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ASPECTS REGARDING SEEDS MORFOLOGY AND GERMINATION PECULIARITIES AT SOME TAXA FROM *SILENE* L. GENERA

IFRIM CAMELIA¹

Abstract: Genus *Silene* L. is represented in the Romanian flora by 37 taxa that have different uses or an endemic species status. Highlighting morphological features of the seeds using light microscope provides very useful information in clarifying some taxonomic issues, but also in setting up a useful database for germplasm preservation. Regarding the germination, of interest were germination percentage and germination speed, plantlets development, the occurrence of the first pair of leaves, etc. The monitoring this process shows that differences between taxa occur in the early stages of development.

Key words: *Silene*, seeds morphology, germination

Introduction

Genus *Silene* L. is represented in Romanian flora by 37 herbaceous perennial species; some of them have of medicinal importance (eg. *S. vulgaris* (Moench) Garcke), others have decorative uses (eg. *S. acaulis* (L.) Jacq.), and *S. dinarica* Sprengel has the status of endemic species to southern Carpathians [CIOCĂRLAN, 2000]. Information on seed morphology in the description of the Romanian flora species is in some cases incomplete (*S. viscosa*) or missing altogether (*S. latifolia* subsp. *alba*, *S. nutans* subsp. *dubia*) [PRODAN, 1953]; for species such as *S. latifolia*, *S. nutans*, *S. vulgaris* more details are provided in the synthesis works [ДОБРОХОТОВ, 1961]. Thorough knowledge of structural features is very useful in clarifying taxonomic issues (especially for a botanical fragmentary material), in preparation for seed storage in germplasm banks or to identify seeds found in archaeological sites [BAŞLI & al. 2009, GÜNER & al. 2009].

The study of seed germination provides information on germination rate and speed which is useful for cultivating medicinal and decorative taxa or for monitoring invasive plant [BLAIR, 2004]. Differences in plantlet morphology arose interest from a theoretical point of view [CSAPODY, 1968; ВАСИЛЬЧЕНКО, 1965], but they also have practical use in agriculture [CIOCĂRLAN, 1975].

Material and methods

The material used in this paper consists of the seeds from seven taxa of the genus *Silene*, collected in 2009. The spontaneous taxa come from different locations in Romania, as follows: Făgăraş Mountains, Sibiu County (*Silene acaulis* (L.) Jacq. subsp. *acaulis*, *Silene dinarica* Sprengel); Iaşi, Iaşi County (*Silene latifolia* Poir. subsp. *alba* (Miller) Greuter & Burdet, *Silene viscosa* (L.) Pers., *Silene vulgaris* (Moench) Garcke subsp.

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vulgaris); Rarău Mountains, Suceava County (*Silene nutans* L. subsp. *dubia* (Herbich) Zapal); Broșteni, Suceava County (*Silene nutans* L. subsp. *nutans*).

For germination study 100 seeds were selected from each taxon and were placed in Petri dishes on filter paper moistened with water and maintained under controlled conditions (21 °C) in the climate chamber in the dark for 35 days (May 27, 2011 - June 29, 2011). All stages of germination were observed, the cracking of the seedcoat, the emergence of root, the cotyledons, and the first three pairs of leaves. Relevant images observed with binocular magnifier were photographed using Canon A540 camera.

The study was conducted in the Laboratory of micropropagation and germplasm preservation of the Botanical Garden, University “Alexandru Ioan Cuza” Iași.

Results and discussions

Most of the seeds of species of the genus *Silene* are of reniform type, but observation of macro-and micromorphology characteristics of the testa show differences from one taxon to another. Seed features description was made by well established parameters used in the literature [FAWZI & al. 2010]. Macromorphological issues pursued were: shape, color, lateral surface and dorsal surface of the seed. Micromorphological features of the testa cells in a frontal view were the outline and shape of the anticline and lateral walls (Fig. 2 A-G). A summary of the light microscope observations is shown in Tab. 1.

Tab. 1. Macro- and micromorphological features for 7 taxa of genus *Silene*

Taxa	Seed shape	Seed colour	Lateral surface	Dorsal surface	Testa cell outline	Anticlinali walls	Periclinal walls
<i>Silene acaulis</i> (L.) Jacq. subsp. <i>acaulis</i>	reniform	brown	concave	convex	elongated polygonal	S-undulated	flat, smooth
<i>Silene latifolia</i> Poir. subsp. <i>alba</i> (Miller) Greuter & Burdet	reniform	brown	convex	convex	polygonal	V-undulated	convex, with tubercle in the central area
<i>Silene dinarica</i> Sprengel	reniform	brown	flat	convex	elongated polygonal	S-undulated	convex
<i>Silene nutans</i> L. subsp. <i>dubia</i> (Herbich) Zapal	reniform	brown	flat	convex	polygonal	V-undulated	convex, with tubercle in the central area
<i>Silene nutans</i> L. subsp. <i>nutans</i>	reniform	brown	flat	convex	polygonal	V-undulated	convex, with tubercle in the central area
<i>Silene viscosa</i> (L.) Pers.	reniform	brown	flat	flat-convex	polygonal	V-undulated	convex, with tubercle in the central area
<i>Silene vulgaris</i> (Moench) Garcke subsp. <i>vulgaris</i>	reniform-circular	brown	flat-concav	flat-convex	polygonal	V-undulated	convex, with tubercle in the central area

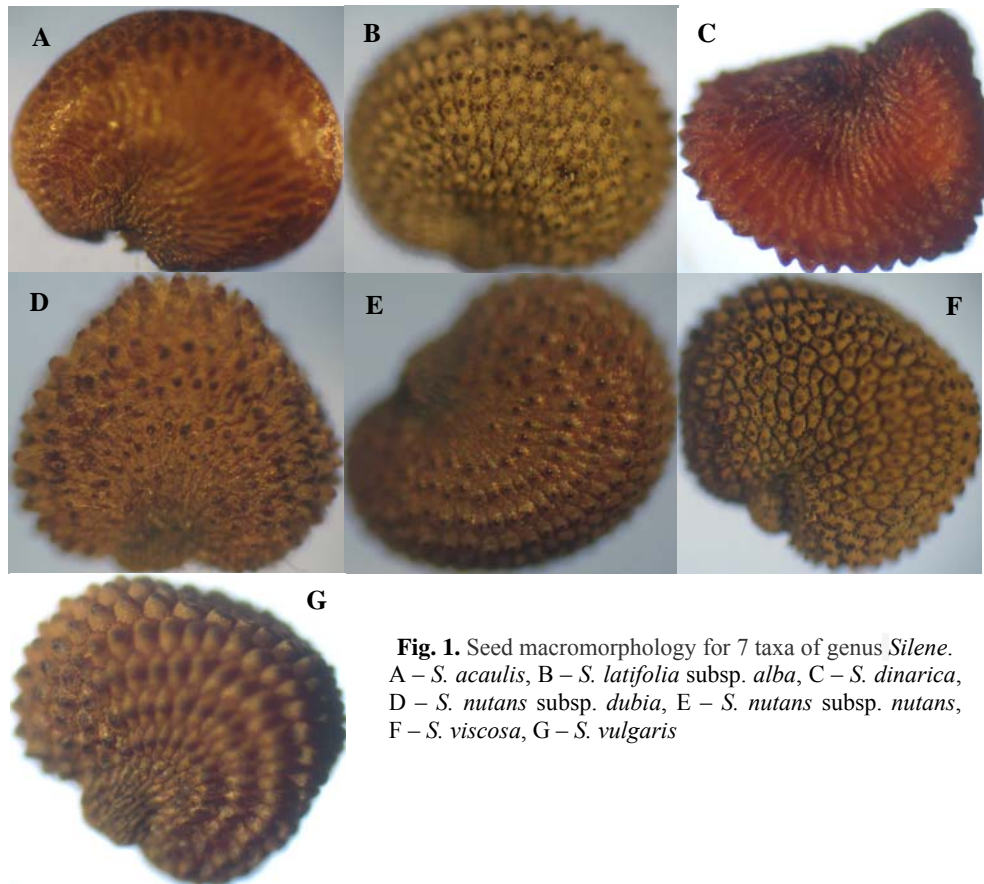


Fig. 1. Seed macromorphology for 7 taxa of genus *Silene*.
 A – *S. acaulis*, B – *S. latifolia* subsp. *alba*, C – *S. dinarica*,
 D – *S. nutans* subsp. *dubia*, E – *S. nutans* subsp. *nutans*,
 F – *S. viscosa*, G – *S. vulgaris*

Observation of the general appearance of the seeds highlights the species *Silene acaulis* for which the impression of smoothness is due to the fact that the grooves next to the anticline wall are shallow and lacking the tubercles on the anticline walls of the testa cells. The arrangement of cells on the surface of the testa shows an ordering in concentric and parallel rows for *S. acaulis*, *S. dinarica*, *S. nutans* subsp. *dubia*, *S. nutans* subsp. *nutans* and *S. vulgaris* (Fig. 1 A, C, D, E, G), concentric, but not parallel in *S. latifolia* subsp. *alba* (Fig. 1 B) and unordered in *S. viscosa* (Fig. 1 G).

Testa cell are approximately the same size across the seed's surface for *S. latifolia* subsp. *alba* and *S. viscosa*, while for the other taxa it changes from outside towards the hilum by tangential elongation. The hilum is embedded in *S. acaulis* and *S. dinarica*, while for the remaining taxa hilum is prominent.

The structure of the seed's testa is of practical "strategic" relevance being closely related to the functions it must fulfil: protection, dissemination and water absorption. The detailed morphological study highlights the theoretical importance of the seeds, as their characteristics can serve as diagnosis tools for taxonomic problems.

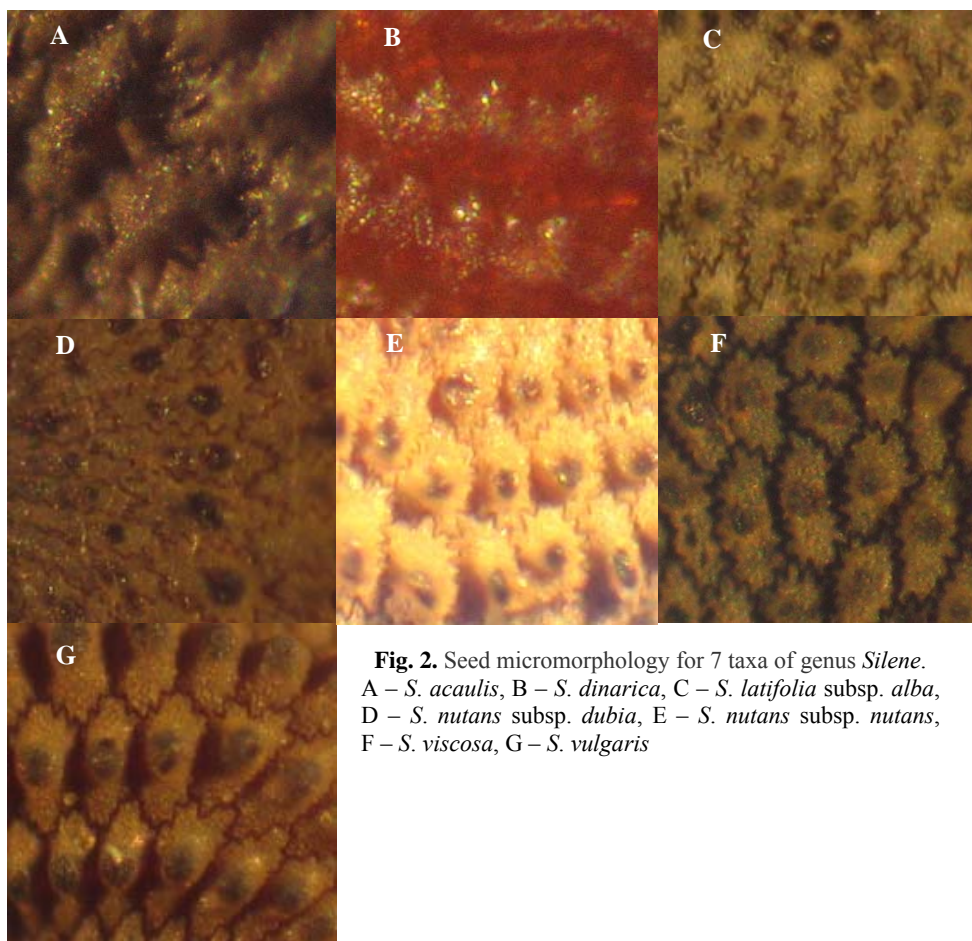


Fig. 2. Seed micromorphology for 7 taxa of genus *Silene*.
 A – *S. acaulis*, B – *S. dinarica*, C – *S. latifolia* subsp. *alba*,
 D – *S. nutans* subsp. *dubia*, E – *S. nutans* subsp. *nutans*,
 F – *S. viscosa*, G – *S. vulgaris*

Ongoing monitoring of the germinating seeds shows differences in the germination rate and speed of the seeds [INDREA, 2007]. Since the seeds were collected during 2009, a low germination rate was expected, but the results show that it varied widely, from 82% in *Silene vulgaris*, to 52% for *S. acaulis* and 8% for *S. nutans* subsp. *dubia*. Germination speed showed very large variations, being high (5 days) in *S. vulgaris* and *S. acaulis*, but reduced (10 days) in *S. nutans* subsp. *dubia* (Fig. 3).

The occurrence of the first pair of leaves was observed after 18 days in *S. acaulis* and *S. latifolia* subsp. *alba*, but only after 25 days in *S. nutans* subsp. *dubia*. The daily observations of the evolution process of germination showed a uniform germination in *S. viscosa* and uneven for *S. acaulis*. This latter taxon had individuals at very different stages of germination towards the end of the experiment (Fig. 4).

Comparison of morphological characteristics of the first pair of leaves reveals notable differences from one taxon to another. Thus, the leaf shape is linear-lanceolate in *S.*

acaulis, ovate la *S. latifolia* subsp. *alba* and *S. vulgaris*, obovate in *S. viscosa*; the tip of the leaf is acuminate in *S. acaulis* and rounded in most taxa. The most significant differences concern the distribution, number, shape and size of trichomes (Fig. 5) on the leaf surface.

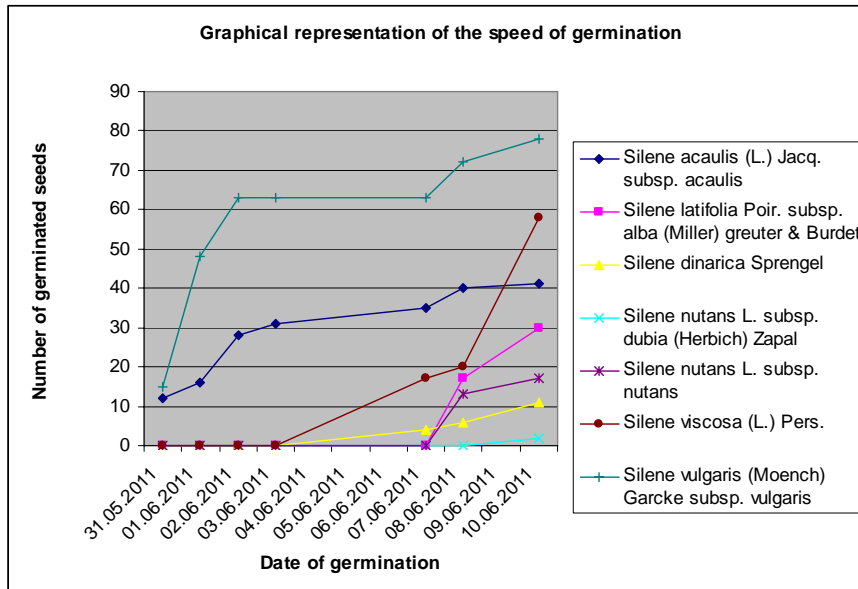


Fig. 3. Graphical representation of the speed of germination for seven taxa of genus *Silene*

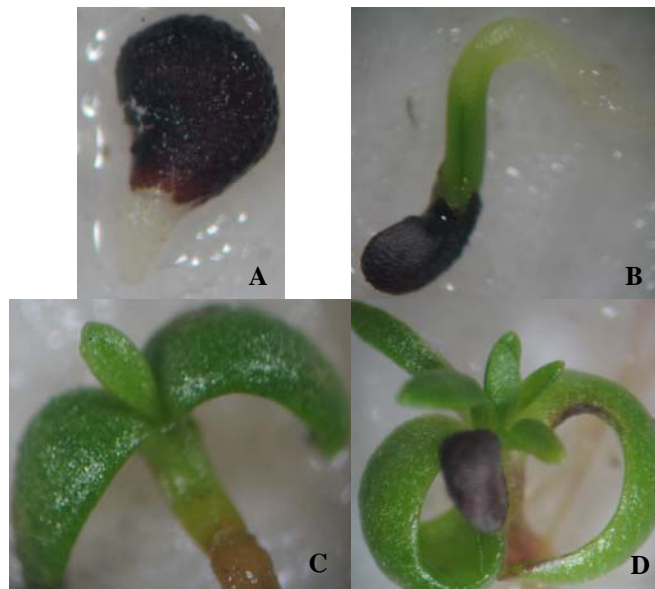


Fig. 4. *Silene acaulis*, June 29, different stages of germination. A – root appearance, B – cotyledons appearance, C – appearance of first pair of leaves, D – plantlet with three pairs of leaves

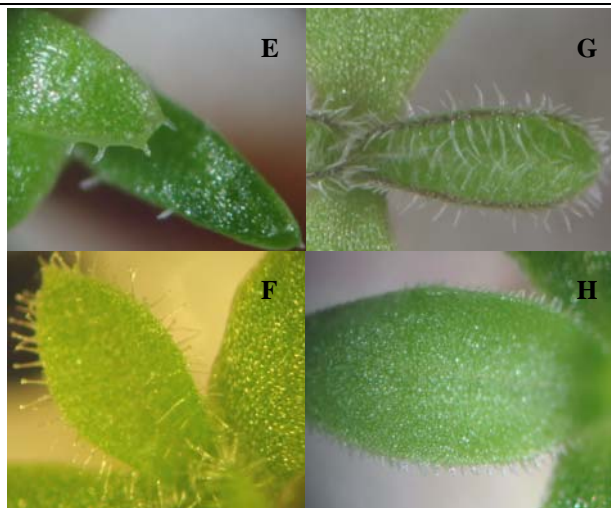


Fig. 5. Morphological characteristics of the first pair of leaves for: E – *Silene acaulis*, F – *S. latifolia* subsp. *alba*, G – *S. viscosa*, H – *S. vulgaris*

Conclusions

Macro- and micromorphological study of the seeds of 7 taxa of the genus *Silene* may provide diagnostic characteristics information useful in solving taxonomy problems.

The evolution of germination process varies from one taxa to another and observed features can be used for obtaining biological material for medicinal, ornamental or ecological restoration use.

Morphology of seedlings (especially the leaves) highlights the differences between taxa from the early stages of development, which is useful in the case of invasive plants or weeds.

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THE CONSERVATION OF SPECIES *BELLEVALIA SARMATICA* (GEORGI) WORONOV BY THE VITROCULTURE METHOD

SEDCENCO MARIA¹, CIORCHINĂ NINA¹, CLAPA DOINA², FIRA ALEXANDRU²

Abstract: *In vitro* bulbification is a method by which bulb formation is stimulated and speeded up in the species that are propagated by bulbs and which need, in the conditions of a classical culture, a longer period for the formation of flower-bearing bulbs. In this article, the results of research is presented, regarding the conservation, multiplication and maintenance of this vulnerable species included in the Red Book of the Republic of Moldova. This study was carried out in order to re-introduce this species into its native habitats. Optimal media were elaborated for the initiation, multiplication, rhizogenesis and the maintenance of this species in *in vitro* conditions.

Keywords: conservation, *in vitro*, multiplication, auxins, cytokinins

Introduction

One of the present problems of contemporary botany is the elaboration of strategies for the conservation of the diversity of plant species that are in danger of extinction.

The most successful strategy for long-term protection of biodiversity is the protection and conservation of the phytocenoses and the populations of the spontaneous flora – in situ maintenance. But many rare species have reached a certain limit and in situ conservation alone does not solve the problems of protection against the more and more frequent anthropogenic factors. In such circumstances the most successful method for the prevention of the extinction of the species is the maintenance and multiplication of the respective taxon in artificial conditions that are similar to the natural conditions.

The introduction of the rare and endangered species into culture ensures conservation, facilitates the study of their biology and development, their ecology, methods for propagation and, at the same time, ensures the obtention, conservation and cultivation of planting material and seeds. These methods are of elementary importance for the repatriation (reintroduction) of these species into their natural ecological niches. The conservation of intact plant populations and phytocenoses and genetic biodiversity of the species in botanical gardens offers a multitude of means for the increase of the number of taxa.

The main and stringent problem related to the conservation of some species is the elaboration of the strategy that should stop the diminishing of the populations of the endangered taxons. More and more scientists have the opinion that the biotechnological methods are extremely efficient for the improvement of the situation, as compared to the traditional methods of regeneration. Many rare species from the spontaneous flora of the

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THE CONSERVATION OF SPECIES *BELLEVALIA SARMATICA* (GEORGI) WORONOV BY ...

