 1997), N. 'Texas Dawn' (Kenneth Landon, 1990), N. 'Little Sue' (Kirk Strawn, 1993) , N. 'Colorado' (Kirk Strawn, 1994), N. 'Perry's Autumn Sunset' (Perry's Water Gardens, 2003), N. 'Orange Sunset' (Slocum, 1996), N. 'Barbara Dobbins' (Kirk Strawn, 1996), N. 'Sunny Pink' (Kirk Strawn, 1997), N. 'Peache and Cream' (Slocum, 1992), N. 'Clyde Ikins' (Kirk Strawn, 1996), N. 'Nefelis' (Protopapas, 2004), N. 'Blushing Bride' (Perry's Water Gardens, 1997), N 'Pink Grapefrut' (Kirk Strawn, 1997), N. 'Peach Lily' (Strawn, 1999), N. 'Almost Black' (Slocum, 1994), N. 'Black Princess' (Slocum, 1998), N.' Greg's Orange Beauty '(Perry Water Garden, 1996), N.' Wanwisa '(Best New Waterlily 2010, Nopchai Chansilpa, Thailand), N.' Siam Purple 1 '(Pairat Songpanich, 2007), N.' Siam Purple 2 '(Pairat Songpanich, 2009).

We used common methods for introduction investigation [3 - 5]. Varietal identification of plants based on reputable sources of information [9, 16, 17].

Results and discussion

Evaluation of ornamental features in water lilies species and cultivars is first of all determined with characteristics based on morphological signs variability. Phenological and economic and biological parameters are among importen indexes, the most significant of which are terms and duration of flowering, flowering productivity, resistance to diseases, pests and unfavourable weather conditions [5].

On the base of the evaluation we found that studied water lilies cultivars are characterized with differences in growth intencity, duration of the flowering period, in the shape and diameter of flower and leaf blade, leaf and corolla color, and in the degree of flowers doubleness.

By growth intencity: pygmy water lilies, with flower diameter 5 - 8 cm: N. 'Perry's Baby Red'; medium height, flower diameter - up to 15 cm, N. 'Red Paradise', N. 'Black Princess', N. 'Barbara Dobbins', N. 'Celebrechion', N. 'Lily Pons', N. 'Yuh Ling', N. 'Red Spaider', N. 'Hidden Violet', N. 'Colorado', N. 'Peache and Cream', N. 'Nefelis', N. 'Peach Lily', N. 'Siam Purple 1', N . 'Siam Purple 2' etc., vigorous (large): N. 'Perry's Double White', N. 'Bernice Ikins', N. 'Mayla', N. 'Fireball', N. 'Perry's Double Yellow', N. 'Yellow Sensation', N. 'Yellow Queen, N.' Lemon Mist ', N.' Inner Light ', N.' Texas Dawn ', N.' Perry's Autumn Sunset, N. 'Wanwisa' etc., very large - flower diameter up to 25 cm: N. 'White Sensation', N. 'Sunny Pink', N. 'Orange Sunset', N. 'Rattana Ubol', N. 'Gold Medal', N. 'Sunny Pink' , N.

'Pink Grapefrut'. The most interesting for exhibition in the ponds of Botanical garden are middle-height and large water lily varieties.

According to duration of flowering period: in the conditions of Simferopol water lilies blooming is mass, starts in the last decade of May and ends in late September, and under prolonged warm autumn it may continue till late October. Cultivars N. 'Inner Light', N. 'Perry's Orange Sunset', N. 'Georgia Peach' demostrated up to 150 days blooming. In the conditions of not deep, fast sun warmed reservoirs with water column height of 0.5 - 0.6 m during the thaws in February water lily N. 'Colorado' blooming was noted sometimes.

Due to leaf colour: compared "retro" varieties, some of which have variegated leaf coloring, in a group of new breeding cultivars multicolored specks appeared on leaves: N. 'Gregg's Orange Beauty' (star-shaped flower, diameter of about 12 cm, with 36 – 37 narrow petals; outer petals are pink, central - are yellow. Leaf is elliptic, dark green, diameter 19 - 21 cm, with many spots of brown and maroon-brown color) (Fig. 1), N. 'Blushing Bride'; water lily cultivars with blue flowers N. 'Siam Purpul 1', N. 'Siam Purpul 2' have purple-brown specks on the ventral side of the leaf blade and bright purple - on its dorsal side.