Republic of Moldova are continuously diminishing because of human activities that lead to the destruction of plant populations and the expansion of invasive species. *Bellevalia sarmatica* (Georgi) Woronov is a bulbous decorative species from the steppe included into the Red Book of the R. of Moldova, protected by the State [NEGRU, 2002]. Until recently, the steppe areas occupied 2/3 of the entire territory of Moldova. Presently, the natural steppe populations are maintained as some small territories as areas protected by the State [TELEUȚĂ, 2002].

The use of *in vitro* cultures is in itself a conservation method, representing a viable and efficient alternative for the protection and maintenance of the genetic fund of rare and endangered species. In bulbous species the increased concentrations of sucrose lead to the more rapid growth of the explants, such reducing the time necessary for the obtention of bulbs and the period until the flowering phase [TAKUO & KOJI, 2004].

The aim of the present study was the testing of the reaction in *in vitro* culture of the explants prelevated from a mature *Bellevalia sarmatica* plant in order to initiate tissue cultures for the medium-term conservation of this species and the induction of the regeneration process for the obtention of viable plants.

Material and methods

The material used for *in vitro* culture consisted of flower-bearing bulb explants taken from mature *Bellevalia sarmatica* plants that were collected in the Budjac Reservation in June 2010.

One of the main conditions for successful microcloning is the sterilization of the explant [CAVALLINI & al. 1987]. The explants prelevated from the donor material (bulb, bulb fragments) were washed under running tap water for 15 minutes. The pre-sterilization was carried out in a solution of KMnO_4 (0.05%) + Tween-20 for 10 minutes. Sterilization was done with diacid (0.01%) solution for 6-7 minutes, followed by 3 rinses with sterile distilled water. After the disinfection of plant material, the explants were inoculated on the culture media, in large or small flasks depending on the size of the prelevated fragments, so as the newly formed bulbs should have enough space for development.

For the study of the reactivity of this species in *in vitro* culture, several variants of culture media were tested, all of them with the macro- and microelements according to the MS [MURASHIGE & SCOOG, 1962] formula, presented in Tab. 1.

The introduction into *in vitro* culture was done in nutritive media with various concentrations of plant hormones. For the initiation of *in vitro* cultures, media with low concentrations of auxins and cytokinins were tested (variants B-1, B-2, B-3). For rapid bulb development and root formation two variants of media were tested (B-6, B-9). As carbon source, sucrose was used. All the culture media were gelled with 6 g/l agar. The pH of the media was adjusted to 5.8 before autoclavation.

Results and discussions

The first evident observations were done after 4 weeks, when it was found that a large number of explants generated small protuberances, similar to 0.5 mm bulbils.

The testing of the three variants of media for culture initiation (Tab. 1) evidenced the fact that the optimal medium for this phase was the one that contained MS (1962) macro- and microelements as basal medium and 1.0 mg/l-6-benzylamilopurine

(BAP) and 0.25 mg/l - α -naphthylacetic acid (NAA) and 30 g/l sucrose (B-1). After 6 weeks of culture, 10-12 bulbs/explant were obtained, with a diameter of about 3.0 mm (Fig. 1, a). On the B-2 medium, with a lower BAP concentration (0.5 mg/l) fewer bulbs were formed (7-9 bulbs/explant). Regeneration also took place on the medium with Kinetin (B-3), but in a lower percentage (4-6 bulbils/explant). The first de novo regenerated bulbils on this medium were noticed after 8 weeks in culture. This fact demonstrates that the presence of BAP leads to a better growth of isolated tissues and better organogenesis as compared to the variants with Kinetin [VOINOV & al. 2009]

The bulbs obtained were transferred onto culture media with higher concentrations of plant hormones and sucrose. On the media with increased concentration of sucrose a more rapid growth of bulbils was observed and there the bulbs had the largest diameters, of 14-17 mm. After two weeks of culture on B-6, B-9 media the bulbs formed roots and leaves. Between the two variants of media, B-6 and B-9 there were no significant differences regarding growth and organogenesis, so that for this stage the B-6 medium with 60 g/l sucrose can be recommended (Fig. 1, b).

The bulbs that were regenerated *in vitro* were acclimated *ex vitro* and successfully transferred to *ex vitro* conditions. Acclimatization was carried out in conditions of controlled climate regarding humidity and temperature, in peat and perlite mixture, for 14 days. The survival rate was of 100% and plant growth and development continued.

The results obtained in this species are promising, so the method of vitrocultures will be extended for other important and endangered taxons from the spontaneous flora, which is constantly diminishing and necessitates protection.

Conclusions

On the basis of our research we can conclude that:

For the sterilization of plant material (bulbs) for the initiation of *in vitro* culture of species *Bellevalia sarmatica* (Georgi) Woronow it is recommended to use disinfection with KMnO_4 (0.05%) + Tween-20 for 10 minutes and diacid (0.01%) solution for 6-7 minutes.

On the basal MS medium supplemented with BAP and NAA at the concentrations of 1.0 mg/l and respectively 0.25 mg/l the best results were obtained regarding the efficiency of microcloning and morphogenic potential.

The optimal medium for rhizogenesis and rapid growth was MS basal medium supplemented with BAP (1.0 mg/l), NAA (1.0 mg/l) and sucrose at the concentration of 60 g/l.

For the acclimation of bulbs the optimal conditions were found and the time for flower-bearing bulb formation was reduced with 1-2 years.

The protocol for micropropagation and medium-term maintenance in *in vitro* culture can be successfully used for the conservation of the endangered species *Bellevalia sarmatica* (Georgi) Woronow. Applying this method ensures the possibility of obtaining homogenous planting material in large amounts in a short period of time and in limited space.

Acknowledgements

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Tab. 1. The composition of the nutritive media for the microclonal propagation of species *Bellevalia sarmatica*

	Variants	Basal medium	Supplementary additives
Media for culture initiation	B-1	MS-100%	1.0 mg/l BAP 0.25mg/l NAA 30 g/l sucrose
	B-2	MS-100%	0.5 mg/l BAP 0.25 mg/l NAA 30 g/l sucrose
	B-3	MS-100%	0.5 mg/l kinetin 0.25 mg/l IAA 30 g/l sucrose
Media for rapid development	B-6	MS-100%	1.0 mg/l BAP 1.0 mg/l NAA 60 g/l sucrose
	B-9	MS-100%	1.0 mg/l BAP 1.0 mg/l NAA 90 g/l sucrose

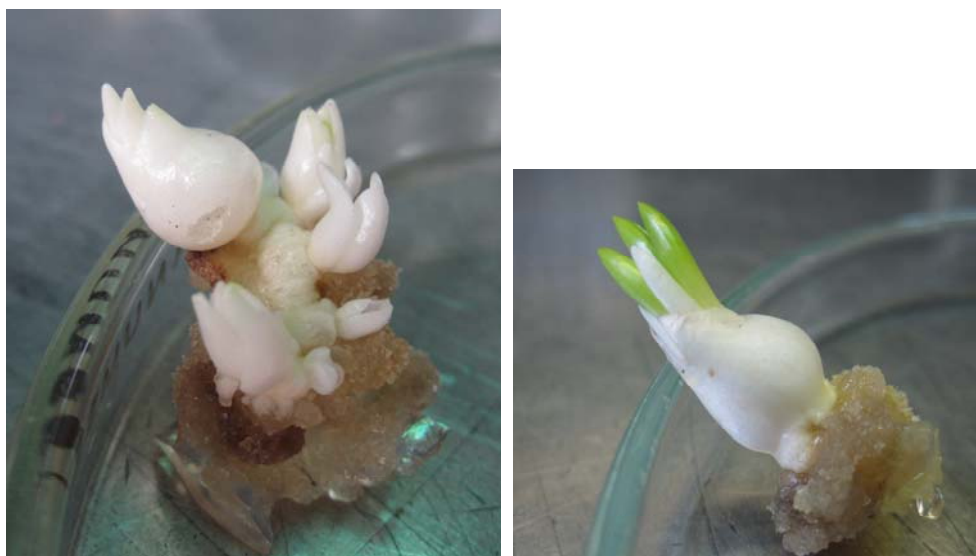


Fig. 1. The *in vitro* culture of species *Bellevalia sarmatica* on B-1 medium (a) and B-6 medium (b)

THE BIOLOGY OF THE PROPAGATION OF SPECIES *SCHISANDRA CHINENSIS* (TURCZ.) BAILL.

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Abstract: The paper presents aspects regarding the possibilities for the propagation of species *Schisandra chinensis* (Turcz.) Baill., as well as its reaction in the pedo-climatic conditions of the Republic of Moldova. Situated in the Lianarium of the Botanical Garden (Institute) AȘM since 1975, *Schisandra chinensis* (Turcz.) Baill. grows, develops and fructifies abundantly every year. It is propagated vegetatively and generatively with some difficulty. In the case of generative propagation, in order to obtain a high germination percentage, the seeds are stratified in three phases, at different temperatures and are sown in spring. Germination percentages of 80-90% were obtained. *Schisandra chinensis* is also propagated by greenwood cuttings, semi-hardwood or hardwood cuttings, by layering or by division. The best results were obtained by using semi-hardwood and hardwood cuttings taken in summer, in June-July, from younger plants. The potential for *in vitro* propagation of this species was also tested. The explants consisting of apical meristems inoculated on MS medium + 0.5 mg/l BAP evolved the best.

Key words: propagation, cutting, climber, medicinal plant, *Schisandraceae*

Introduction

The pedoclimatic conditions of the Republic of Moldova are relatively favourable for non-traditional fruit shrubs which, as they easily adapt to the environment they can be successfully introduced into culture. Also, on the market in the republic of Moldova there is an increasing interest for the introduction into culture of some new plant species from the spontaneous flora. One of these species is the Magnolia vine – *Schisandra chinensis* (Turcz.) Baill., a perennial climber from Family *Schisandraceae*.

Utilization. This species is utilized as an ornamental and medicinal plant. As an ornamental plant it is a decorative climber used for decorating balconies, terraces and buildings.

The majority of vegetative and generative organs contain many biologically active substances, but the most important one is schizandrine and its derivatives. In the leaves and fruits there are, in a higher amount, the vitamins C and B (580 mg/%), catequins, organic acids, ketones (18%); tannic substances; sugars (15%). The seeds contain 33% oils. The fruits, leaves, bark and seeds are used. The infusion obtained from leaves, shoots and fruits is used for stimulating the vitality of the body as a whole, for stimulating the activity of the heart, for calming the nervous system. The extract, decoction, tincture prepared from fruits and seeds is used for treating tuberculosis, bronchitic asthma, gastritis, hepatitis, kidneys, dysentery in children and other diseases that cause the weakening of the organism. The juice, the fresh fruits as well as the tincture from the leaves, fruits and shoots is used as an

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immunomodulator of the body, for increasing intellectual as well as physical working capacity, for eyesight and for strengthening the body. The tea from leaves and shoots has a pleasant flavour similar to that of lemon and it has a slightly sour taste.

The essential oils extracted from this plant are used for decreasing the glucide content of the blood. The use of *Schisandra chinensis* fruits in small amounts is recommended for people that suffer from insomnia, who have psychological problems or high arterial blood pressure and dysfunctions of heart activity.

The fruits are also used in the cosmetic industry and food industry (confectionery, marmelades, chocolates), in the production of soap and detergents.

Spread. The Magnolia vine can be found mostly in mixed forests in the Far East, mostly on the river banks in China, Japan, Sachalin Islands, and in the Republic of Moldova in Soroca, Chişinău. It is cultured by some amateurs and it can be found in several botanical gardens worldwide.

Ecological requirements. It withstands low temperatures and shaded places. It grows fast. It can be cultured on sandy soils. On soils rich in humus it develops better. It does not withstand acidic, dense, compact soils.

Morpho-biological peculiarities. *Schisandra chinensis* is a climber that can reach heights of 14-15 m and 1.5-2 cm in thickness. The roots are branched, situated close to soil surface. The shoots, the roots and the fruits are smelling. The stem of this climber is dark brown in colour and can reach 2 cm in thickness and winds around the trellis in clockwise direction. The leaves are altern, ovate, 5-10 cm in length, the petiole is reddish. The flowers are white, aromatic, they have a pinkish nuance towards the end of flowering. The fruits are of an intense red colour, juicy, sour, spherical, 5-10 mm in length, usually with one seed. The buds are mixed, oval in shape, a little lengthened and pointed. Flowering and fruiting takes place on the annual shoots (Fig.1).

Propagation. The Magnolia vine is propagated vegetatively and generatively with great difficulty. It is propagated by cuttings, layering, division and by seeds [GLUKHOV & al. 2000; KHROMOVA, 1980; TITLYANOV, 1969]. The seeds are sown quickly in the autumn after harvest or in spring after stratification.

There are few scientific papers regarding the micropropagation of *Schisandra chinensis*. Micropropagation has been done by using nodal explants [STANIENĖ & STANYS, 2007], cotyledonary nodes from seeds germinated in vitro [HONG & al. 2004], somatic embryogenesis starting from zygotic embryos [CHEN & al. 2010, SMÍŠKOVÁ & al. 2005] and embryogenesis starting from unopened flower buds [YANG & al. 2011].

Material and methods

The plant material used in the experiments was in the Lianarium of the Botanical Garden (Institute) AŞM, where *Schisandra chinensis* (Turcz.) Baill. grows, develops and fruits abundantly every year, after its introduction into the collections in 1975 and also from the experimental field of the Laboratory of Embriology and Biotechnology.

Experiments were carried out regarding the possibility for the propagation of this species by seeds, by cuttings and by micropropagation.

Observations were done regarding the growth and development of the species in the conditions of the Republic of Moldova as well as experiments regarding the possibilities for the propagation of this species.

For propagation by seeds, before sowing the seeds were stratified in 3 phases: the first phase for 30 days at $t = 18-20\text{ }^{\circ}\text{C}$, then 30 days at $t = 3-5\text{ }^{\circ}\text{C}$ and the third phase, for two months at $t = 8 - 10\text{ }^{\circ}\text{C}$.

For vegetative propagation the collecting and making of cuttings was carried out in summer, at the end of June and at the beginning of July when the shoots of the mother plant start to lignify. Lignified as well as semi-hardwood cuttings were made using well sharpened instruments, and the time for harvesting the shoots was in the morning. The cuttings had 12-15 cm in length, with 2-3 nodes.

For propagation by hardwood cuttings the cuttings were made in February and March before the beginning of the vegetation period. The cuttings were kept in sand and sawdust until the danger of frost at ground level passed and then they were planted into cold frames.

The cuttings were subjected to treatment with a weak (pink in colour) solution of KMnO_4 and with growth regulators, with IBA at 0.005% for a period of 16 hours or with IAA at 0.01% for a period of 5 hours [HROMOVA, 1980]. They were planted into cold frames into two substrates: sand or sand + peat in a ratio of 1:1 and minimum 24% artificial mist. After one year the cuttings were planted into containers or in the open field.

Having in view that by generative propagation some qualities specific to the mother plant appear or disappear and vegetative propagation presents some difficulty, the initiation of *in vitro* cultures was tested for this species.

The plant material consisted in various explant types: apical meristems, lateral meristems of the 2nd, 3rd, 4th, 5th and 6th degree, fragments of juvenile leaves, shoot fragments from the apical part, shoot fragments with lateral meristem, Fragments of young leaves with veins, ovules, ovary with a fragment from the stem, stem fragment with the apical meristem. For culture initiation nine experimental variants were tested, with MS (1962) basal medium and various concentrations of plant hormones (Tab. 1).

The operations were carried out according to the standard laboratory procedures.

Results and discussions

The research and observations carried out in a period of several years in various ecological conditions show that *Schisandra chinensis* develops differently according to the zone. It was found that *Schisandra* prefers rich, humid, loose soil and zones of shadow. In the sunny places in the Republic of Moldova growth is inhibited. It does not withstand drought and high air temperatures in the period of vegetation. It is resistant to frost. It withstands temperatures even as low as $-45\text{ }^{\circ}\text{C}$. In the drought-stricken years, irrigation and soil loosening is necessary. Fertilization or the addition of chernozem to the roots of the plants is recommended. Growth per decade is about 20 cm.

The root system is superficial and can reach to depths of 25 cm. The young shoots are greenish-grey and, as they mature, they become reddish-brown. The plant is monoecious, the flowers are monosexuate. In the conditions of the Republic of Moldova it flowers in May-June. Since the beginning of bud development until their sprouting there is a period of 11-17 days. The flowering period is of 15-19 days. The fruits fully mature in 14-21 days. The flowers that have strong flavour are small, up to 1.5 cm, grouped 3-5 at the axils of the leaves, on flexible, thin peduncles 1-4 cm in length. The flowers are white, with pink nuances and they develop on the annual shoots. The flower formula is $\text{♂}^*\text{P}_{3+3+3}\text{A}_{\infty}[4]$,

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or ♀♂ *P^{Co}₆₋₉ A₍₃₋₇₎ G₃₀₋₄₀^[5]. The Magnolia vine sets fruit starting from the age of 4-5 years.

Schisandra chinensis (Turcz.) Baill. is characterized by rapid shoot growth in June-July and towards the end of August growth diminishes. Its leaves fall down right from the beginning of September. In the meteorological conditions of the Republic of Moldova the species has a period of profound dormancy, which confers resistance to low temperatures during winter and to late frosts in spring. In late spring the plant enters the vegetation period, which has a duration of 175-190 days depending upon the meteorological conditions of the year (sum of temperatures and amount of precipitations). Another factor that favours the development of this species is the presence of phreatic water at shallow depths.

Propagation by seeds. 1000 seeds weigh 25 g. Seed stratification in the three phases at different temperatures ensures a germination percentage of 80-90%. The plants obtained in this way have well developed roots and will set fruit at the age of 4-5 years (Fig. 2). 2-3 year old seedlings need protection during winter. But in *Schisandra chinensis* (Turcz.) Baill. as well as in other fruit shrubs obtained by generative propagation some specific qualities of the mother plant may appear or disappear.

In order to keep the characteristics and qualities of the cultivar, vegetative propagation by cuttings is recommended (Fig. 3). The advantage of propagation by cuttings is that the cuttings are selected from healthy, vigorous and productive plants and plant material with the same characteristics is obtained.

The rooting capacity of the cuttings also depends on the biological features of each species, soil conditions and the special interventions for stimulating the cuttings. Such, in the process of root formation, some hormones like auxin stimulate growth, which can also be achieved artificially by treating the cuttings with stimulating substances, for example heteroauxin. The success of the culture of cuttings depends upon the amount of nutritive substances that they contain and the conditions offered to them when planting – a well prepared, loosened, fertile, well aerated soil and sufficient humidity.

The roots of the cuttings result from the root rudiments, which are groups of meristematic cells localized in the contact point of the medullary rays with the cambium. The root rudiments are formed long the axis of the shoot, with higher densities at the base of the shoot, close to the axillary buds.

As a result of the cut made by making the cutting from the harvested shoot, a primary parenchymatic tissue named callus is formed, which has the role of cicatrizing the wound and which, later, forms a cambium with adventitious buds from which the roots develop. Until the formation of roots, the cuttings consumes the nutritive substances from its own reserves.

The cuttings are made from the sunny part of the plant, from the lower and middle part of the vine. The leaf lamina is shortened with 1/3-1/2 in order to decrease transpiration. A higher percentage (with 10-12%, as compared to the untreated ones) of rooting was obtained in the cuttings treated for 24 hours with 0.01% heteroauxin and stuck into the substrate to a depth of 2-3 cm. In the first 3-4 weeks the cuttings should be watered 2-3 times a day, and then once a day, then 1-2 times a week. The rooting percentage of the cuttings was of 40-50%. It was found that the rooting percentage of the cuttings taken from young plants was of 57-60 %, whereas from older plants 45-50%.

Propagation by hardwood cuttings (winter cuttings) in cold frames needs a well processed substrate, loosened to the depth of 40 cm, with rich aeration and humidity. The

cuttings were harvested in February and March before the beginning of the vegetation period. The cuttings were kept in sand and sawdust until the danger of frost at ground level passed, then they were planted in cold frames. During the insertion of the cuttings into the substrate, the cutting has to adhere well to the particles of the substrate and for this purpose the substrate has to be well prepared and loosened so as to prevent the bruising and wounding of the cutting.

Burying the cuttings into the substrate is done vertically, with the buds upwards and at 1-2 cm below substrate level, so as to prevent the drying out of the tips of the cuttings. Harvesting the shoots for making the cuttings is done from special cultures of mother plants. One year old shoots are harvested, from which the cuttings are made with a well-sharpened knife. The cuttings should be straight, well formed, they should have at least 2 buds, without mechanical lesions. The length of the cuttings was of minimum 15-20 cm and the thickness 8-20 mm at the upper end. For making the cuttings, the upper part of the shoots, which is not sufficiently lignified, should not be used. The cuts should be smooth, perpendicular on the axis of the shoot, without cracks and bark exfoliations. The cut at the upper end should be at 1-2 cm above the bud.

After being cut, the cuttings should be put into KMnO_4 solution and then immediately fixed into sand in vertical or slightly bent position, leaving the upper end at 1 cm above the sand, placed into the greenhouse or cold frame, where humid air should be provided to them, as well as the free access of oxygen to their basal end and an optimal regime of heat and light. These conditions can be ensured by the correct construction of the cold frame, by inserting the cuttings at 0.5-1.0 cm into the sand, by moderate and gradual watering of the sand and by providing a constant temperature of 20-26 °C.

For constructing the cold frame, one should take care that between the surface of the sand and the window of the cold frame there should be 12-15 cm of space. Before inserting the cuttings the sand should be watered abundantly with boiling water and with KMnO_4 solution. The cuttings should be put at distances of 6-10 cm between the rows and 4-5 cm in the row. Immediately after inserting the cuttings, fine spraying is applied and the frame is covered completely.

The cold frames should be shaded in such a way that only diffuse light should enter (sparsely knitted mats or staves are used, which should cover 1/3 of the surface of the frame). The cuttings should be sprayed 3-5 times in the sunny days and 1-2 times in the cloudy days and in the evening the weeds and the blackened cuttings should be pulled out.

After rooting, the frames should be opened gradually, so that the plantlets get accustomed to the outer environment and then kept open permanently and watering should be done until October. The cuttings which have grown good roots until October should be taken out and transferred into the nursery or into containers and they should be watered and shaded during warm days. If they do not have well developed roots in October, the cuttings should be kept in the cold frame, covered with sawdust, until spring.

The root system of the cuttings is relatively poorly developed and one should keep in mind that the roots reach to a depth of just 15-20 cm under ground level.

The length of the first-order root system in the plants obtained from semi-hardwood cuttings reach to 2-5 cm in length from summer till next spring.

In the conditions of the Botanical Gardens, propagation by softwood cuttings did not give good results, only 1% of the softwood cuttings rooted. In the case of propagation by root cuttings, the resulting plants are poorly developed.

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In vitro propagation. In Tab. 1 the nine variants of nutritive media are presented, all of them with 100% MS as basal medium [MURASHIGE & SKOOG, 1962].

In Tab. 2 are presented the results of testing various explants under the influence of auxins and cytokinins present in the nutritive media. The explants consisting of apical meristems had the best reaction on the variants MS -100% with 0.5 mg/l BAP, resulting 44.40% viable plantlets and MS-100% + 0.5 mg/l BAP, 0.1 mg/l NAA, resulting 36.30% viable plantlets (Fig. 4).

The following explant types also reacted positively: shoot fragment from the apical part, shoot fragment with apical meristem. The reaction of all the explant types on the nine variants of media is presented in Tab. 2.

Conclusions

Schisandra chinensis (Turcz.) Baill. is a species that has adapted to the pedoclimatic conditions in the republic of Moldova.

It can be propagated generatively or vegetatively.

In the case of propagation by seed it is recommended to stratify the seeds in three phases: I - for 30 days at $t = 18-20$ °C, II- 30 days at $t = 3-5$ °C and III- 60 days at $t = 8-10$ °C and then the seeds should be sown, such ensuring 80-90% germination.

As a result of the process of propagation by hardwood and semi-hardwood cuttings, uniform genetic material is obtained, healthy, vigorous plantlets that possess the features and qualities of the mother plant. The rooting of the cuttings depends on several factors: the quality of the cuttings and of the substrate, The conditions for the growth and development of the mother plants, respecting the optimal timeframes for propagation by cuttings and correctly applying the technology of propagation by cuttings, the density of the cuttings in the cold frame etc. For obtaining a higher rooting percentage of *Schisandra chinensis* (Turcz.) Baill. It is necessary to select the shoots from young plants, which were also obtained by vegetative propagation, in the 20th of June-10th of July, which coincides with the end of flowering and the beginning of fruit set.

The optimal substrate for the vegetative propagation of *Schisandra chinensis* (Turcz.) Baill. is made up of sand + peat 1:1.

Among the rhyzogenesis stimulators used for rooting the semi-hardwood *Schizandara chinensis* (Turcz.) Baill. cuttings, it was established that the stimulators IBA and IAA-0,01% plus sucrose at 10 g/l concentration stimulate rooting.

The plants obtained from cuttings as well as the ones obtained from seeds are very sensitive to low temperatures in the spring in the first years, hence the plants must be protected by leaves.

Among the 10 types of explants tested for *in vitro* culture initiation in *Schisandra chinensis* (Turcz.) Baill, the apical meristems reacted the best, especially on the variant with MS medium + 0.5mg/l BAP (44.44% viable plantlets obtained). The explants consisting of shoot fragments from the apical part and shoot fragments with apical meristem also had positive reaction.

Schisandra chinensis (Turcz.) Baill. can be recommended in the range of species used for setting up green areas, as it is a robust climber, with decorative value during the whole year and also used as a medicinal plant with multiple active and stimulating substances, with a wide range of applicability.

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a



b

Fig. 1. *Schisandra chinensis* (Turcz.) Baill. a) The flowering phase; b) The fruiting phase



Fig. 2. Propagation by seeds



Fig. 3. Propagation by cuttings

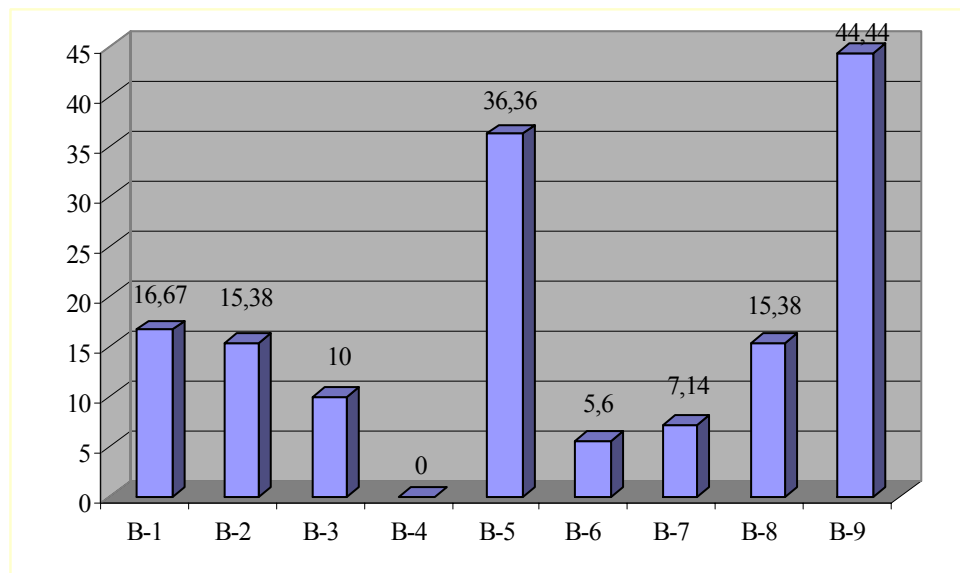


Fig. 4. The reaction of the apical meristems on the nine variants of media (%)

Tab. 1. The composition of the nutritive media for the microclonal propagation of species *Schisandra chinensis* (Turcz.) Baill.

Variants	Basal medium	Supplementary additives
B-1	MS-100%	Kin - 0.5 mg/l NAA- 0.1mg
B-2	MS-100%	Kin -1.0 mg/l IAA – 0.5 mg/l
B-3	MS-100%	Kin – 0.5 mg/l IAA – 0.1 mg/l
B-4	MS-100%	Kin – 2.0 mg/l IAA– 0.5 mg/l
B-5	MS-100%	BAP – 0.5 mg/l NAA -0.1 mg/l
B-6	MS-100%	BAP – 0.5 mg/l GA ³ – 0.5 mg/l
B-7	MS-100%	BAP – 0.5 mg/l IAA – 0.1 mg/l
B-8	MS-100%	BAP – 0.5 mg/l IAA – 0.5 mg/l
B-9	MS-100%	BAP – 0.5 mg/l

Tab. 2. The initiation and *in vitro* reaction of various types of explants in *Schisandra chinensis*

Explant Medium	Apical meristem	Lateral meristems of the 2 nd , 3 rd , and 4 th degree	Lateral meristems of the 5 th and 6 th degree	Fragments of juvenile leaves	Shoot fragments from the apical part	Shoot fragments with lateral meristem	Fragments of young leaves with veins	Ovules	Ovary with a fragment from the stem	Stem fragment with the apical meristem
1	2	3	4	5	6	7	8	9	10	11
B-1	+			~				~	~	
B-2	+	~	~	~	+	+	+	~		
B-3	+	~	~	~	-	+		~		
B-4	-	-	-	+	+					
B-5	+	~	~		+	+		~		+
B-6	+									
B-7	+	~			+	+	~	~		
B-8	+		~	~	+	+	~	+	+	+
B-9	+	~	~	~	~	~	~	~		

+ progress; - necroses; ~ without changes

SOME ASPECTS REGARDING THE CULTIVATION OF SPECIES WITH DECORATIVE VALUE *ACONITUM DEGENII* Gáyer

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BÎRSAN CIPRIAN¹

Abstract: *Ex situ* conservation is the most important way through a Botanic Garden contributes to biodiversity conservation. One of the multiple directions of *ex situ* conservation is the diversification of the collections with spontaneous cormophytes presenting decorative value. *Aconitum degenii* Gáyer is an herbaceous, perennial and toxic species with the areal in Alps and Carpathian Mountains, sporadically met at forest's margins. This species has been studied in 2009-2011 period in order to observe its behavior in the environmental conditions characteristic to the Botanic Garden of Iași. To accomplish this aim seeds, rhizomes and individuals have been collected from the wild and introduced in experimental fields. Comparisons between flowering periods, qualitative (color of the flowers) and quantitative (number of the flowers, leaves and ramifications, plants height, rhizomes length) decorative characters of both cultivated and spontaneous individuals have been realized. It was observed that *Aconitum degenii* Gáyer individuals are keeping (almost the same quantitative characteristics) and even improve (longer flowering period) their decorative characteristics without being deteriorated or diminished. From the morpho – anatomical perspective none significant differences have been observed.

Key words: decorative species, conventional cultivation

Introduction

The *Aconitum* genus includes approximately 300 species distributed mainly in the Northern Hemisphere [LUO & al. 2005]. In Romania, there were identified 10 species with 5 atypical subspecies [CIOCĂRLAN, 2000] and it seems that the richest areas in *Aconitum* species are represented by the Northern half of the Oriental Carpathians and the Eastern part of the Southern Carpathians; in the Apuseni Mountains, *Aconitum* species appears less frequently [MIHOK & al. 2005]. These are herbaceous perennial plants, mainly cultivated for their tubers, used in medicinal and pharmaceutical purposes. Various active constituents produced from the roots of various species of *Aconitum* are used to cure a wide range of diseases [NIDHI & al. 2010]. Some species are also cultivated in ornamental purposes.

Aconitum degenii Gáyer (syn. *Aconitum paniculatum* Lam. nom. illeg.) is a species presenting a cylindrical, branched rhizome and tall stems (60-150 cm). The leaves are alternately arranged, palmate divided and the flowers are blue-purple colored, with long pedicels and arranged in wide, thinned and richly branched inflorescences. It grows at the mountain forests edges. It is an herbaceous, perennial species (hemicryptophyte), with areal in Alps and Carpathians Mountains. It prefers full light, cool mountain areas, relative humid, neutral and rich in nitrogen soils (L₆T₂C₄U₆R₇N₇) [ELLENBERG, 1992]. It can be found in 6430 – Hydrophilous tall-herb fringe communities of plains and of the montane to

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alpine levels habitat type [GAFTA & MOUNTFORD, 2008], growing on fertile soils (*Betulo – Adenostyletea*) [CHIFU & al. 2006]. It is a diplo-polyploid species, spread sporadically in Romania (beech – fir vegetation levels) [CIOCĂRLAN, 2000].

The species is known and used for medicinal purposes since antiquity. It is a toxic species that can be grown either alone or in groups in parks and gardens, being decorative by flowers.

Material and methods

In order to set up the experimental fields and to favor the growth, flowering and fructification of *Aconitum degenii* individuals, a flat, sunny and protected from strong currents of air land located between the greenhouses of the Botanical Garden of Iasi has been chosen. Soil preparation was done in the preceding autumn of planting and consisted of weed removal and destruction of their roots [CIREAȘĂ, 1993]. Before planting, in the spring, the land was worked with a cultivator. Fertilizers were not applied in advance.

Multiplication has been realized by seeds (Fig. 1) presenting good germination, about 80%, of which seedlings are produced (autumn sowing in pots, in November, because seeds lose germination capacity) and rhizomes (vegetative). Seedlings were obtained in pots using a mixture of earth, organic natural fertilizer, neutral peat and sand (1 : 2 : 1 : 1). The planting was done in spring, at the end of April. The plants were subjected to the process of mud and planted at 70 cm between rows and 50 cm distance from each other. The vegetative propagation by rhizomes has been tried. To do this, only the young and healthy rhizomes were used. Rhizomes were planted with the buds facing up, at the same distance intervals as the seedlings. The seeds collection has been realized after the fruit maturation (the second half of September). The harvesting of rhizomes has been made both during flowering period (June-August) and after the flowering period (September). Only young rhizomes have been collected. Waterings were applied each day (the first month after planting) to maintain soil moisture continuously. Weeds removal had a very important role because weeds absorbed the moisture and food of *Aconitum* plants and, in the early stages of development overshadowed the seedlings, with negative effects on their development. In culture conditions, *Aconitum degenii* plants grow well, vigorous, but do not tolerate the presence of weeds.

Results and discussions

Natural populations from Eastern Carpathians (Stânișoarei, Ceahlău, Bistriței, Călimani and Nemira Mountains) of *Aconitum degenii* have been studied in areas with altitudes varying between 750 and 900m, characterized by a relative cold climate (2-4 °C yearly average), abundant precipitations (750-1000 mm/m²/year), increased relative humidity of atmosphere (≈ 80-85%) and neutral-weak acid soils (pH 6-7). From the natural habitats (*Vaccinio – Piceetea* forests edges) seeds and individuals have been collected in order to cultivate them in the Botanic Garden from Iasi (characterized by a temperate continental climate with annual precipitations average of approximate 518 mm, annual temperatures average by 9,6 °C, relative humidity of the atmosphere ≈ 70% and neutral soils – pH ≈ 7).

The seeds have initiated the germination process after 3 weeks. The cultivated plants produced flowers and viable seed in the first year of growth. Cultivated plants, grown from seeds and tubers in Botanic Garden from Iasi, generally produced one to two daughter tubers

by the end of the growing season. The transplanted seedlings collected from the wild and grown at low altitude presented a decrease of the plants characteristics (Fig. 3).

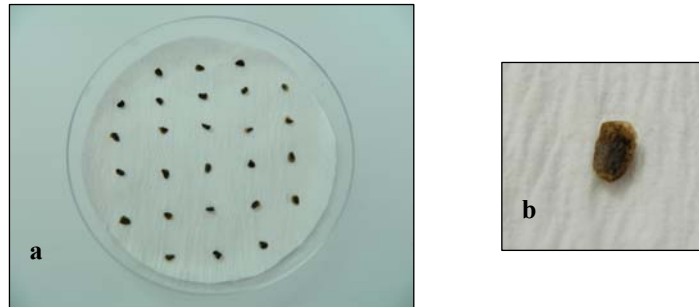


Fig. 1. a) Seeds of *Aconitum degenii* – germination capacity determination
b) seed – detail

In culture conditions the *Aconitum degenii* individuals present cylindrical, branched rhizomes, with an average length of 8.21 cm, less with approximate 2 cm on average comparing with the specimens studied in natural habitats. Individuals average height is 77.28 cm, lower than the height of the individuals from spontaneous flora (average 86.46 cm).

The leaves of *Aconitum degenii* are palmate divided (Fig. 2b), are alternately arranged and are less in the cultivated plants comparing to the specimens studied in natural habitats (an average of 19.83 leaves on a stem compared to 27.9) which is correlated with height decrease under culture conditions (Tab. 1).

The flowers are numerous and are arranged in branched rich inflorescences (average 12.32 branches / plant). The flowers (Fig. 2a) are light blue - purple (size, color and intensity are kept) and presents long pedicels. Regarding the flowering period it was observed that the most of the cultivated individuals bloom at the same time as in natural habitats (July - September).

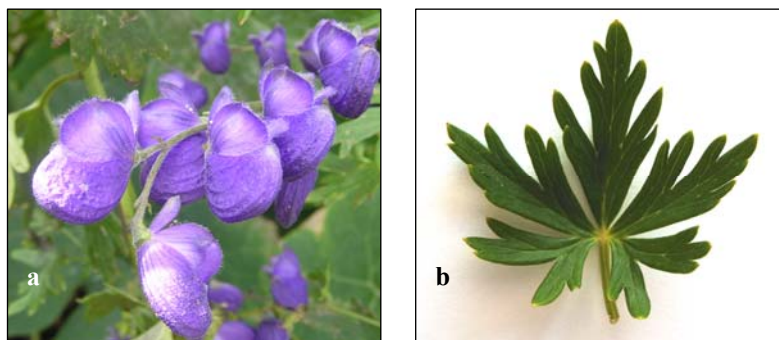


Fig. 2. The main ornamental characteristics of *Aconitum degenii* Gáyer
a – flowers
b – leaf

Tab. 1. Comparison of the morphometric characteristics of *Aconitum degenii* in natural habitats and culture conditions

POPULATION	ECOLOGIC CONDITIONS	COENOTIC AMBIANCE	MORPHOMETRIC CHARACTERS	NATURAL HABITATS AVERAGE	TRANSPLANTATION PLACE / ECOLOGIC CONDITIONS	MORPHOMETRIC CHARACTERS (2009-2011)
Stănișoarei Mountains	Altitude: 750 m. pH soil: ≈ 6.5 . Annual precipitations average: 800 mm. Annual temperatures average: 4 °C. Relative humidity of the atmosphere: $\approx 85\%$.	<i>Leucanthemo waldsteinii-Piceetum</i> : <i>Leucanthemum waldsteinii</i> , <i>Picea abies</i> , <i>Hypericum maculatum</i> , <i>Digitalis grandiflora</i> , <i>Veronica urticifolia</i> etc.	Plant height: 90,1 cm. Rhizomes length: $\approx 10,3$ cm. Ramifications number: 10,6. Leaves number/plant: 26,4. Flowers number/plant: 38,6.	Plant height: 86,46 cm. Rhizomes length: 10,12 cm. Leaves number/plant: 27,9. Ramifications number: 12,375. Flowers number/plant: 30,25.	Iasi Botanical Garden Altitude: 150 m. pH soil: ≈ 7 . Annual precipitations average: 518 mm. Annual temperatures average: 9,6 °C. Relative humidity of the atmosphere: $\approx 70\%$.	Plant height: 77,28 cm. Rhizomes length: 8,21 cm. Leaves number/plant: 19,83. Ramifications number: 12,32. Flowers number/plant: 35,55.
Bistriței Mountains	Altitude: 810 m. pH soil: ≈ 7 . Annual precipitations average: 900 mm. Annual temperatures average: 4 °C. Relative humidity of the atmosphere: $\approx 85\%$.	<i>Hieracio transsilvanici-Piceetum</i> : <i>Picea abies</i> , <i>Luzula luzuloides</i> , <i>Brachypodium sylvaticum</i> , <i>Sanicula europaea</i> , <i>Epipactis helleborine</i> etc.	Plant height: 84,94 cm. Rhizomes length: 9,6 cm. Leaves number/plant: 26,48. Ramifications number: 14,1. Flowers number/plant: 31,2.			
Călimani Mountains	Altitude: 900 m. pH soil: ≈ 6 . Annual precipitations average: 1000 mm. Annual temperatures average: 2 °C. Relative humidity of the atmosphere: $\approx 80\%$.	<i>Hieracio transsilvanici-Piceetum</i> : <i>Picea abies</i> , <i>Oxalis acetosella</i> , <i>Lilium martagon</i> , <i>Streptopus amplexifolius</i> , <i>Dryopteris filix-mas</i> etc.	Plant height: 90,50 cm. Rhizomes length: 11,22 cm. Leaves number/plant: 30,2. Ramifications number: 10,8. Flowers number/plant: 24,4.			
Nemira Mountains	Altitude: 780 m. pH soil: ≈ 7 . Annual precipitations average: 750 mm. Annual temperatures average: 4 °C. Relative humidity of the atmosphere: $\approx 85\%$.	<i>Hieracio transsilvanici-Abietetum</i> : <i>Abies alba</i> , <i>Picea abies</i> , <i>Lonicera xylosteum</i> , <i>Viola reichenbachiana</i> , <i>Geranium robertianum</i> etc.	Plant height: 80,3 cm. Rhizomes length: 9,36 cm. Leaves number/plant: 28,54. Ramifications number: 14. Flowers number/plant: 26,8.			