Flower shape: together with traditional "Nymphaeaceae" form of the flower other well recognized shapes appear: spherical - in N. 'Firebal' (flower is of spherical shape, 14 - 18 cm in diameter, with 48 – 50 red petals, light outside and darker in the center of the flower. Leaf is round, dark green, about 26 cm in diameter, with closed section) (Fig. 2); cupped: N. 'Black Princess' (flower intense dark red, cup-shaped, 14 cm in diameter, with 34-36

Fig. 1 Nymphaea 'Gregg´s Orange Beauty'

Fig. 2 Nymphaea 'Fireball'

narrow petals. To date - this is one of the most dark-coloured cultivars. Leaf is almost round, dark green, 20 cm in diameter, with a slightly open incision) (Fig. 3), N. 'Red Paradise', N. 'Orange Sunset', N. 'Siam Purpul 1'; N. 'Siam Purpul 2'; tulip-shaped: N. 'Little Sue'; goblet-shaped: N. 'Burgundy Princess'; chrysanthemum-shaped: N. 'Lily Pons'; peony-shaped: N. 'Blushing Bride'; stellate: N. 'Sunny Pink', N. 'Gregg's Orange Beauty', N. 'Lemon Mist', N. 'Virginia'.

Flower doubleness degree: more than 30 petals such cultivars have: N. 'Black Princess', N. 'Peaches and cream', N. 'Siam Purpul 2'; more than 40 petals: N. 'Fireball', N. 'Mayla' (flower is chrysanthemum-shaped, 13 - 17 cm in diameter, with 40 broadly lanceolate petals of bright pink color. Leaf is round, olive green, smooth, 18 - 20 cm in diameter) (Fig. 4.) N. 'Blushing Bride'.

Fig. 3 Nymphaea 'Black Princess'

Fig. 4 Nymphaea 'Mayla'

Flower color: together with the traditional white, pink, yellow and red water lily flowers new varieties with dark red flowers (considered black) appear: N. 'Black Princess', N. 'Almost Black'; bicolor (outer petals are bright pink, inner ones - cream): N. 'Peaches and cream' (32 - 37 lanceolate petals form a stellate flower, diameter 15 - 20 cm. The outer petals are bright pink, inner are of light yellow color. Flowers rise above the water surface. Leaf is nearly round, green, with reddish-brown elongated spots, 19 - 22 cm in diameter, with a small incision blade) (Fig. 5), N. 'Sunny Pink', N. 'Greg's Orange Beauty '; Peach: N. 'Blushing Bride', N. 'Barbara Dobbins', N. 'Georgia Peach' (flower during the blossom rises up to 10 cm above the water surface, its diameter 15 - 18 cm. In the early blooming flowers are pale pink with yellow tinge and further flower becomes more saturated yellow with pink tinge. Leaves are nearly round, dark green, young leaves with reddish-brown spots, diameter 15 - 18 cm. Cultivar has abundant flowering) (Fig. 6) ; colorful petals: N. 'Wanwisa' (Flower is stellate, diameter 12 - 13 cm. Petals are red with yellow strokes. Sometimes a few flower petals are yellow. Leaf is elliptic, diameter of 16 - 17 cm. Young leaves are bright purple, adult leaves have clearly visible marble pattern of brown colour) (Fig. 7). Blue-violet corolla color previously typical only for tropical water lilies – presents in N. 'Siam Purpul 1', N. 'Siam Purpul 2' (32-36 bright blue-violet petals and orange stamens form a cup-shaped flower, diameter 12 - 13 cm. Leaf is round, green, 18 cm in diameter, dark red-brown with more marbling on the low side) (Fig. 8).

Fig. 5 Nymphaea 'Peaches and cream'.

Fig. 6 Nymphaea 'Georgia Peach'

Fig. 7 Nymphaea 'Wanwisa'

Fig.8 Nymphaea 'Siam Purpule 2'

Conclusions

According to the estimation of the International Water Lily Society, modern world assortment of cold-resistant water lilies has about 300 cultivars [16] that can satisfy the variety of aesthetic preferences of water gardening enthusiasts, but if the collections of amateur gardeners can be formed under the influence of their tastes and preferences, fashion trends, market demand, then for supplement of water lilies collection in BG TNU named after V.I. Vernadsky number of criteria are proposed to use. Formation of the collection should be based on the principles of existing standard classification of varieties, with the advice of botanical gardens and other organizations engaged in water lilies cultivation. Collection should demonstrate the diversity of world assortment of the culture and include both "retro" and modern cultivars. Cultivars that are grade-winners of special exhibitions (for complex of signs) and ones interesting for breeding programs (possessing certain genotypes) should be exhibited