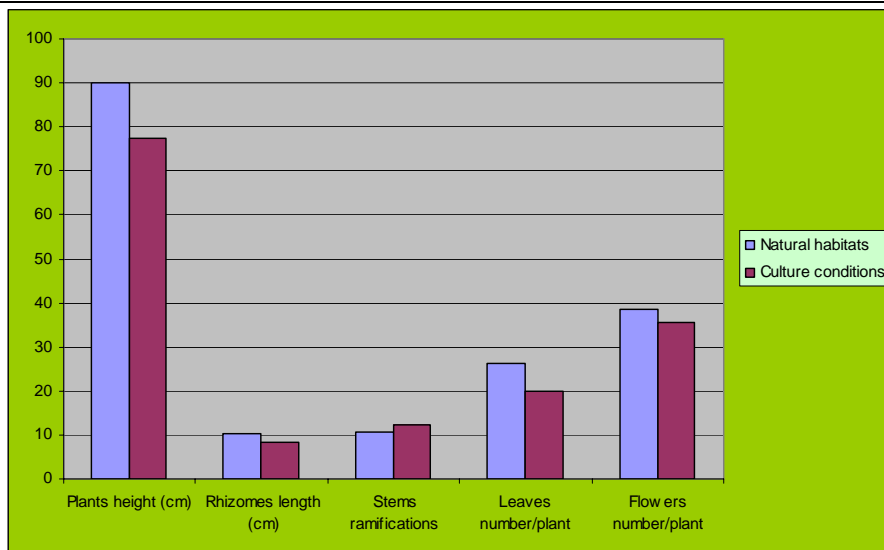


Fig. 3. Comparison between of some morphometric characters of *Aconitum degenii* individuals in natural habitats and under culture conditions

In the ecological conditions of the Botanic Garden of Iasi the plants have developed well both in partially shaded areas and full sunned areas as long as the soil was kept moist. The vegetation period of the *Aconitum degenii* individuals is about 160 days per year. The growth and the development of the plants in the first year of culture is slower but in the next years (the second and third) is more intense.

This species does not raise special issues of growth in the ecological conditions from Iași. It can be easy replicated by seedlings (from seeds) and rhizomes. Also, there have been not recorded any diseases or other pests during those three years of cultivation.

The *Aconitum degenii* inflorescences can be used as cut flowers (with caution because the species is toxic) or they can add a blue color to a shaded garden. These plants grow well in damp, rich soils and the beautiful blue or blooms is very attractive from the early to late summer in the garden. To determine the plant to form another round of flowers in the late summer or early autumn, the first inflorescences must be cut in order to not allow the plant to make seeds. Also, the flowers of *Aconitum degenii* resist a relative long time as cut flowers (about 10 days) during which the flowers continue to open.

The species could be planted under trees, in near wild gardens, among shrubs and in borders. It can fill vacant spaces in the garden when the earlier flowering plants have past. The *Aconitum degenii* individuals should be planted in masses (spots with numerous individuals). All species of *Aconitum* are very toxic (they contain aconitine, other alkaloids etc.), and,

consequently, they should never be planted in or too close to the vegetable gardens or the playing places of the children.

Conclusions

In the studied species (*Aconitum degenii*) the main decorative element is the flower. Secondly, the plant height, the number of stem's branches, the size, the shape and the arrangement of leaves and number of flowers (or inflorescences) are other characters that justifies the introduction of this species in culture.

Comparisons between the flowering periods, between a series of qualitative characters (such as flower color) and quantitative (number of flowers, leaves and stems branches, plant height, length of rhizomes) both in individuals obtained in culture or from the natural habitats showed that, under culture conditions, plants remains (approximately the same quantitative characters) and even improve (a longer period of flowering) decorative characters without to be deteriorated or diminished.

We consider that *Aconitum degenii* is well suited to the purpose of introducing in the culture because it proves to be adapted quite well (even acclimatized, because they produced flowers and fruits) in the environmental conditions from the Botanical Garden of Iași.

Acknowledgements

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**RESEARCH REGARDING THE INTRODUCTION OF A LEAST
KNOWN VEGETABLE SPECIES IN CULTURE,
IN TRANSYLVANIAN TABLELAND AREA;
THE POSSIBILITY OF CULTIVATING CHINESE CABBAGE IN
EARLY SPRING IN OPEN FIELD**

LACZI ENIKÓ¹, APAHIDEAN ALEXANDRU SILVIU¹

Abstract: The research concerning the possibility of cultivating Chinese cabbage (*Brassica campestris* var. *pekinensis*) took place in the spring of 2011, in the experimental field which belongs to the Vegetable Growing Department from the University of Agricultural Sciences and Veterinary Medicine from Cluj-Napoca. A collection of varieties and hybrids belonging to this species was established, within which a variety (Granat) and four hybrids (Michihli, Kingdom 80, Nepa F1 and Vitimo F1) were used.

During the vegetation period measurements were made regarding the growing and the development of these plants in Transylvanian Tableland specific conditions. The processing of data recorded at harvest shows that the obtained yield varied between 41.00 and 63.15 t/ha, the Vitimo F1 hybrid reaching the highest yield.

The obtained yields are satisfying, considering that the culture was an ecological one, no chemical products such as fertilisers or substances for prevention and control of pests and diseases were used.

Keywords: Chinese cabbage, organic culture, Chinese cabbage varieties

Introduction

Chinese cabbage, *Brassica campestris* var. *pekinensis* (syn. *Brassica rapa* var. *pekinensis*) is a less known vegetable in our country and it is cultivated mostly by amateur gardeners. Unfortunately, in this moment, it isn't defined a well-established culture technology in our specialty literature.

The development of this species in China was parallel with the European cabbages in Europe. Both belong to the same genus, *Brassica*, both evolved by cultivation from wild ancestors, both have been important foods since the remote past, and both now exist in numerous varieties which can be bought almost all year round [DAVIDSON & TOM, 2006].

Chinese cabbage has a short vegetation period, and it belongs to that group of plants which are the fastest growing of all leafy vegetables, in good conditions heads can be cut ten weeks after sowing; loose-headed types two to three weeks sooner, while seedlings four to five weeks after sowing.

The headed types of Chinese cabbage form a barrel-shaped, rounded or tall cylindrical head of closely folded leaves, usually creamy to light green in color, with a crinkled texture, prominent white veining and white midribs broadening out at the base [LARCOM, 2003].

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Material and method

The research took place in the experimental field belonging to the Vegetable Growing Department of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, in the spring of 2011.

The main purpose of this experiment was the establishment of a culture technology, which allows the cultivation of this new species in the Transylvanian Tableland specific conditions. There were tested five varieties of Chinese cabbage in early spring ecological cultures.

To achieve the objectives of this experiment a collection of varieties was organized, which involved the following varieties:

- Michihli
- Kingdom 80
- Granat
- Nepa F1
- Vitimo F1

Each variety was placed into three repetitions.

The seeding started in 25th February, the seeds being sown, one by one, in small nutrient pots and were transplanted in bigger pots in stage of 3-4 true leaves, in 26th of March. Planting was realized in 4th of April, in the experimental field.

During the vegetation period there weren't made any treatments or fertilizations. Harvest was realized in 1st of June at Granat variety and Nepa F1 and Michihli hybrids, and in 10th of June at Kingdom 80 and Vitimo F1 hybrids.

During growing season observations were made regarding plants growth and development (these were made at planting, at one month after planting and at harvesting), and on obtained production to.

Results and discussions

Plants height evolution from planting to harvest. At planting, the highest seedlings (15.33 cm) were those from Granat variety, while at harvest those from Nepa hybrid, with an average height of 52.50 cm.

It can be observed that at four of the five variants the plants height is increasing constantly, but at the last one, represented by Vitimo hybrid, plants average height is decreasing in the last few weeks of the vegetation period, from 31.37 cm to 29.33. This fact can be explained by the head formation, where every leaf has a very important role.

At the remaining variants the height increasing, from planting to a month after planting, varied between 7.83 cm (at Kingdom 80 hybrid) and 26.83 cm (at Michihli hybrid), while until harvest the average height has grown with 3.17 cm (at Kingdom 80 hybrid) and 16.17 cm (at Nepa F1 hybrid) (Fig. 1).

Plants diameter evolution from planting to harvest. The seedlings diameter varied between 13.33 cm (at Vitimo hybrid) and 19.00 cm (at Kingdom 80 hybrid), while at harvest the measured diameters had values between 47.33 cm (at Vitimo hybrid) and 66.50 cm (at Nepa hybrid).

Unlike the plants height, their diameter was increasing constantly at all variants from planting to harvest. So, in one month from planting the plants diameter increasing

varied between 24.67 cm (at hybrid Michihli) and 33.33 cm (at Kingdom 80 hybrid), while until harvest the increasing had much lower values, varying between 1.17 cm (at Kingdom 80 hybrid) and 17.83 cm (at Nepa hybrid) (Fig. 2).

Leaf number evolution from planting to harvest. In Fig. 3 it can be observed that the highest number of leaves was registered at Kingdom 80 hybrid, not only in seedling stage (when it had an average of 7.67 leaves), but at the measurements made at one month after planting (with 19.83 leaves) and at harvest to (when plants were formed in average from 35.67 leaves). This hybrid was closely followed by Vitimo F1, which had an average of 34.67 leaves.

The lowest increasing of leaves number from planting to harvest was registered at Michihli hybrid, which formed only 14.50 leaves in the vegetation period.

Correlation between total number of leaves and total weight. The correlation coefficient between total number of leaves and total weight had a value of 0.73, which is lower than the value of $p(5\%)=0.88$, for the five cases studied, so between these characters there is no statistically supported correlation (Fig. 4).

Cabbage head development at maturity. Data from Table 1 shows that the longest heads (with an average length of 46.83 cm) and largest diameter (with an average diameter of 41.00 cm) were registered at Michihli hybrid. The highest weight of plants (0.73 kg) was noticed at Vitimo hybrid, while the plants belonging to Kingdom 80 hybrid had the highest number of leaves.

Comparison between total plant and head weight. Total head weight varied between 0.56 and 0.88 kg, while the head weight between 0.47 and 0.73 kg. The lowest difference between the two characters was registered at Michihli hybrid (a difference of only 80 g), and the highest one at hybrid Vitimo, where the heads were easiest with 150 g than the plants (Fig. 5).

Correlation between total and head weight. Fig. 6 presents the correlation between total and head weight, the coefficient of correlation between this two characters, being 0.97, which is higher than the theoretically value for $p(1\%) = 0.96$, for the five studied cases, so between total and head weight exists a distinct significant positive correlation.

Leaf layout. The leaf layout is a very important characteristic of Chinese cabbage, because the leaves from rosette are in most cases removed, and only the cabbage head is used. The rosettes were formed, in average, from 5.67 leaves (at Michihli hybrid) and 8.17 leaves (at Kingdom 80 hybrid), while the number of leaves from the heads varied between 14.83 leaves, at Granat variety and 27.50 leaves at Kingdom 80 hybrid, followed closely by Vitimo hybrid, with 27.17 leaves (Fig. 7).

Results regarding the bolting percentage. The measurements made at one month after planting shows that at Michihli hybrid, the bolting percentage was 8.33%, at Granat and Nepa was 12.5%, while at the last two hybrids (Kingdom 80 and Vitimo) no plants had been bolted until this moment, the average bolting percentage being 6.67%.

Until harvest, the average bolting percentage increased, reaching the value of 18.33%, the lowest bolting percentage (8.33%) was registered at Vitimo hybrid, while the highest (29.17%) at Granat variety (Fig. 8).

The influence of variety upon the yield of Chinese cabbage. The data from Table 2 shows that the yields varied between 41.00 and 63.15 t/ha. The lowest yield was registered when Granat variety was used, while the highest one was observed when Vitimo hybrid was cultivated. If Granat variety was took as control variant, a distinct significant

RESEARCH REGARDING THE INTRODUCTION OF A LEAST KNOWN VEGETABLE SPECIES ...

difference was observed at Kingdom 80 hybrid, which has a yield of 49.07 t/ha, with 14.80% more than at Granat variety. In addition to this difference two more very significant differences were observed at Nepa and Vitimo hybrids, where the yields were higher than at the witness variant with 28.48% and 54.02%, their yields being 52.67 and 63.15 t/ha.

If the average yield of the five varieties was considered the control variant, there was registered a distinct significant difference at Granat variety, where the yield was lower with 8.66 t/ha than the average one. Beside this, a significant negative difference was registered at Michihli hybrid (with a lower yield of 7.26 t/ha), and a very significant positive one, at Vitimo hybrid, which had an increased yield with 13.49 t/ha compared with the control variant.

Conclusions

The highest plants with largest diameter, at harvest, were those from Nepa hybrid, which had an average height of 52.50 cm and an average diameter of 66.50 cm.

The plants belonging to Kingdom 80 hybrid, had the highest number of leaves, 35.67, which were followed closely by the plants from Vitimo hybrid, with 34.67 leaves.

Even if between total leaf number and total weight there was no statistically supported relationship, between head and total weight exists a distinct significant positive correlation.

The bolting percentage had an average value of 18.33%, at harvest, with a minimum number of bolted plants at Vitimo hybrid.

The yields varied between 41.00 t/ha (at Granat hybrid) and 63.15 t/ha (at Vitimo hybrid).

The most suitable hybrid, for cultivation in Transylvanian Tableland area is Vitimo F1 (due to its high production and low bolting percentage), followed by Nepa F1 (due to its high yield and good plant development).

In conclusion it can be said that Chinese cabbage can be cultivated in Transylvanian Tableland area, even in early spring ecological cultures, in open field, without using special measures, adding fertilizers or making treatments with synthetic products.

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Tab. 1. Cabbage head development at maturity

Variant		Cabbage head			
No.	Variety / Hybrid	Lenght (cm)	Diameter (cm)	Weight (kg)	Number of leaves
1	Michihli	46,83	41,00	0,48	16,17
2	Kingdom 80	35,00	34,50	0,59	27,50
3	Granat	44,33	27,50	0,47	14,83
4	Nepa F1	42,00	38,67	0,66	19,83
5	Vitimo F1	28,67	37,17	0,73	27,17
<i>Average</i>		39,37	35,77	0,59	21,10

Tab. 2. The influence of variety upon the yield of Chinese cabbage

Variant	Average yield (t/ha)	Relative yield (%)	Difference (t/ha)	Significance	Relative yield (%)	Difference (t/ha)	Significance
Hybrid							
Granat	41.00	100,0	0,00	Mt.	82.56	-8.66	oo
Kingdom 80	49,07	114.80	8.07	**	94.78	-0.59	-
Michihli	42.40	103.41	1.40	-	85.38	-7.26	o
Nepa F1	52,67	128.48	11.67	***	106.06	3.01	-
Vitimo F1	63.15	154.02	22.15	***	127.16	13.49	***
<i>Average</i>	49.66	-	-	-	100,0	0,00	Mt.

LSD (p 5%)

6,09

LSD (p 1%)

8.05

LSD (p 0,1%)

11.25

FIGURE CAPTIONS

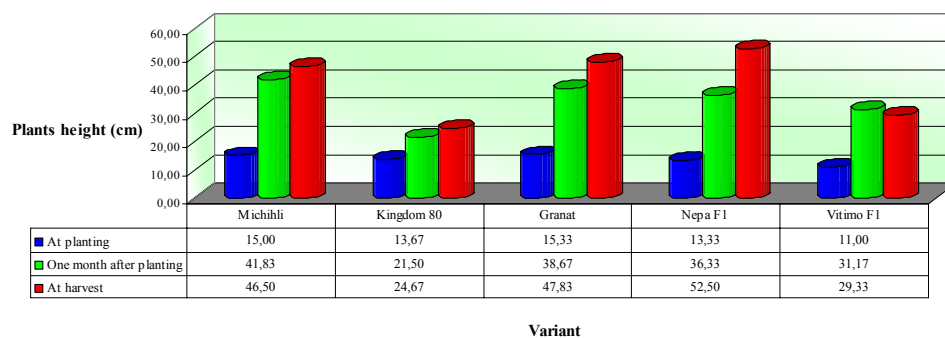


Fig. 1. Plants height evolution from planting to harvest

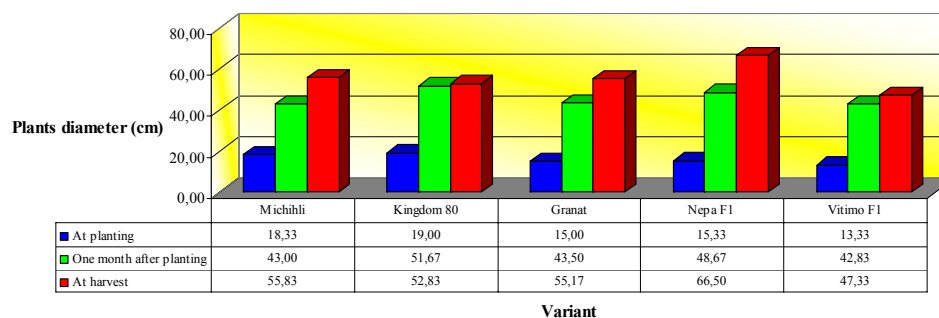


Fig. 2. Plants diameter evolution from planting to harvest

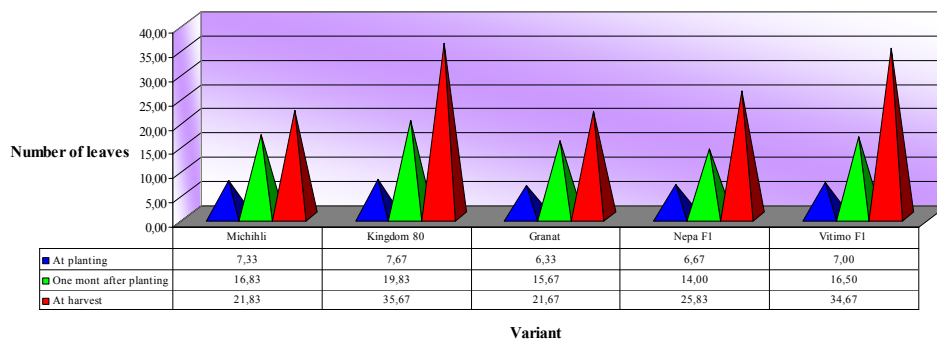


Fig. 3. Leaf number evolution from planting to harvest

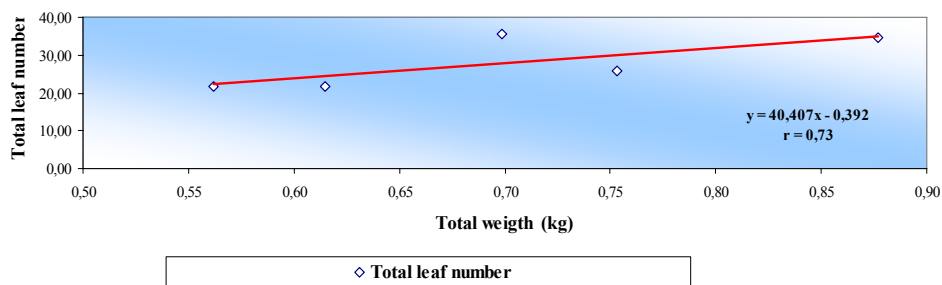


Fig. 4. Correlation between total number of leaves and total weight
 n=5, p(5%)=0.88, p(1%)=0.96

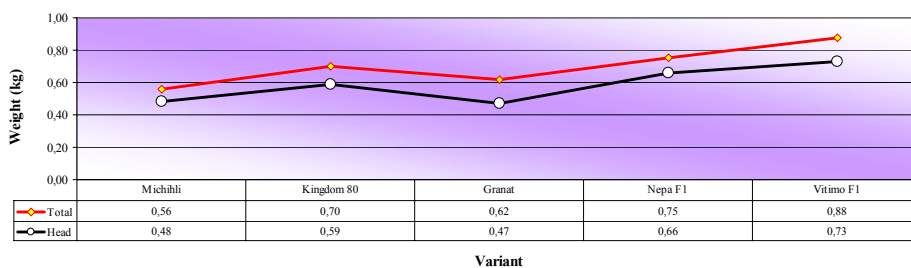


Fig. 5. Comparison between total plant and head weight

RESEARCH REGARDING THE INTRODUCTION OF A LEAST KNOWN VEGETABLE SPECIES ...

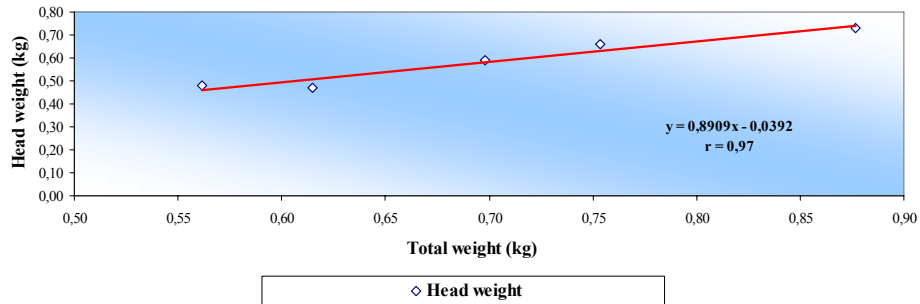


Fig. 6. Correlation between total and head weight
 $n=5$, $p(5\%)=0.88$, $p(1\%)=0.96$

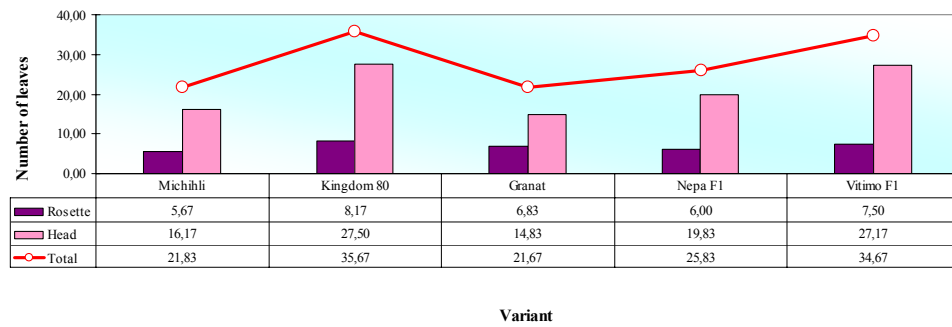


Fig. 7. Leaf layout

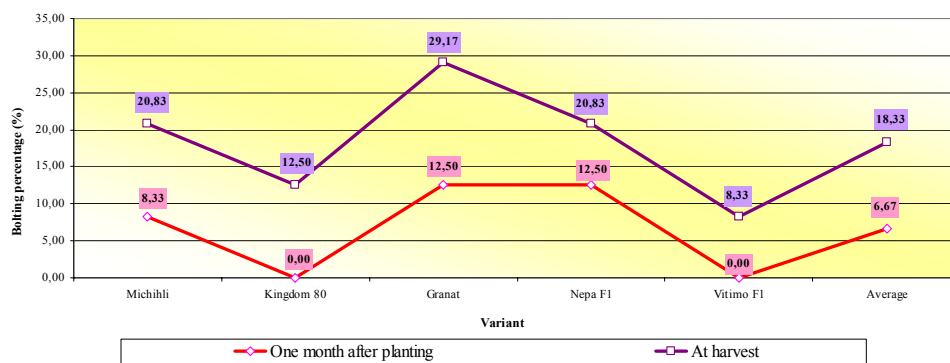


Fig. 8. Bolting percentage

COMPARATIVE BIOCHEMICAL AND PHYSIOLOGICAL RESEARCH ON TAXA OF *MENTHA* L. GENUS

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ATOFANI DOINA¹, COISIN MAGDA¹, ZAMFIRACHE MARIA-MAGDALENA¹

Abstract: The *Mentha* L. genus has many aromatic and medicinal taxa with a large area in our country. These taxa prefer flooded, swampy areas and wetlands, but they can also grow in moderate dry areas. Biochemical characteristics were obtained for 7 taxa from *Mentha* L. genus, wild or cultivated plants. The studies concerning the assimilative pigments, the hydric content, and the dry matter were determined for each vegetation stage. We used the gravimetric method for the hydric content and dry matter and the spectrophotometric method for estimation of the assimilative pigments. The results of the experiments are not the same for each taxon because of different harvesting periods and the ecological conditions of each taxon area.

Key words: *Mentha*, assimilative pigments, dry matter, water

Introduction

Mentha genus includes herbaceous plants, perennial, aromatic, with a pungent odor characteristic, due to the volatile oil they contain. The Genus *Mentha* L. has a complex taxonomy, which makes it difficult to identify the species because of its phenotypic plasticity, genetic variability and because most species are able to produce hybrids by crossing. For example, the delimitation of the species *Mentha spicata* L. is problematic due to hybridization and doubling the number of chromosomes, especially when introgressive hybridization appears between some species in certain areas [HARLEY, 1972].

Due to their properties, these plants are used in pharmaceutical cosmetic and food industries. Photosynthetic pigments are known for their physiological role of protection against physical agents, such as blue and ultraviolet radiation, but also against biological agents [HOPKINS, 1985].

Water provides an environment for vital biochemical reactions. In metabolic processes it makes the enzyme activity and is involved in both biosynthesis and anabolic processes to catabolic processes of degradation [TOMA & JIȚĂREANU, 2000].

Materials and methods

The material used in this paper is represented by seven taxa of the genus *Mentha*, cultivated or from spontaneous vegetation, collected in three phenophases: vegetative, flowering and senescence, during the vegetation period of 2010. The cultivated taxa are: *Mentha spicata* L., *Mentha piperita* var. *black* Mitcham. and *Mentha x piperita* var. *columna* L. The spontaneous taxa were collected from the following locations: Caraorman,

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Tulcea County (*Mentha aquatica* L. and *Mentha pulegium* L.), Negrești and Cioatele, Vaslui County (*Mentha longifolia* (L.) Huds.).

For determination of foliar assimilatory pigments we used the spectrophotometric method and for determining the content water and dry matter the gravimetric method was used [BOLDOR & al. 1983].

Research has been conducted in the Laboratory of Plant Physiology of the Faculty of Biology, “Alexandru Ioan Cuza”, Iași.

Results and discussions

The research conducted on the biosynthesis and accumulation of assimilatory pigments in the studied taxa, reveals the following results (Tab. 1):

Tab. 1. Assimilatory pigment content in species of the genus *Mentha* during vegetative stage in 2010

Taxon	Vegetative stage	Chlorophyll <i>a</i> (mg/g fresh matter)	Chlorophyll <i>b</i> (mg/g fresh matter)	Carotenoidic pigments (mg/g fresh matter)	Chlorophyll <i>a</i> / Chlorophyll <i>b</i>
<i>Mentha longifolia</i> (L.) Huds. (Negrești)	Vegetative	1.130	0.404	0.0003	2.797
	Flowering	1.353	0.451	0.0004	3.000
	Senescence	1.012	0.283	0.0003	3.575
<i>Mentha longifolia</i> (L.) Huds. (Cioatele)	Vegetative	0.961	0.311	0.0002	3.090
	Flowering	0.909	0.339	0.0002	2.681
	Senescence	1.252	0.360	0.0003	3.477
<i>Mentha aquatica</i> L. (Caraorman)	Vegetative	0.455	0.198	0.0001	2.297
	Flowering	0.495	0.178	0.0001	2.780
	Senescence	0.641	0.315	0.0002	2.034
<i>Mentha pulegium</i> L. (Caraorman)	Vegetative	0.779	0.237	0.0001	3.286
	Flowering	0.905	0.365	0.0002	2.479
	Senescence	1.127	0.401	0.0003	2.810
<i>Mentha x piperita</i> var. <i>columna</i> L. (Vaslui)	Vegetative	1.841	0.590	0.0005	3.120
	Flowering	1.532	0.450	0.0004	3.404
	Senescence	1.340	0.452	0.0004	2.964
<i>Mentha piperita</i> var. <i>black</i> Mitcham. (Piatra Neamț)	Vegetative	1.510	0.558	0.0004	2.706
	Flowering	2.081	0.739	0.0001	2.815
	Senescence	1.898	0.675	0.0002	2.811
<i>Mentha spicata</i> L. (Piatra Neamț)	Vegetative	1.101	0.387	0.0003	2.844
	Flowering	2.294	0.755	0.0006	3.038
	Senescence	2.178	0.643	0.0003	3.387

On the taxa taken into study one may note an upward trend of assimilatory pigments in plant during the growing season. From the quantitative point of view there are significant differences throughout the growing season: the content of assimilatory pigments in cultivated taxa (*M. x piperita* var. *columna*, *M. x piperita* var. *black*, *M. spicata* is obviously higher than that of spontaneous taxa (*M. longifolia*, *M. aquatica*, *M. pulegium*). These taxa were collected in the same phenophase, so the process of photosynthesis is greater in the leaves of cultivated taxa, compared with those collected from spontaneous taxa.

It was recorded a significant quantitative increase of chlorophyll *a* from the vegetative phenophase to senescence in the case of *M. aquatica* and *M. pulegium* taxa. At three of the taxa studied (*M. longifolia*, (Negrești), *M. x piperita* var. *black*, *M. spicata* the maximum amount of chlorophyll *a* was recorded at flowering and at the other two (*M. longifolia* (Cioatele), *M. x piperita* var. *columna* the maximum amount of chlorophyll *a* was recorded in vegetative phenophase.

In the case of chlorophyll *b* was found a similar dynamic, the values recorded being obvious lower. The variations recorded for chlorophyll *a* and chlorophyll *b* change the relationship between the two fractions of chlorophyll, the highest value being reached during the flowering period of the plant (3.404 mg/g fresh matter) at the *M. x piperita* var. *columna* taxon.

In all three vegetative phenophases is observed that the ratio of chlorophyll *a* and chlorophyll *b* varies from 2.034 to 3.404; the lowest value was registered at senescence, while the lowest value was reached in the flowering phenophase. We see therefore that the ratio of 3/1 expressed in specialty literature for many species is not recorded constantly throughout the period analyzed [BURZO & al. 1999; TOMA & JIȚĂREANU, 2000; ZAMFIRACHE, 2005].

Carotenoid pigments are found in small quantities, 0.0001 to 0.0006 mg/g fresh matter, compared with chlorophylls *a* and *b*. The maximum value, 0.0006 mg/g fresh matter for carotenoid pigments was recorded during the flowering period for *M. spicata* taxon.

The intensity of photosynthesis varies throughout the year. With the increase in leaf size the number of chloroplasts and the amount of chlorophyll also increases, the photosynthesis process becoming more intense [BĂDULESCU, 2009]. If we analyze the foliar tissue indicators at the studied taxa, it appears that the process of photosynthesis records significant increases throughout the growing season in proportion to the chlorophyll content. The intensity of this process stays constant thereafter.

Tab. 2. Dry foliar matter and water content at species of the genus *Mentha* L. during the growing season in 2010

Taxon	Vegetative stage	S.U. (g%)	H ₂ O (g%)
<i>Mentha longifolia</i> (L.) Huds. (Negrești)	Vegetative	27.21	72.79
	Flowering	28.36	71.64
	Senescence	32.81	67.19
<i>Mentha longifolia</i> (L.) Huds. (Cioatele)	Vegetative	29.87	70.13
	Flowering	28.02	71.98
	Senescence	39.11	60.89
<i>Mentha aquatica</i> L. (Caraorman)	Vegetative	19.35	80.85
	Flowering	27.12	72.88
	Senescence	27.75	72.25

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<i>Mentha pulegium</i> L. (Caraorman)	Vegetative	19.18	80.82
	Flowering	22.59	77.41
	Senescence	28.20	71.80
<i>Mentha x piperita</i> var. <i>columna</i> L. (Vaslui)	Vegetative	20.51	79.49
	Flowering	21.68	78.32
	Senescence	23.28	76.72
<i>Mentha x piperita</i> var. <i>black</i> Mitcham. (Piatra Neamt)	Vegetative	16.61	83.39
	Flowering	30.11	69.89
	Senescence	31.78	68.22
<i>Mentha spicata</i> L. (Piatra Neamt)	Vegetative	15.73	84.25
	Flowering	29.35	70.65
	Senescence	35.60	64.40

Water content: from research conducted at the studied taxa we noticed that in the vegetation period it was recorded the highest water content, which decreased gradually towards fructification (Tab. 2). The highest value was registered in the vegetative phenophase - 84.25 g% in *M. spicata* species, and the lowest value of 60.89 g% was obtained from the species *M. longifolia* collected from Cioatele area, Vaslui County; we consider this water content to be sufficient for the physiological processes in normal parameters at the plants investigated. The observed values for water content of taxa analyzed, range from 70.13 to 84.25 g% for vegetative phenophase 69.89 to 78.32 g% for flowering and from 60.89 to 76.72 g% senescence.

Dry matter accumulates during the development of the plant, its highest value occurring in senescence 39.11 g% of the species *M. longifolia* collected from the area Cioatele. The values obtained in the vegetative phenophase range from 15.73 to 29.87 g% at flowering from 21.68 to 29.35 and from 23.28 to 39.11 g% at senescence.

At the studied taxa a similar trend is observed concerning the water content and dry foliar matter throughout the whole vegetation season; as an exception we report the situation of *M. longifolia* taxon collected from the area Cioatele, which has the lowest content of dry foliar matter at flowering phenophase (28.02 g%).

Therefore it is observed a quantitative increase in dry matter content throughout the vegetation period inversely proportional to the decrease of water content. This was also observed by other authors as ZAMFIRACHE & al. (1997, 2005), BURZO & al. (1999), STRATU (2002) etc., at many other species. All these results indicate that the investigated plants have a higher metabolic rhythm in the phenophase of vegetation, and afterwards it decreases gradually towards fruition.

Conclusions

The taxa taken into account, according to the foliar investigated indicators, show that the intensity of photosynthesis varies throughout the whole vegetation season, this process increasing proportionally with the quantity of assimilative pigments.

The content of foliar water and dry matter of the analyzed taxa suggests that the physiological processes take place at an alert pace during phenophase and decrease progressively towards senescence.

Acknowledgements

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OBSERVATIONS ON THE FOLIAR ASSIMILATING PIGMENTS CONTENT FOR WILD AND GARDEN ROSES

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OLTEANU ZENOVIA², BOZ IRINA²

Abstract: The study of foliar assimilating pigments (chlorophyll a and b, carotenoid pigments) during the ontogenetic development for the wild and the cultivar species of roses indicate from interesting aspects on the ratio chlorophyll a and b, which is more than a unit in the case of species and in most cases, less than a unit for the cultivars. The ratio chlorophyll and carotenoid pigments, reaches thousands in the case of species and units (tenths) for cultivars. We mention here that the cultivars are the result of multiple and introgressive hybridizations conducted for hundreds of years [ADUMITRESEI & STĂNESCU, 2009; KRÜSMAN, 1986].

Key words: wild and cultivated roses, chlorophyll, carotenoids from the leaf

Introduction

The *Rosa* genus represents, through the spontaneous species spread in the holarctic region, a botanical entity of wide scientific, fundamental and applied interest; despite all this, information about the fundamental research on its biology is still scarce [BURZO & al. 2005; JITĂREANU, 2007; SIHNA, 2004; ZAMFIRACHE, 2005, 2006].

As far as its behaviour towards sunlight is concerned, the speciality literature mentions that the species of the *Rosa* genus are mostly heliosciophyte having a transition character between the sun and the shade species [KRÜSSMAN, 1986].

From a physiological point of view, for the representatives of the *Rosa* genus the more intensely studied under a functional aspect are the two distinct photo systems and less other characteristics of photo assimilating pigments [TAIZ, 2002].

Material and methods

The study of assimilating pigments content from 9 spontaneous species of the *Rosa* genus, 5 aboriginal ones (*R. canina* L., *R. gallica* L., *R. glauca* Pourr., *R. pimpinellifolia* L., *R. rubiginosa* L.) and 4 alochtonous ones (*R. damascena* Mill., *R. multibracteata* Hemsl. et Wills., *R. multiflora* Thunb. and *R. rugosa* Thunb.) was conducted during vegetation period, observing chlorophyll and carotenoid pigments content at vegetative status, blooming fructification and fruit ripening. In order to emphasize the same compounds 8 types of garden roses ('Cocktail', 'Laminuette', 'Luchian', 'M-me A. Meilland', 'Perla d'Alcanada', 'Président Briand', 'Pristine' and 'Rose Gaujard') were observed.

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The fresh material of foliar assimilating pigments determinations was done in the Vegetal Physiology Laboratory of the Biology Faculty, "Alexandru Ioan Cuza" University of Iași by spectrophotometric method.

Results and discussions

In the case of species, during the vegetative stage, there can be observed a higher chlorophyll a content compared to chlorophyll b, of a ratio ranging around the value of 3/1 in all the cases.

Slightly higher values of chlorophyll were registered for the species *R. damascena* (1.897 mg), *R. glauca* (1.576 mg) and *R. pimpinellifolia* (1.448 mg), with a minimum value for the *R. multibracteata* (1.016 mg) species (Fig. 1).

High values of chlorophyll b are present in the species *R. damascena* (0.617 mg), followed by *R. glauca* (0.538 mg) and *R. canina* (0.516 mg), while the lowest quantities are to be found in *R. rubiginosa* (0.319 mg) and *R. multibracteata* (0.306 mg).

The minimum values of the chlorophyll a : chlorophyll b ratio are observed in *R. pimpinellifolia* (2.998/1), *R. glauca* (2.929/1), while *R. rubiginosa* and *R. multibracteata* have ratios that are superior to the values 3.3/1.

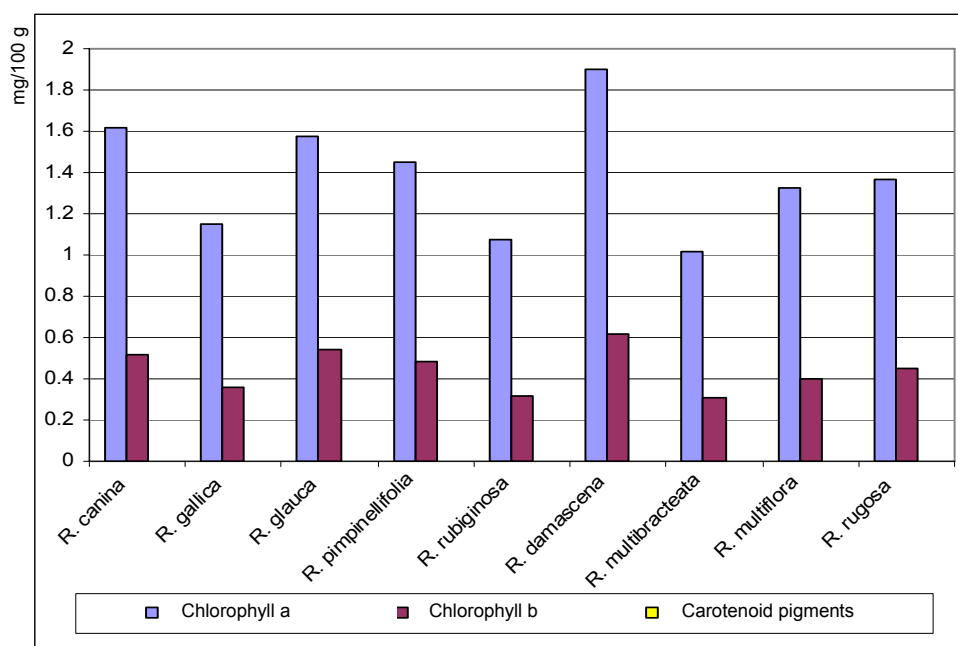


Fig. 1. The assimilating pigments content variation for the investigated species of *Rosa*, vegetative stage

Carotenoid pigments have comparable values for all the taxons, varying between 0.0004 mg (*R. multibracteata* and *R. gallica*) and 0.0006 mg (*R. damascena*).

One must notice that the relatively high content of chlorophyll pigments in the *R. glauca* species, which are macroscopically shielded by antocianic pigments, giving it the colour naming the species.

The carotenoid pigments content is extremely low in all the species, and the ratio of chlorophyll and carotenoid pigments varies between 3305/1 in the case of the *R. multibracteata* species and 4266 for the *R. canina* species.

It is possible, that the usual role of protective screen of the carotenoid pigments may be taken over by the antocianic pigments present in large quantities in the leaves as well as in the young *Rosa* shoots

The already mentioned observations for the vegetative development fenophase are generally true for the **blooming fenophase**, with the sole observation that there is a decreasing tendency of the chlorophyll content (a and b) for the majority of species, except for *R. pimpinellifolia*, where chlorophyll a content (2.215 mg compared to 1.448 mg in the previous stage), as well as chlorophyll b (0.772 mg compared to 0.483 mg) is going up, while for the exotic species *R. multibracteata* and *R. multiflora* there is only a significant increase in chlorophyll a content (2.361 mg compared to 1.016 mg and 2.083 mg respectively, compared to 1.328 mg).

In this fenophase, the ratio of these two types of chlorophyll slightly modifies its values, slightly superior to the 4/1 level in *R. gallica* among all the other aboriginal species and *R. damascena* among the alochtonous species. Most the species have a ratio between 3 and 4/1. *R. rubiginosa* clearly distinguishes itself with a 1/0.141 ratio of chlorophyll a and b, where we can also notice on the one side a reduction in the total content of assimilating pigments (0.525 mg compared to 1.396 mg in the previous stage). On the other side, there is also a reverse in the ratio of the two types of chlorophyll in favour of chlorophyll b (Fig. 2).

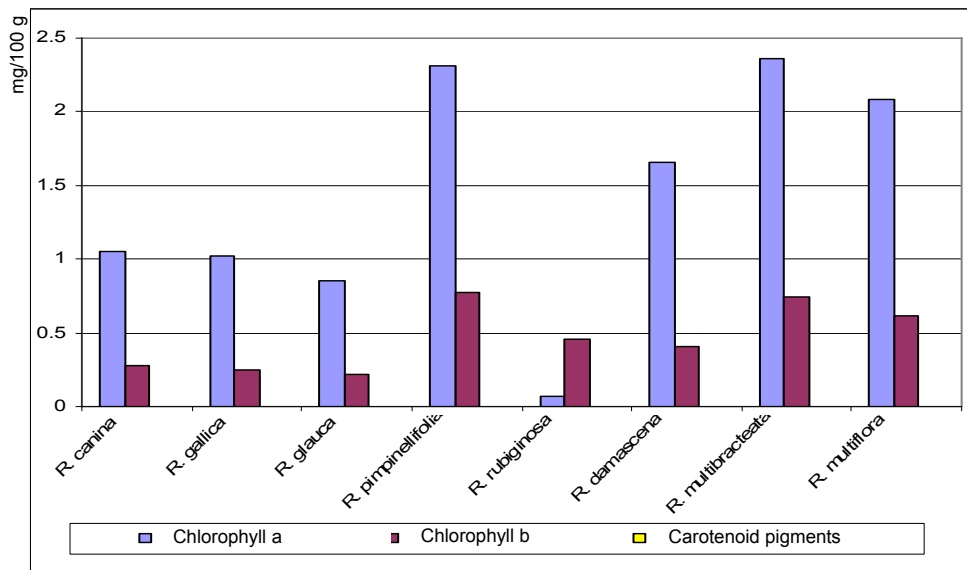


Fig. 2. The assimilating pigments content variation for the investigated species of *Rosa* at the blooming fenophase stage

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We must mention that during this phenophase the volatile substances content in the *R. rubiginosa* leaf is at its peak.