Perspectives for development of water lilies collection fund in Botanical Garden of Tauride National University named after V.I. Vernadsky we observe on the basis of its further supplement with water lilies cultivars according to the complex analysis of their decorative characteristics on the identified features. Among presented assortment water lilies we can recommend: N. 'White Sensation', N. 'Sunny Pink', N. 'Orange Sunset', N. 'Rattana Ubol', N. 'Gold Medal', N. 'Sunny Pink', N . 'Pink Grapefrut', N. 'Red Paradise', N. 'Black Princess', N.' Barbara Dobbins', N. 'Celebrechion', N. 'Lily Pons', N.' Red Spaider ', N.' Hidden Violet ', N.' Colorado ', N.' Peache and Cream ', N.' Nefelis ', N.' Peach Lily ', N.' Siam Purple 1 ', N.' Siam Purple 2 ', N.' Perry's Double White ', N.' Bernice Ikins', N. 'Mayla', N. 'Fireball', N. 'Perry's Double Yellow', N. 'Yellow Sensation', N. 'Yellow Queen, N.' Lemon Mist ', N.' Inner Light ', N.' Texas Dawn ', N.' Perry's Autumn Sunset, N. 'Wanwisa'.

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The article covers the main morphological characteristics of the flower and leaf of new varieties of cold-water lilies of Simferopol collection. The possibility to replenish the water lilies collection of BS TNU named after Vernadsky V.I. by new modern varieties was studied as well.

Key words: collection of water lilies varieties, new varieties, the Botanical Garden, the ornamental characteristics, economically valued characteristics, the introduction.

CONTENTS

Koba V.P., Plugatar Yu.V.
Problem of natural populations of types of sort Pinus L. protection in Mountain Crimea **3**
Plugatar Yu.V., Ilnitsky O.A., Kovalyov M.S., Korsakova S.P.
A dynamic model of the water regime of some bush species of the lower canopy in parks phytoclimate conditions on the South Coast of the Crimea……..... **11**
Bagrikova N.A., Bondareva L.V., Ryff L.E.
Distribution features Opuntia humifusa (Raf.) Raf. in the Sebastopol……. **29**
Bagrikova N.A., Ryff L.E.
Invasive species of Opuntia lindheimeri Engelm. on the Southern Crimea…….. **43**
Nikiforov A.R., Korzhenevsky V.V.
Types of shoot formation of the plants in glareophyton of upper belt of Mountain Crimea…… **63**
Sarkina I.S.
New types of the mikobiota at the nature reserve "Cape Martyan": macromycetes…… **68**
Korsakova S.P.
The review of adverse meteorological phenomena in Nikitskij Botanical Garden area... **74**
Rabotyagov V.D., Shibko A.N.
Investigations of essential oil component composition in Hyssopus officinalis …….. **88**
Paliy A.E., Grebennikova O.A., Rabotyagov V.D., Paliy I.N.
Biologically active substances of heady aromatic and medicinal plants from Nikitsky Botanical garden…… **101**
Tikhomirov A.A.
Principles of essential oils using for medical purposes. A Review……... **109**
Shevchenko S.V.
Some morphobiological characteristics of Glaucium flavum Crantz (papaveraceae)….. **120**
Myroshnichenko N.N.
Special features of reproductive biology of some species of sort Campanula L…….. **129**
Brailko V.A., Gubanova T.B.
Potential frost resistance of species of sort Lonicera L. and analyses of factors which limits their winter hardiness……... **140**
Isikov V.P.
Annotated checklist of tree-inhabiting fungi of the Black Sea Coast of the Caucasus… **149**
Opanasenko N.E., Kostenko I.V., Yevtushenko A.P.
Agroecological zoning of the steppe and piedmont Crimea for fruit-bearing plants at the present stage…… **159**
Klymenko O.E., Klymenko N.I.
Elements of innovative technology of seedlings Spiraea x vanhouttei (Briot) Zab. growing in industrial nursery……..... **170**
Vlasava N.B., Michener D.C., Yukhimuk A.N., Gaishun V.V., Bryant R.,
Agabalaeva E.D., Spiridovich E.V.
Genetic differentiation of historic cultivars of herbacious Paeonia based on SRAP markers: documentation and conservation of botanic collections……..... **177**
Spiridovich E.V., Vlasova A.B., Yukhimuk A.N., Reshetnikov V.N.
Assessment of genetic divergence of lilac (Syringa L.) cultivars of Belarusian selection based on integrated application of RAPD- and ISSR-markers…… **191**
Khalyavina S.V., Kashyrskaya Y.K.
New cold-resistant varieties of water lilies in artificial reservoirs landscaping (Simferopol)..... **199**

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