At the start of the fructification one can notice an increase in the assimilating pigments content for the majority of the species combined with a decrease of the chlorophyll a and b ratio under the 3/1 value. The value remains superior to this level only for *R. canina* (3.043/1). The content of carotenoid pigments slightly increases, reaching values between 0.0006 and 0.0007 mg for the species *R. pimpinellifolia*, *R. rubiginosa*, *R. glauca* and *R. rugosa* (Fig. 3).

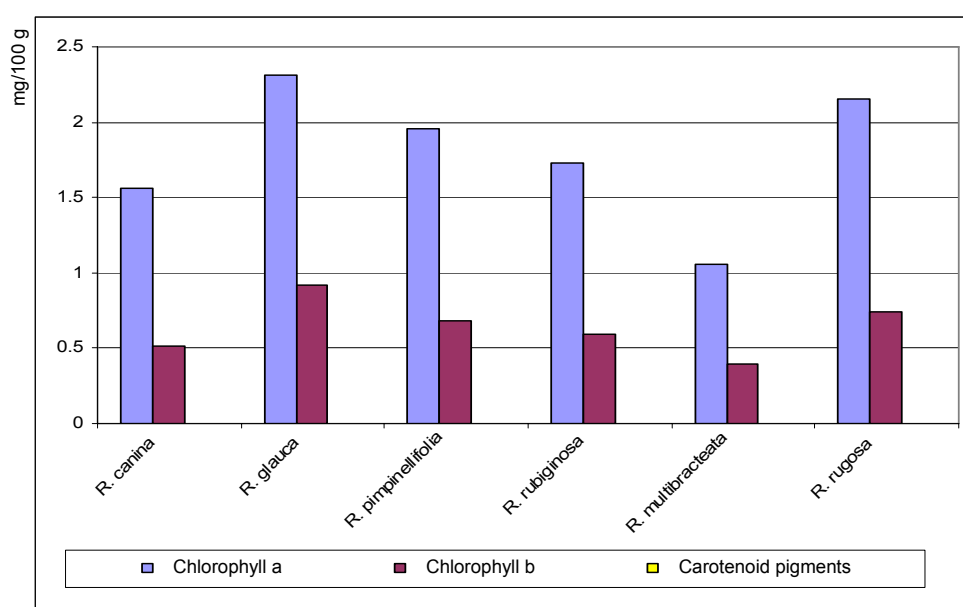


Fig. 3. The assimilating pigments content variation for the investigated species of *Rosa* at the start of the fructification stage

The ratio of assimilating and carotenoid pigments also displays extremely high values ranging from 3873/1 for *R. rubiginosa* and 6913/1 for *R. canina*.

At the **ripening of the fruits** we notice a decrease in the content of assimilating pigments, with a ratio of assimilating pigments varying between 1.612/1 for *R. multibracteata* and 2.820/1 for *R. rubiginosa*. The ratio of assimilating pigments and carotenoid ones register high values between 3644/1 for *R. gallica* and 4411/1 for *R. multibracteata* (Fig. 4).

The analysis of foliar assimilating pigments for cultivars at vegetative and generative stage (blooming) reveals a series of significant differences compared to the spontaneous species.

First of all, there is a much higher chlorophyll b content than chlorophyll a at the majority of the taxons, at both harvestings. Secondly, the cultivars display larger quantities of carotenoid pigments compared to other species.

The chlorophyll a and chlorophyll b ratio, as well as the one for assimilating and carotenoid pigments presents itself as having totally different limits.

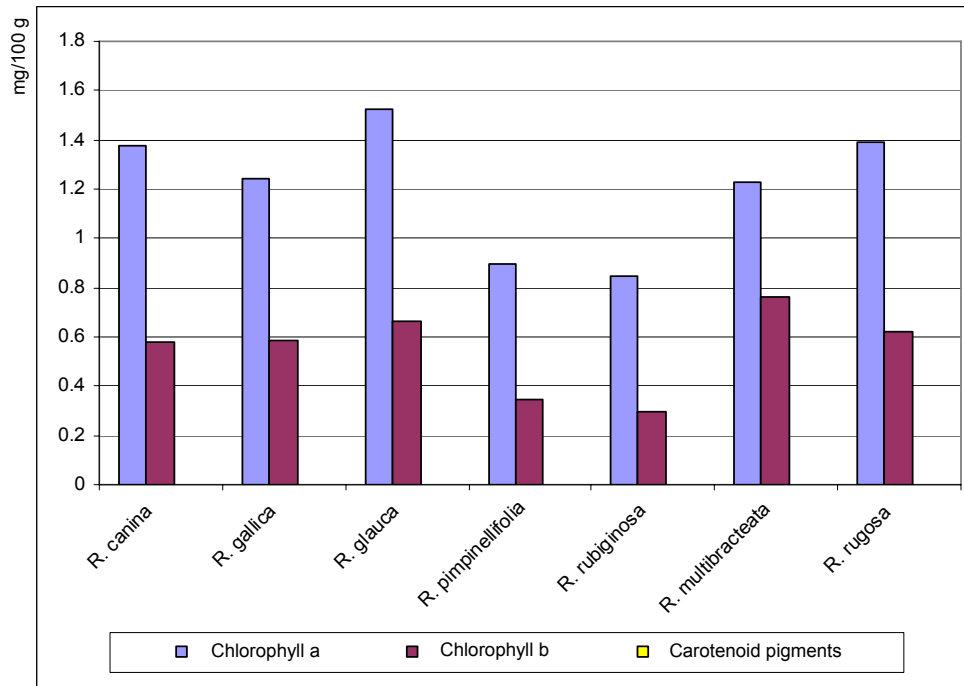


Fig. 4. The assimilating pigments content variation for the investigated species of *Rosa* at the ripening stage

For the **cultivars at vegetative stage** there is a low content in chlorophyll a varying between 0.048 mg for the cultivar ‘Cocktail’ and ‘M-me A. Meilland’ as well as ‘President Briand’ (0.076 mg), with values closer to 0.1 mg for cultivars ‘Laminuette’, ‘Rose Gaujard’ and ‘Pristine’, raising over 0.2 and 0.3 mg respectively for cultivars ‘Perla d’Alcanada’ (0.202 mg) and ‘Luchian’ (0.0308 mg). As far the chlorophyll b content is concerned, small quantities in absolute values (in these cases lower than in chlorophyll a) can be observed in the cultivars ‘Perla d’Alcanada’ (cu 0.016 mg), ‘Rose Gaujard’ (0.054 mg) and ‘Luchian’ (0.225 mg). The other cultivars contain between 1.265 mg in the case of the cultivar ‘M-me A. Meilland’ and 1.691 mg in the case of cultivar ‘Pristine’ (Fig. 5).

The chlorophyll a and chlorophyll b ratio displays less than a unit values in the case of most cultivars, ranging from 0.038/1 for the cultivars ‘M-me A. Meilland’ and ‘Cocktail’ and 0.071/1 for the cultivars ‘Laminuette’ and ‘Pristine’. Ratios over the unit can be seen in the cultivars ‘Luchian’ (1.369/1), ‘Rose Gaujard’ (2/1) and a much higher value for ‘Perla d’Alcanada’ (12.625/1).

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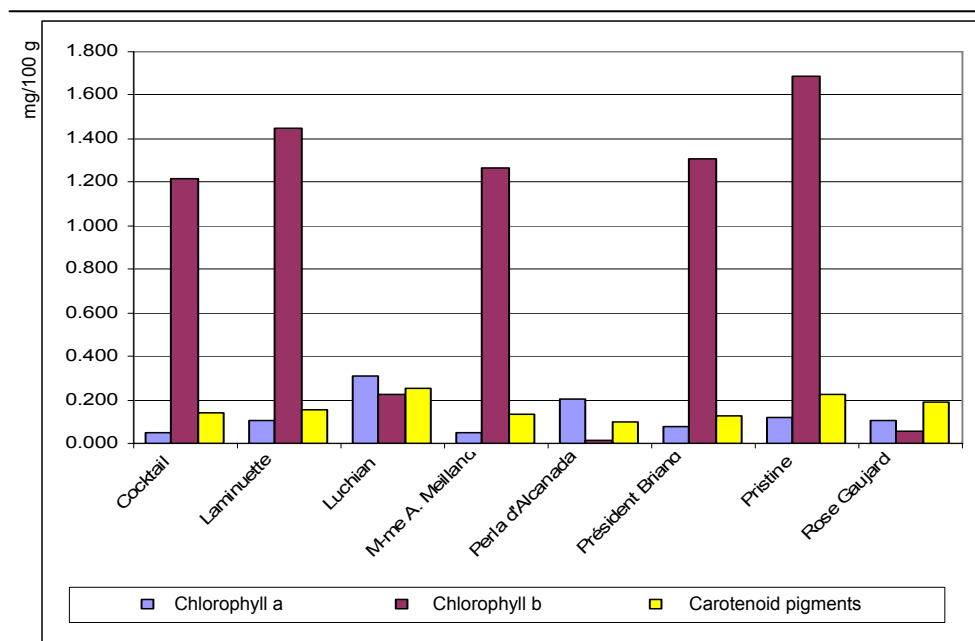


Fig. 5. The assimilating pigments content variation for the investigated species of *Rosa* at the vegetative stage

The ratio of assimilating and carotenoid pigments comes in the case of cultivars close to the values mentioned by classic physiology, varying between (0.866/1) for the 'Rose Gaujard' cultivar and 10.836/1 for the 'Président Briand' cultivar, while the other cultivars are aiming at one of these two poles: 'Luchian' (2.098/1) and 'Perla d'Alcanada' (2.247/1) and between 8 and 9/1 respectively, in the case of the cultivars 'Pristine', 'M-me A. Meilland', 'Laminuette' and 'Cocktail'.

At the **blooming fenophase** there is, first of all, a high chlorophyll b content, which varies between 1.297 mg for the 'Perla d'Alcanada' cultivar and 2.264 mg for the 'Rose Gaujard' cultivar.

Secondly, there is a low chlorophyll a content in all the examined cultivars, between the limits: 0.050 mg for 'Perla d'Alcanada', 0.075 mg for the 'Pristine' cultivar and 0.272 mg for the 'Cocktail' cultivar.

Finally, there is a remarkable carotenoid pigments content, which in most cases overpasses the chlorophyll a content with two exceptions, the 'Cocktail' (with 0.272 mg chlorophyll a compared to 1.1 mg of carotenoid pigments) and 'Rose Gaujard' cultivars (chlorophyll a 0.216 mg, and carotenoid pigments 0,196 mg), but even in these cases the differences are small [TĂMAȘ & NEAMȚU, 1986].

As a consequence, the chlorophyll a and b ratio is places less than a unit with extremely low values (between 0.039 for the 'Perla d'Alcanada' cultivar and 0.198 for the 'Cocktail' cultivar).

As it in vegetative stage, the ratio of chlorophyll and carotenoid pigments has values close to those mentioned in classic physiology textbooks (between 9.258 for the

'Cocktail' and 'Laminuette' cultivars and 12.713 mg for the 'M-me A. Meilland' cultivar) (Fig. 6).

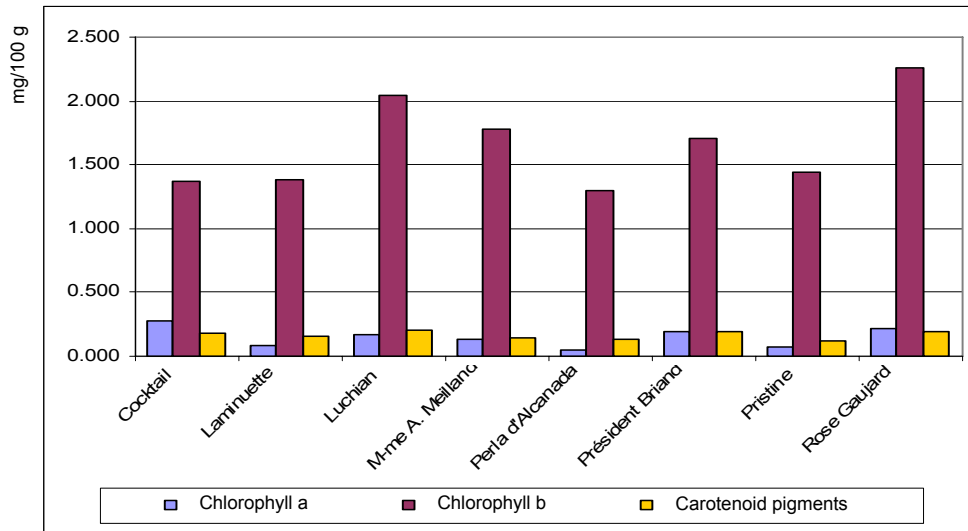


Fig. 6. The assimilating pigments content variation for the investigated cultivars of *Rosa* at the blooming phenophase stage

The higher content of chlorophyll b compared to chlorophyll a is, according to concepts of the classic physiology, something particular of the shade plants, but the roses planted vegetated really well in full sunlight. It is possible that the cultivated roses need a much consistent protection screen since they bloomed better there than in the shade [ARGATU, 1989; WAGNER, 1978; KRÜSSMAN, 1986].

According to the modern physiology concepts the chlorophyll and carotenoid pigments selectively absorb the radiations of light. Chlorophyll a presents a maximum absorption of the radiations with a wavelength of 700 and 435 nm respectively. Chlorophyll b has a maximum absorption of the radiations with the wavelength of 644 and 453 nm respectively, while the carotenoid pigments have a maximum absorption of the radiations within the wavelengths 400-480 nm. With this reality as a starting point, one may observe that the current roses cultivars have a more intense absorption of blue radiation [JITĂREANU, 2007 according to AUDERIRK & AUDERIRK, 1993].

Conclusions

In the case of spontaneous species chlorophyll a is predominant, the chlorophyll a and b ratio varying between 1.6-4/1, similar to the one described by classic physiology as being specific to plants that love sunlight.

For the hybrid origin cultivars, chlorophyll b is predominant with an a/b ratio varying between 0.37-2/1, which would suggest that, according to classic physiology, these cultivars love shade. However, the field observations contradict it. According to the modern physiology concepts [JITĂREANU, 2007 according to AUDERIRK & AUDERIRK, 1993]

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the chlorophyll and carotenoid pigments selectively absorb the light radiations. This is how the garden roses display a more intense absorption of blue radiations.

The carotenoid pigments content is extremely low for spontaneous species (under ppm per mg/100 g fresh material), while for the cultivars it ranges within normal limits, with the values of 0.1-0.2 mg/100 g per fresh material.

Most probably, together with the carotenoid pigments acting as protectors of the chlorophylls, there are other anthocyanic pigments present in large quantities in species and cultivars as well in all of the ontogenesis stages.

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INTERRELATIONS BETWEEN THE MYCORRHIZAL SYSTEMS AND SOIL ORGANISMS

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Abstract: The mycorrhizae are largely spread in natural ecosystems, and the proportion of plants that realise mycorrhizas is overwhelming, this relation involving advantages for both partners. The presence or absence of mycorrhizae, the rate and intensity of mycorrhiza formation are aspects with ecological importance, but also present importance in modern agriculture. The research results published on international literature which views the principal relations between mycorrhizae and soil microbiota, the way in which these relations affect the intensity of mycorrhizae formation and also the efficiency of mycorrhizae under the influence of soil organisms are synthesized and commented in this paper. The relations between mycorrhizae and different categories of bacteria, protozoa or microfungi, as well the influence of invertebrates through interactions of them with microorganisms are also being analyzed.

Key words: mycorrhizae, interrelations, rhizosphere

Introduction

The presence of mycorrhizae makes possible the coexistence of symbiotic organisms in hostile environment or in places where the competition is very strong. In this way, the mycorrhizal partners present advantages and benefits that allow them to develop or reproduce in underoptimal conditions or to become competitive, being able to survive. These are natural constant mutualistic associations between the roots of plants and soil fungi. The purpose of these relations is to obtain anorganic nutrients by the plants and organic nutrients by the fungi in an easily and efficiently way. Fungal species that form mycorrhizae are taking up to 25% from photosynthesis products of plants and they can contribute with P and N up to 80% of plant necessary [MEYER & al. 2010].

Mycorrhizae are morphological and physiological different, and the interactions between mycorrhizal species are also different. The mycorrhizae types and the distribution of them in terrestrial bioms are being influenced by the climatic factors, soil composition and participant species, as well by the composition of soil organisms communities. The influence of mycorrhizae over the distribution in ecosystems of plant species is major, playing an active role in qualitatively and quantitatively modelling the ecosystems structure. In nature, the mycorrhizal species establish extremely complex relations with soil organisms, the formation of mycorrhizae often leads to qualitative and quantitative modifications of soil biota, the process being reciprocal, so soil organisms may play a decisive role on the way and intensity of mycorrhiza formation. As plants are a valuable source of nutrients for many categories of

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soil organisms, they represent the centre of different types of interrelations, competition or cooperation, in order to gain access to these nutrients.

The fungal symbiont occupies a special position in these relations, its presence in rhizosphere leading to profound modifications of microrrhizosphere community structure. According to the effects of soil microorganisms on mycorrhiza formation and functioning, they can be beneficial, neutral or they can negatively affect the functionality of mycorrhizae.

There is a possibility of using some microorganisms that can significantly reduce negative effects of the pathogens or microorganisms that directly or indirectly stimulate plants growth and development (through stimulation of mycorrhiza formation). In order to use it, it is necessary to elucidate the complex mechanisms established between mycorrhizal species and other categories of microorganisms on a hand, and on the other hand to elaborate the efficient schemes and methods of utilisation such beneficial microorganisms in agriculture, forestry, ecological reconstruction etc.

The researches of Roumanian specialists concerning mycorrhizae were initiated half century ago [ŞESAN & al. 2010]. These researches approached the symbiosis in general and mechanisms determined by mycorrhizae (ALDEA, ZARNEA, ZAMFIRACHE, TOMA, MAXIMILIAN, CARASAN etc.). Some authors (CHIRA, IORDACHE, NEAGOE etc.) were highlighting particular aspects of ecto- or endomycorrhizae. ALDEA, CHIRA, BRĂILOIU etc. had studied the mycorrhizal impact to the economical important plants.

The concerns of researchers regarding the interactions between mycorrhizal species and soil microorganisms had generally targeted the plants protection and the reduction of frequency and severity of the phytopathogens attack [IACOMI & al. 2010]. It has been discovered that some microorganisms are frequently associated with mycorrhizae, their action being positive in relation with fungal symbiont, protecting the plant against the pathogens. In the last decades, the interest concerning elucidation of the mechanisms involved in establishing the complex interactions of the mycorrhizosphere and their role in protecting and stimulating plant development had increased.

The mycorrhizal systems – clarification

The mycorrhizae are symbiotic association, during which specific fungi are colonizing plants rootlets. In this type of relation, pathogenity and lesion of root structures are normally missing and the fungal invasion is blocked by the plant. Mycorrhizal plants are better developed than non-mycorrhizal plants. In this relation, plants are providing organic compounds for the fungal symbiont, and receive, in exchange, anorganic nutrients absorbed by hyphae. Due to the small size of hyphae, the absorptive surface and the explored volume of soil are very large, and the formation of root hairs is no longer necessary. Thus, the functions of mycelium become complementary to the root function.

The mycorrhization represents a common phenomenon in ecosystems and it is characteristic for very different taxa, from the bryophytes to angiosperm. It is estimated that over 90% of terrestrial plants realise vesicular-arbuscular mycorrhizae [ENE & al. 2010], adding to them the plants that realise other types of mycorrhizae. A few groups of vascular plants do not realise mycorrhizae at all, these usually living in wet habitats. The fungal invasion is limited by the plant, being located to the cortex level, in intercellular position (at ectomycorrhizae) and with intracellular ramification (at endomycorrhizae).

The hyphae are not colonizing tannins or calcium oxalate containing cells neither the organs apices. In the case of endomycorrhizae, arbuscules, vesicles and even hyphae are frequently lysed, their content being spilled in the cells of the host [ZAMFIRACHE &

TOMA, 2000]. Some mycorrhizal species are considered common and spread in different habitats, and other species considered rare are forming mycorrhizae only with some host plants [FODOR & al. 2010].

Interspecific signals in the mycorrhizal systems

The functions of the root can be influenced by mycorrhizae, in response to the rhizosphere action and soil fertility. These factors control the root architecture, reducing the ramification level and growing the dependency of plant for the symbiotic fungi [ZAMFIRACHE & TOMA, 2000].

The mycosymbiont becomes associated to the plant root and avoids the defence mechanisms. These processes are being initiated by the exchange of specific signals between both partners. The signalling is a process remarkably complex, involving different molecular mechanisms. In the early stages of the mycorrhiza formation, H_2O_2 plays a signalling role, and in a similar manner, the efflux of Cl^- and K^+ and the influx of Ca^{2+} and extracellular alkalization [HEBE & al. 1999]. The higher concentrations of monosaccharides at the root-soil interface are leading to the activation of some physiological modifications in the fungal metabolism which play a signalling role. On the other hand, the presence of nitrogen compounds with fungal origins induces some modifications in the radicular metabolism [HAMPP & al. 1999]. The degradation rate of the organic compound with N is controlled by the plants through the C resources given to fungi for extracellular enzymes synthesis [TALBOT & TRESEDER, 2009].

After these preparatory mechanisms of metabolic activation, is following a specific recognition phase mediated by phytohormones secreted by both the plants and the fungi, the process being bidirectional. Transport inhibitors of auxine and the compounds that release ethylene are activating the root ramification, a process that can be stopped by the ethylene synthesis inhibitors. In the mycorrhizal formation processes morphological modifications of root cells are interfering, modifications which are controlled through gene activity regulation by fungal or plant phytohormones (auxines, ethylene, abscisic acid). The root exudates secreted during fungal inoculation, induce defense mechanisms against the pathogens [DUCHESNE, 1989].

REQUENA & al. (2007) proved that some flavonoidic compounds from the root exudates are increasing the spores germination and the hyphal growth and development. Also, during the formation of the mycorrhiza, the fungi are influencing the expression of genes involved in phenylpropanoids, flavonoids and isoflavonoids radicular metabolism.

The rutin induces hyphal growth, and hypaphorine, an auxine analogous indolic compound, inhibits the root hairs elongation [NEHLS & al. 1998].

In the infective phase, proteosynthesis modifications take place, at least 50% from the both symbiotic partners proteins being synthesised in concentrations that differ from the concentration in which they are synthesised in a separate development of symbionts [DUCHESNE, 1989]. Some polyamines produced by fungal mycelium, have roles in the plants germination processes [NEHLS, 1998].

The hyphal adhesion is influenced by some hyphal wall compounds, as hydrophobines, cysteine-reached proteins, α -tubulin and actin [TIMONEN & al. 1996].

During the penetration of the root by the hyphae, low defensive responses are activated in plant organism, such as peroxidases production and proteins phosphorylation modifications. During a root infection made by a pathogenic fungal species, the plant reaction is strong and invariable, by contrary, the root infection made by a mycorrhizal

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species is permitted by the plant. In physiological stress conditions, the production of such compounds increases, the fungal or plant metabolism being modified by them, with the purpose to adapt to the new conditions. The indol acetic acid of fungal origins controls the root morphological changes [GAY & al. 1994].

The types of mycorrhizae

The morphology of mycorrhizae can deeply vary along with the type of relations between plant-host and the fungal species and with the environmental condition. The endomycorrhizae do not have a varied external morphology, but the ectomycorrhizae have different colours, shapes, sizes, which are characteristic to the participating species. A particular type is represented by peritrophic mycorrhizae, this being a stable relation between partners, in which the fungi develop around the root and form a mycelial network without having a direct contact with them.

The ectomycorrhizae are symbiotic associations in which the fungal mycelium develops in strong contact with the roots, forming a mantle that covers the apice of the root. They are characteristic for many trees. There has been proposed different ectomycorrhizae classification systems based on morphological characteristics: colour (yellow, orange, red, brown, violet, black etc.) sizes, ramification types (non-ramificated, dichotomic branched, coralloids etc.) the sizes and shapes of rhizomorphs etc. A recent proposed criteria is based on the exploring type of substrate by the extramatrical mycelium, this having ecological importance [AGERER, 2001].

The ectoendomycorrhizae represent intermediate forms between ectomycorrhizae and endomycorrhizae, some authors placing them in the latter group.

The endomycorrhizae are formed on the young rootlets, presenting only intercellular hyphae, which have well developed haustoria in root cortical cells. The haustoria can be twisted or divided, being named arbuscules. In some cases, mycelial hyphae can get through the entire organism of a plant.

There are different categories of endotrophic mycorrhizae, some of them being characteristic for specific groups of plant: monotropoid mycorrhizae are present at *Monotropa hypopitys*, ericoid mycorrhizae found at species from Ericaceae and Epacridaceae, arbutoid mycorrhizae characteristic for species from Pyrolaceae and some species from Ericaceae, orchidean mycorrhizae present at Orchidaceae species. The vesicular-arbuscular mycorrhizae are being formed by the most of the plants, the involved fungal species having a siphonal structure.

The influence of the mycorrhizae on soil organisms

The modification process of soil properties and of soil microbiota is bidirectional, based on "first to come" rule. The inhibition mechanisms are represented by the competition for the C and energy sources, and also by the production of antibiotics or other inhibitory compounds. If the mycorrhizal species do not find in the environment, in pre-infective phases, beneficial microorganisms, the chances of survival and colonizing a host decrease.

According to ALBERTSEN & al. (2006), associated bacteria play an important role in vesicular-arbuscular fungi development in the organic matter. The process is bidirectional, because the teluric microorganisms respond to the mycorrhizal species extramatrical mycelium growth.

According to RAIESI & GHOLLARATA (2006), the glomaline released by this fungi has negative effects on microbial respiration, this leading to the decrease of organic matter degradation rate from soil.

By secreting mixtures of selective substances, plants will create selective conditions for developing the rhizosphere organisms. The plants are exuding a variety of chemical compounds and anorganic ions, mucilages, also antimicrobial compounds with role in defending the host. Also, the mycorrhizal species mycelium releases some exudates that contain organic compounds that stimulate the development of a hyphospheric microbiota, but in a lower quantity than that produced by the plants [ANDRADE & al. 1997].

In this manner, the host plant and its symbiotic partner mycelium are “selecting” bacteria that are beneficial for their relation [TARKKA & al. 2009].

After this selections, microbial communities will be dominated by some bacterial groups [HRŠELOVÁ & al. 1999; WELSH & al. 2010]. These bacteria stimulate the mycorrhizae development, but are also complementary to the functions of mycorrhizae, as nutrients absorption and biological control of the host plant [FREY-KLETT & al. 2007; cited by TARKKA & al. 2009]. The bacterial diversity in hyphosphere seems to be lower compared to the free soil [GRYNDLER & al. 2000], the gram negative bacteria prevailing [VOSÁTKA, 1996; cited by BAREA & al. 2002].

However, BIANCIOTTO & BONFANTE (2002) observed that there is a big specific diversity in the rhizosphere of mycorrhizal plants compared to the rhizosphere of non-mycorrhizal plants. The mycosphere participates to P recycling process from organic or anorganic compounds [BAREA & al. 2002]. The mycorrhizal associated microorganisms modify, also, the composition of mycorrhizosphere microbiota. In this way, *Streptomyces* Ach505, which colonizes the mycelium of ectomycorrhizal species *Amanita muscaria*, produces auxofuran, an antibiotic that modifies hyphospheric microbiota [RIEDLINGER & al. 2006, cited by HARTMANN & al. 2009].

The *Bulkholderia cepacia* species is frequently present as free in mycorrhizosphere, but it has not been isolated from free soil or from the non-mycorrhizal plants rhizosphere.

Interrelations between the mycorrhizal systems and the soil microbiota

Between soil microbiota, the mycorrhizal species and plants are establishing extremely complex relations, with positive effects [AZCÓN-AGUILAR & BAREA, 1985] or negative effects [LARSEN & al. 2009] over the mycorrhization rate and over these processes efficiency. The root exudates are a valuable nutritive resource for rhizospheric microorganisms, qualitative and quantitative properties of these root exudates are influencing those interrelations established between organisms from this level.

Many soil microorganisms can be considered as being neutral, because they do not bring a benefit nor a loss for the plant host or the mycorrhizal species. However, these organisms influence the soil activity and properties, contributing to organic matter mineralization or can be involved in different physico-chemical processes. Although these organisms do not directly interact with the plants, the processes in which they are involved might have influence over the plant development. The rhizospheric bacteria are bacteria already present in the soil and, as a result of the soil conditions modification (roots development) they find favourable niches to abundantly develop. The rhizospheric microorganisms can have different activities: pathogenic activity, plant protection, antibiotics productions etc.

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Many categories of rhizospheric bacteria have the capacity of stimulating the plant development beyond the presence of the mycorrhizal species. For these species, it was used the acronym PGPR (Plant Growth Promoting *Rhizobacteria*) by LINDERMAN [1992, cited by AZCÓN-AGUILAR & BAREA, 1997], including both free and nodulating nitrogen fixing bacteria, soil phosphate solubilising bacteria, as well the bacteria that produce plant growth stimulators or pathogens inhibitors. But when they are both present, the mycorrhizal species and PGPR present complementary functions.

The mechanisms by which some microorganisms can inhibit mycorrhizal development are diverse: they can compete for nutrients [MIRANSARI, 2009] both with mycorrhizal species in preinfective phase, sometimes even in postinfective phase, and with favourable microorganisms. On the other hand, non-favourable microorganisms can directly inhibit the fungi by releasing the antifungal toxins, or indirectly by releasing the phytotoxines, bactericidal or bacteriostatic substances and by modifying the soil properties (pH modification or ratio between different substances in the soil) or they can be pathogenic for fungi and for plants. Other microorganisms are attached to the fungal spores or to the hyphae surfaces [MIRANSARI, 2011], using them as vectors for colonizing the root plants (BIANCOTTO & al. 2000; cited by BAREA & al. 2002).

Bulkholderia, *Ralstonia* and *Pandora* are endobacteria of some vesicular-arbuscular fungi. It has been discovered that these bacteria are constantly present at Gigasporaceae [RUIZ-LOZANO & BONFANTE, 2001; BIANCIOTTO & BONFANTE, 2002] and that fungal species without endosymbiont develop abnormally.

Although there have been made many studies regarding the composition of microbial community from rhizosphere, up to 90% of rhizospheric microorganisms remain unstudied [GOODMAN & al. 1998; cited by BUÉE & al. 2009], as a result of the unrecovery on artificial media. By means of modern techniques, recently developed, this number has been reduced.

FULTHORPE & al. [2008; cited by BUÉE & al. 2009] have analyzed soil samples, by sequencing some nucleic acid molecules, soil samples that have been collected from different biogeographical regions, proving that there are other dominant taxonomical groups of bacteria than previously proposed (through isolation on artificial media). The bacterial communities from rhizosphere fluctuate with root growth, and by that, the densest communities will be found in root hairs regions [BUÉE & al. 2009].

Interrelations between the mycorrhizal systems and the nodulating nitrogen fixing bacteria

Many studies have been focused over the double inoculation of plant with mycorrhizal species and nodulating nitrogen fixing bacteria [AZCÓN-AGUILAR & BAREA, 1997; SIVIERO & al. 2008; GUTIÉRREZ-MICELI & al. 2008; BAREA & al. 2002], because of its applicability in agriculture. These bacteria colonize the roots of some nodules forming plants, and at their level, in different biochemical processes, nitrogenous is synthesized in compounds available by plants using molecular nitrogen. For the plants, this colonization is beneficial, but this involves the reduction of saccharides available for fungi, the effect being the reduction of mycorrhization rate. Nevertheless, a good development of the plants implies an increase of the exudation rate by root plants which lead to a better fungal development. This hypothesis was confirmed by double inoculation, with mycorrhizal species and *Rhizobium* strains [TOBAR & al. 1996; SIVIERO & al. 2008], the authors reported an increase of mycorrhizal units number. TIAN & al. (2003) found a better

development of *Robinia pseudacacia* plantlets by triple inoculation: with endomycorrhizal species, ectomycorrhizal species and *Rhizobium* strains, compared to the double inoculation or simple inoculation.

Interrelations between the mycorrhizal systems and free nitrogen fixing bacteria

The free nitrogen fixing bacteria have positive effects on plants development. One might say that their activity could negatively influence mycorrhiza formation by increasing nitrogenous compounds available for plants meaning the reduction of plant dependency for mycorrhizae, but experimental results obtained by GUTIÉRREZ-MICELI & al. (2008) infirmed this assumption, being noticed positive effects on the mycorrhization rate. The co-inoculation of *Azospirillum* and *Glomus mosseae* made the two species act synergistically, offering nutrients to plants, nutrients which contain same quantity of N and P as by administration of artificial fertilizers. Some strains of *Azospirillum* and *Paenibacillus* have stimulated the vesicular-arbuscular mycelium growth and the mycorrhiza formation [BAREA & al. 2002; BIACIOTTO & BONFANTE, 2002]. However, contradictory results obtained by ZUBEK and colabs. (2009) proved that these processes depend on involved organisms genome.

The *Burkholderia* species are bacteria capable of fixing molecular nitrogen, often isolated from mycorrhizosphere. These bacteria are able to stimulate plant growth and contribute to mineral resources bioavailability [KOELE & al. 2009]. The mycorrhizal species and some bacteria cooperate in soil transformation. These mechanisms imply the roots presence which improve nutrient content in rhizospheric microhabitat and sustain the bacterial inoculum stability [SIVIERO & al. 2008].

The mycorrhizal species increase the surviving rate of these bacteria in rhizosphere, thus *Azotobacter paspali* develops better in *Paspalum notatum* rhizosphere when plants are mycorrhizated [BAREA & al. 1983]. Similarly, nitrogen fixing bacteria isolated from *Drosera villosa* rhizosphere stimulated the rice roots and stalks growth when they were co-inoculated with *Glomus claroideum* [GUTIÉRREZ-MICELI & al. 2008].

Interrelations between the mycorrhizal systems and the mycorrhiza promoting bacteria

Some of bacteria can directly stimulate the mycorrhiza formation by releasing some stimulatory compounds as auxines, gibberellines and cytokinins, substances that influence the root morphology and physiology and contribute to the qualitative and quantitative modification of the root exudates, with direct effects on fungi. These bacteria also produce vitamins and organic acids that stimulate spores germination. Helper bacteria produce hypaphorine type phenolic compounds that increase fungal aggressivity [GARBAYE, 1994, cited by DUPONNOIS & PLENCHETTE, 2003]. However, the effect depends on the inoculum size, if the dose is suboptimal there are not any beneficial effects, if the dose exceeds the optimal values there are negatively effects, possible due to antibiosis effect or resources consumption [FREY-KLETT & al. 1999]. Some fungal species colonize the roots only in extreme habitats by reason of microorganisms competition lack [BOWEN & THEODORU, 1978].

Some authors [VIVAS & al. 2003; MARULANDA & al. 2006] reported positive effects of *Bacillus thuringiensis* inoculation over the mycorrhizal species extra- and

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interradicular development, increasing the mycorrhizal intensity and the extramatrical mycelium growing rate. For these bacteria it is used the acronym: MHB – mycorrhizal helper bacteria. Similarly, co-inoculation with *Azospirillum brasilense* or *Bacillus amyloliquefaciens* [OANCEA & al. 2010] as well as coinoculation of *Enterobacter agglomerans* with *Glomus etunicatum* [KIM & al. 1998] led to a better mycorrhizal species development.

In savannas, the mycorrhization rate was high when microclimatic conditions were favorable for rhizospheric bacteria development [LÓPEZ-GUTIÉRREZ & al. 2004]. The inoculation with ectomycorrhizal species success depends on fungal surviving in soil during preinfective phase, and this may be correlated to the helper bacteria presence.

Many studies confirmed the efficacy in stimulating and potentiating of mycorrhization effects of helper bacteria from *Pseudomonas*, *Ralstonia* and *Bacillus* genera: *Pseudomonas fluorescens* [DUNSTAN & al. 1998; BRULÉ & al., 2001; GAMALERO & al. 2004, cited by HAMEEDA & al. 2007], *Pseudomonas putida* [KOZDRÓJ & al. 2007], *Pseudomonas aeruginosa* [KOTHAMASI & al. 2006], *Pseudomonas monteillii* [REDDELL & WARREN, 1986, cited by DUPONNOIS & PLENCHETTE, 2003], *Bacillus subtilis* [DUNSTAN & al. 1998; BRULÉ & al. 2001], *Ralstonia* sp. [KATAOKA & FUTAI, 2009; HRYNKIEWICZ & al. 2010].

Similar effects have been reported in the case of actinomycetes. These organisms stimulated *Amanita muscaria* mycorrhization concomitantly with the inhibition of the pathogen: *Armillaria obscura* and *Heterobasidion annosum* [MAIER & al. 2004], or hyphal growth induction realised by *Streptomyces* strains [SCHREY & al. 2007]. Also, some oxalobacteriaceae stimulated *Glomus mosseae* in vitro growth [MIRANSARI, 2011].

There are some spores associated to the bacteria as *Stenotrophomonas* and *Arthrobacter*, with favourable effects on the spores germination [BHARADWAJ & al. 2008].

Some saprotrophic fungi, as *Trichoderma hartzianum* [IACOMI & al. 2010] can stimulate helper bacteria effects, showing in this manner a multilevel synergistical effect.

There is a strong correlation between rhizospheric microbial species and mycorrhizal ones [DUNSTAN & al. 1998], the helper bacteria beneficial effects over the mycorrhizae being fungi-specific. In the studies realised by PIVATO & al. (2009), *Pseudomonas fluorescens* differently stimulated the development of some *Glomus* species.

Different studies [FRANCO-CORREA & al. 2010; CALVARUSO al. 2007] revealed the fact that many actinomycetes species isolated from mycorrhizosphere had the capacity to solubilise Phosphorus from organic and anorganic soil sources, as well the capacity to produce siderophores or to fix Nitrogen. These bacteria have a more abundantly development in mycorrhizosphere comparing with their development in non-mycorrhizal plants rhizosphere.

ASPARY & al. (2006) showed a high level of dependency between the helper bacteria strains and different mycorrhizal species. These results have been confirmed by other researchers [AZCÓN, 1989]. Some *Bacillus subtilis* strains stimulate *Suillus granulatus* development, but inhibit the development of *Rhizopogon* [KATAOKA & al. 2009]. Also, in a research realised by XIAO & al. (2008), *Bacillus subtilis* significantly decreased *Zea mays* roots colonization frequency by the mycorrhizal species (from 75% at 55.6%), inhibiting spores germination and hyphal growth, contrary to VIVAS & al. (2003) observations.

Interrelations between the mycorrhizal systems and phytopathogens

The mycorrhizal species directly protect the host plant by releasing some compounds toxic to pathogens [ZARNEA, 1994], mechanical protection of the root [ZAMFIRACHE & TOMA, 2000] or by activating host plant defence mechanisms through modulating the salicylic acid and jasmonate metabolism [MIRANSARI, 2011] or flavonoids metabolism [NEHLS & al. 1998], or indirectly by alteration of the microbial community structure due to the induction of the qualitative and quantitative changes of root exudates and also due to the stimulation of some favourable antagonistic microorganisms.

Negative correlations between vesicular-arbuscular fungi and the rhizospheric pathogens have been observed [WEHNER & al. 2010]. There are some variations of soil pathogens inhibiting capacity depending on fungal species.

Some mycorrhizal species [BOWEN & THEODORU, 1978] or the associated helper bacteria [LI & al. 2007; SIASON & al. 2009, cited by WEHNER, 2010] produce antibiotics against phytopathogens like: *Phytophthora cinnamomi*, *Pythium aphanidermatum* or *Gaeumannomyces graminis* var. *tritici*. *Pseudomonas putida* is antagonistic to *Cylindrocarpon destructans*, *Pythium ultimum* & *Rhizoctonia solani* [GU & MAZZOLA, 2003, cited by BUÉE & al. 2009].

According to AZCÓN-AGUILAR & BAREA (1997) the bacteria from *Rhizobacterium* genera can be used as biocontrolling agents. Similarly, *Bacillus subtilis* and *Pseudomonas fluorescens* strains have antagonistic reactions against pathogens, being stimulated by the presence of mycorrhizae [SCHELKLE & PETERSON, 1996; AZCÓN-AGUILAR & BAREA, 1997; NEERAJ & SINGH, 2010].

Some *Streptomyces* strains that colonize Norway Spruce ectomycorrhizae, protect the plant against the attack of *Heterbasidion annosum* [LEHR & al. 2007; cited by HARTMANN & al. 2009]. The bacteria often have the capacity of degrading the toxins produced by phytopathogenic fungi or viral factors of them [COMPANT & al. 2005].

Interrelations between the mycorrhizal systems and saprotrophic fungi

The competition for nutritive resources is the most frequent relation established between mycorrhizal species and soil saprotrophic fungi. In McALLISTER & al. (1994) experiments, the inoculation of *Lactuca sativa* rhizosphere with *Trichoderma koningii* or *Fusarium solani* strains before inoculation with *Glomus mosseae* lead to development inhibition of the last. These effects have not been observed in the case of initial inoculation with *Glomus mosseae*. As mycorrhizal species are colonizing the hosts roots, the relations with saprotrophic fungi are changing, acting often synergistically for making bioavailable some minerals needed by plants. Many telluric species of fungi have strong reactions against phytopathogenic fungi.

AZCÓN-AGUILAR & BAREA (1997) reported synergistical relations between *Glomus* and *Trichoderma* species concerning inhibiting *Fusarium* attacks at tomatoes or *Pythium* attacks at potatoes. By influencing soil microbiota, mycorrhizal species influence, also, the saprotrophic fungal activity, through inhibition processes [TIUNOV & SCHEU, 2005] or through direct or indirect stimulation.

Interrelations between the mycorrhizal systems and protozoans

In the rhizosphere, the protozoans are able to release nutrients with the consumption of the microorganisms. By consuming preferentially some bacteria, the protozoans change the bacterial community structure which leads to modifications in protozoans community, therefore a fast feed-back [RØNN & al. 2002].

The presence of the mycorrhizae can negatively influence protozoans community, indirectly by alteration the soil bacterial community in an unfavourable way for the protozoans or directly by production of some inhibitory compounds [RØNN & al. 2002].

The presence of protozoans has opposite effects to mycorrhizal species, stimulating the root ramification [BONKOWSKI & al. 2001].

Both microbial systems are beneficial and complementary for the plants, because the ectomycorrhizal species increase bioavailability of Phosphorus, and protozoans increase bioavailability of Nitrogen. However, the presence of both categories of organisms leads to an increasing competition for the plant secreted carbohydrates and their numerical reduction [TIMONEN & al. 2004; cited by HERDLER & al. 2008]. Different studies [HERDLER & al. 2008; OLSSON & al. 1996, cited by BONKOWSKI & al. 2001] revealed the fact that double inoculation, with protozoans and with fungi, strongly stimulated the biomass production of plants, concomitantly with the population significantly reduction of both categories of organisms.

Interrelations between the mycorrhizal systems and invertebrates

Although, the direct interactions between mycorrhizal species and the soil fauna are limited, they do exist. Among the soil animals that interact with mycorrhizal species and their host plant, there are different categories of invertebrates such as: insects, nematodes, annelids, mites etc. Many insect species (often larval stages) as well as nematodes, consume or attack both plant roots and mycelium (colemboles), affecting in this way the mycorrhizal symbiosis.

A particularly interesting relation is represented by earthworms. The colonization rate has been better when plants were inoculated with mycorrhizal species in the presence of earthworms from *Pheretina* [ZAREA & al. 2009], their action being beneficial for mycorrhizae through several mechanisms: earthworms can produce phytohormones, and their excrement may contain ten times more propagules than the soil [GANGE, 1993, cited by ZAREA & al. 2009]. Adding earthworms lead to increase the harvest of *Trifolium* by improving the soil chemical properties and by producing the plant regulatory compounds due to microbial activity stimulations by earthworms [QUAGGIOTTI & al. 2004, cited by ZAREA & al. 2009]. The earthworms increase the number of free nitrogen fixing bacteria by qualitative modifications of the soil (modification of porosity and aggregation) and by improving plant water and oxygen uptake. The earthworms, also, stimulate the production of exudates and create microhabitats [ZAREA & al. 2009].

Many species of colenbolas feed with mycorrhizal species, although they prefer saprotrophic fungi [GANGE, 2001; cited by TIUNOV & SCHEU, 2005]. Colembolas feed with mycorrhizal species only when insects population reach high density levels. By feeding with saprotrophic fungal species, colembolas destabilize the soil fungal community, making them more susceptible to be influenced by the mycorrhizal species [TIUNOV & SCHEU, 2005].

Conclusions

Mycorrhiza forming species strongly modify the structure and dimension of rhizospheric microorganisms, either by direct interactions, or indirectly by influencing the release of the root exudates in rhizosphere.

The mycorrhizae exercise, generally, a strong selective pressure on rhizospheric habitats, stimulating the development of mutualistic or comensal microbiota.

The mycorrhizae influence all the relations established between different categories of organisms in rhizospheric microhabitats under late succesional stages, and in young rhizospheric microhabitats the mycorrhizations success depends on the microbial community already established.

The plant benefits from all of mutualistic relations established between mycorrhizal species and the soil organisms, while the fungal partner often competes with different soil organisms for the plant carbohydrates.

The elucidation of the intimate mechanisms that underline the structure of microbial community and the processes that influence the mycorrhizal intensity and rate are premises in the elaboration of the efficient ecological reconstruction strategies or for the sustainable agriculture development.

There are needed some extensive researches concerning signal phase prior to tripartite mutualistic relations development and the involved factors, in order to use and optimize them in the purpose of integrated pest management strategies development.

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NEW CHARACTERISTICS FOR MORPHOTAXONOMY OF *GIGASPORA* SPECIES BELONGING TO ARBUSCULAR MYCORRHIZAL FUNGI

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Abstract: New characteristics for morpho-taxonomy were devised to support the species concept in genus *Gigaspora* belonging to arbuscular mycorrhizal fungi. Three species viz. *G. margarita*, *G. decipiens* and unidentified *Gigaspora* sp. were studied for various characters viz. bulbous suspensor, sporophore, germ tube, presence of septum and presence or absence connecticle. The term “Connecticle” is newly introduced and is a region present between the base of bulbous suspensor and septum overlying the sporophore. The term “Germ pore” is also newly introduced. The present study reported that germ tube in *Gigaspora* is always attached to the spore through a pore which is now named as germ pore. In all, eight types of subtending hypha were recorded during the present study with variations in shape of bulbous suspensor and sporophore along with presence or absence connecticle. Presence of germ pore, septum and germ tube was common feature in *Gigaspora* species undertaken for the study. The location of septum was another new character devised for taxonomy in the present study. Thus the present study upholds the species concept in *Gigaspora* based on morpho-taxonomy.

Key words: arbuscular mycorrhizal fungi, bulbous suspensor, connecticle, germ pore, germ tube, *G. margarita*, *G. decipiens*, morpho-taxonomy, septum, sporophore, unidentified *Gigaspora* sp.

Introduction

Traditionally, Glomeromycotan taxonomy of arbuscular mycorrhizal (AM) fungal group has been based on the morphology of the spores. The way the spore is formed on the hypha (“mode of spore formation”) has been important to circumscribe genera and families, and the layered structure of the spore wall is used to distinguish species [WALKER, 1983; MORTON, 1988]. Glomeromycotan taxonomy is relatively young. Among the glomeromycotan fungi, the *Gigasporaceae* (*Scutellospora* and *Gigaspora*) members are distinguished by the formation of their spores on a “bulbous suspensor” and are well supported by molecular data. Recently, OEHL & al. (2008) revised family *Gigasporaceae* on the basis of morphological spore characters and 18S and 25S rRNA gene sequences. In the family *Gigasporaceae*, 36 *Scutellospora* species were reorganized in three new families including five new genera: *Scutellosporaceae* (*Scutellospora*), *Racocetraceae* (*Racocetra*, *Cetraspora*) and *Dentiscutataceae* (*Dentiscutata*, *Fuscutata*, *Quatunica*). The family *Gigasporaceae* now remains with a single genus *Gigaspora*.

The group *Gigaspora* is the smallest group since member species were transferred into erected *Scutellospora* Walkers & Sanders primarily on the basis of presence of sub-cellular structures associated with germination [WALKERS & SANDERS, 1986]. With this, species level differences in *Gigaspora* rested upon seemingly small morphological differences in spore colour, size and wall thickness [BENTIVENGA & MORTON, 1995].

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NEW CHARACTERISTICS FOR MORPHOTAXONOMY OF *GIGASPORA* SPECIES BELONGING ...

Gigaspora poses a problem since spore of all species differentiate only a spore wall and diverge in characteristics of that spore wall [BENTIVENGA & MORTON, 1995]. Research workers questioned the fact that, do small morphological differences within the genus constitute adequate criteria for delimitation of species? Advanced studies in taxonomy viz. ontogeny define relationships between the characters while a phylogenetic study provides information on relationship between *Gigaspora* and other fungi in *Glomeromycota* [BENTIVENGA & MORTON, 1995]. Thus the validity and importance of morphological characters in establishing taxonomic species are of considerable importance to construct workable system of identification. In view of the above, the present paper throws light on new characteristics to be incorporated to boost the traditional morpho-taxonomy in *Gigaspora* species.

Material and methods

Extraction of AM fungal spores. Spores of AM fungi associated with *Carica papaya* L. plants from Goa, India were isolated directly from rhizosphere soil samples by wet sieving and decanting method [GERDEMANN & NICOLSON, 1963]. All the available healthy turgid spores were isolated from the rhizosphere soil. Repeated samplings were carried out in monsoons (rainy season) when the spores sporulated newly in the soil. The host plant was same for all the samplings. This plant was grown in monoculture and mulching practices carried out in the field and because of this there were no weeds. Therefore the samplings were carried out under same environmental conditions and from the rhizosphere soil of the replicates of single host plant viz. papaya.

Identification of AM fungi. Diagnostic slides containing intact and crushed spores of AM fungi were prepared in polyvinyl alcohol lactoglycerol [KOSKE & TESSIER, 1983]. Spore morphology and wall characteristics were considered for the identification of AM fungi and these characteristics were ascertained using compound microscope, Leica WILD MP 3 and Nikon E 800. Arbuscular mycorrhizal fungi were identified to species level using bibliographies provided by SCHENCK & PEREZ (1990), SCHÜBLER & al. (2001) and OEHL & al. (2008).

Results

The genus *Gigaspora* consists of azygospore with subtending hyphae. This hypha is attached to spore through a pore (Fig. 1). The subtending hypha consists of terminal swollen sporogenous cell called bulbous suspensor and sporogenous hypha called sporophore (Fig. 1). A septum is present at the base of the swollen portion separating the bulbous suspensor from the sporophore (Fig. 1). In some cases, the subtending hypha at the base of the bulbous suspensor metamorphoses to produce various shaped structure. This part of the subtending hypha connecting the bulbous suspensor to the sporophore which is overlined by the presence septum is called a connecticle (Fig. 1). In the present study, the type of bulbous suspensor, sporophore and connecticle varied in *Gigaspora* species.

In all, eight types of subtending hypha were recorded during the present study (Table 1); (Fig. 2). 1) thin walled clavate bulbous suspensor with funnel shaped sporophore (Fig. 2a), 2) thin walled clavate bulbous suspensor with straight sporophore (Fig. 2b), 3) thick walled clavate bulbous suspensor with recurved sporophore (Fig. 2c), 4) thin walled clavate bulbous suspensor with recurved sporophore (Fig. 2d), 5) subglobose bulbous

suspensor with funnel shaped sporophore (Fig. 2e), 6) globose bulbous suspensor with funnel shaped connecticle and curved sporophore (Fig. 2f), 7) globose bulbous suspensor with vase shaped connecticle and curved sporophore (Fig. 2g), 8) globose bulbous suspensor with elongated connecticle and curved sporophore (Fig. 2h).

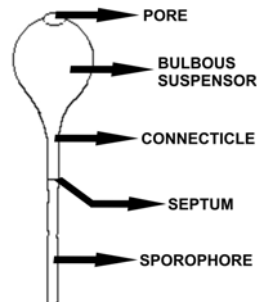


Fig. 1. Diagrammatic representation of characteristics of subtending hypha in *Gigaspora* species.

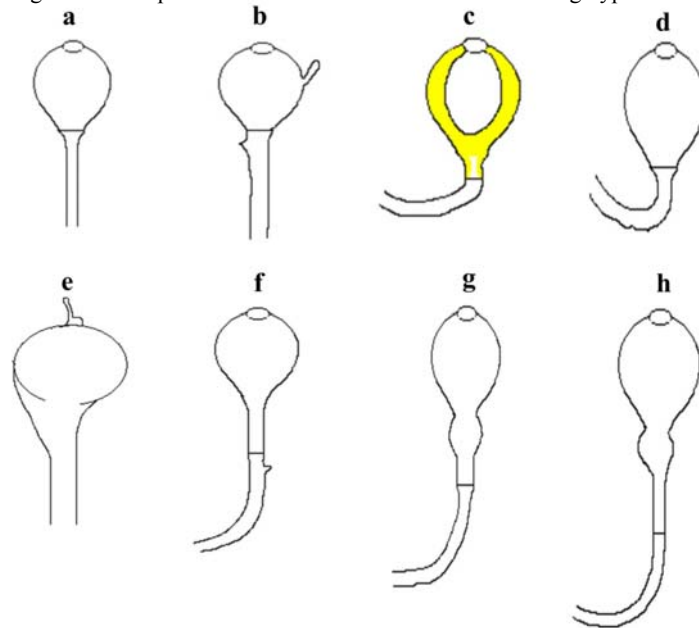


Fig. 2. Diagrammatic representation of variations in subtending hyphae of *Gigaspora* species.

- a) Thin walled clavate bulbous suspensor with funnel shaped sporophore.
- b) Thin walled clavate bulbous suspensor with straight sporophore.
- c) Thick walled clavate bulbous suspensor with recurved sporophore.
- d) Thin walled clavate bulbous suspensor with recurved sporophore.
- e) Sub-globose bulbous suspensor with funnel shaped sporophore.
- f) Globose bulbous suspensor with funnel shaped connecticle and curved sporophore.
- g) Globose bulbous suspensor with vase shaped connecticle and curved sporophore.
- h) Globose bulbous suspensor with elongated connecticle and curved sporophore.

Tab. 1. Characteristics of subtending hypha in *Gigaspora* species

*Type of subtending hypha	Occurrence in	Bulbous suspensor (Bs)	Connecticle	Sporophore (Sp)	Pore	Location of septum
Thin walled clavate Bs with funnel shaped Sp (Fig. 2a, Fig. 3)	<i>G. margarita</i>	40–70 µm wide; 50–320 µm long; 2–10µm thick wall	Absent	30µm wide at the base of Bs; 15–20µm wide, away from the base of Bs	Present	At the base of Bs
Thin walled clavate Bs with straight Sp (Fig. 2b, Fig. 8)	<i>G. decipiens</i>	80–200 µm wide; 170–350 µm long; 2–10µm thick wall	Absent	30–40µm wide at the base of Bs; 15–20µm wide, away from the base of Bs	Present	At the base of Bs
Thick walled clavate Bs with recurved Sp (Fig. 2c, Fig. 9)	<i>G. decipiens</i>	200–350 µm wide; 270–400 µm long; 20–65µm thick wall	Absent	30–60µm wide at the base of Bs; 20–30µm wide, away from the base of Bs	Present	At the base of Bs
Thin walled clavate Bs with recurved Sp (Fig. 2d, Fig. 10)	Unidentified <i>Gigaspora</i> species	150–250 µm wide; 180–300 µm long; 2–10µm thick wall	Absent	30–50µm wide at the base of Bs; 20–30µm wide, away from the base of Bs	Present	At the base of Bs
Subglobose Bs with funnel shaped Sp (Fig. 2e, Fig. 4)	<i>G. margarita</i>	60–150 µm wide; 80–300 µm long; 20–50µm long lateral hypha; 2–10µm thick wall	Absent	100–50 µm wide at the base of Bs; 20–40 µm wide, away from the base of Bs	Present	At the base of Bs
Globose Bs with funnel shaped connecticle and curved Sp (Fig. 2f, Fig. 5)	<i>G. margarita</i>	60–140 µm diam. 2–10µm thick wall	40–60 µm wide at the base of Bs; 20–30µm near septum 250–320 µm long	20–30µm wide	Present	At the end of connecticle
Globose Bs with vase shaped connecticle and curved Sp (Fig. 2g, Fig. 6)	<i>G. margarita</i>	60–130 µm diam. 2–10µm thick wall	30–40 µm wide at the base of Bs, inflated at the centre, 40–60 µm wide, narrow near septum, 20–30 µm wide; 200–350 µm long	20–30µm wide	Present	At the end of connecticle
Globose Bs with elongated connecticle and curved Sp (Fig. 2h, Fig. 7)	<i>G. margarita</i>	60–250 µm diam. 2–10µm thick wall	Constricted at the base, 30–40 µm wide, swollen below, 50–80µm wide, below the swollen portion straight 40–60 µm wide; 350–500 µm long	30–40µm wide	Present	At the end of connecticle

*Bs = Bulbous suspensor; *Sp = Sporophore

Three species of *Gigaspora* with eight types of subtending hypha were recorded during the present study (Tab. 1); (Fig. 3 – Fig. 10). In *G. margarita* Becker & Hall, five types of subtending hypha were observed viz. thin walled clavate bulbous suspensor with funnel shaped sporophore (Fig. 3), subglobose bulbous suspensor with funnel shaped sporophore (Fig. 4), globose bulbous suspensor with funnel shaped connecticle and curved sporophore (Fig. 5), globose bulbous suspensor with vase shaped connecticle and curved sporophore (Fig. 6) and globose bulbous suspensor with elongated connecticle and curved sporophore (Fig. 7). In *G. decipiens* Hall & Abbott, two types of subtending hyphae were observed viz. thin walled clavate bulbous suspensor with straight sporophore (Fig. 8) and thick walled clavate bulbous suspensor with recurved sporophore (Fig. 9). In unidentified *Gigaspora* species, one type of subtending hypha was observed viz. thin walled clavate bulbous suspensor with recurved sporophore (Fig. 10).

Types of subtending hypha in *Gigaspora margarita*

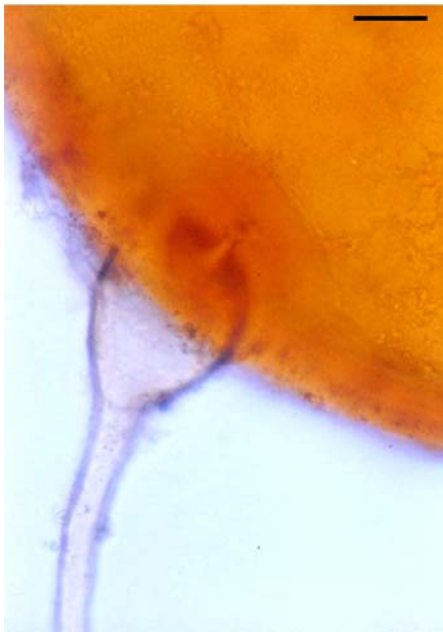


Fig. 3. Thin walled clavate bulbous suspensor with funnel shaped sporophore (Bar = 30 μ m).

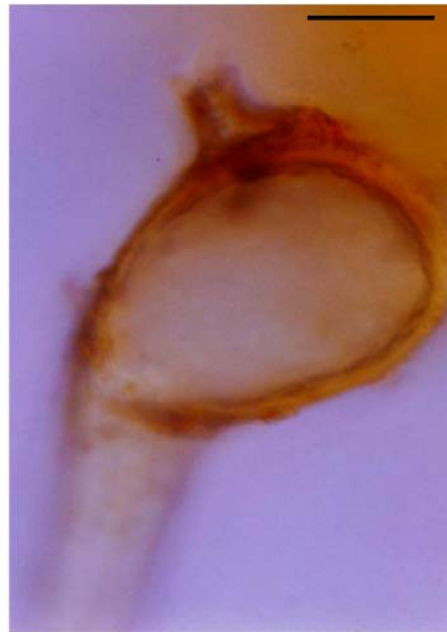


Fig. 4. Sub-globose bulbous suspensor with funnel shaped sporophore (Bar = 20 μ m).

Types of subtending hypha in *Gigaspora margarita*



Fig. 5. Globose bulbous suspensor with funnel shaped connecticle and curved sporophore (Bar = 100µm).



Fig. 6. Globose bulbous suspensor with vase shaped connecticle and curved sporophore (Bar = 100µm).



Fig. 7. Globose bulbous suspensor with elongated connecticle and curved sporophore (Bar = 50 µm).

[* Note the presence of pore (arrow) in Fig. 5-7]

Types of subtending hypha in *Gigaspora* species

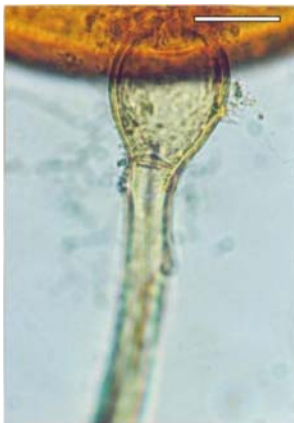


Fig. 8. Thin walled clavate bulbous suspensor with straight sporophore in *Gigaspora decipiens* (Bar = 20µm).

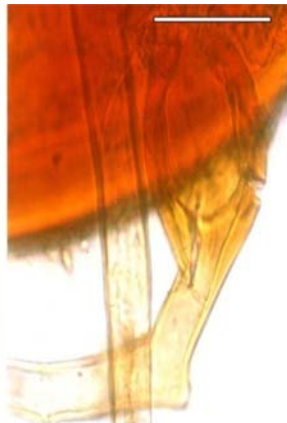


Fig. 9. Thick walled clavate bulbous suspensor with recurved sporophore in *Gigaspora decipiens* (Bar = 100µm).

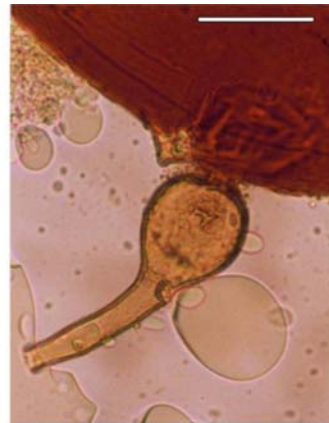


Fig. 10. Thin walled clavate bulbous suspensor with recurved sporophore in unidentified *Gigaspora* sp. (Bar = 100µm).

In *Gigaspora* the innermost wall known as the germinal wall in the close proximity of bulbous suspensor, produces germ tube at the time of spore germination. This germ tube is attached to the spore through germ pore (Fig. 11-14). No variation was observed in the type of germ pore. However, in the present study the type of germ tube varied in different *Gigaspora* species. In *G. margarita*, numerous “warts” or “papillae” were present on the inner surface of germinal layer and they were especially concentrated in regions where germ tube originated (in close proximity to the suspensor cell) (Fig. 7, Fig. 11). In this species, the germ tube was curved and germ pore was present at the point of attachment to the spore wall (Fig. 11). In *G. decipiens*, the warts were absent on the germinal wall in the vicinity of bulbous suspensor and here the germ tube was straight and attached to the spore through germ pore (Fig. 12). In unidentified *Gigaspora* sp., the germinal wall produced several centimeters long coiled germ tube with presence of germ pore at the point of attachment to the spore wall (Fig. 13, Fig. 14).

Germ tubes in *Gigaspora* species



Fig. 11. Curved germ tube in *Gigaspora margarita* (Bar = 25 μ m)

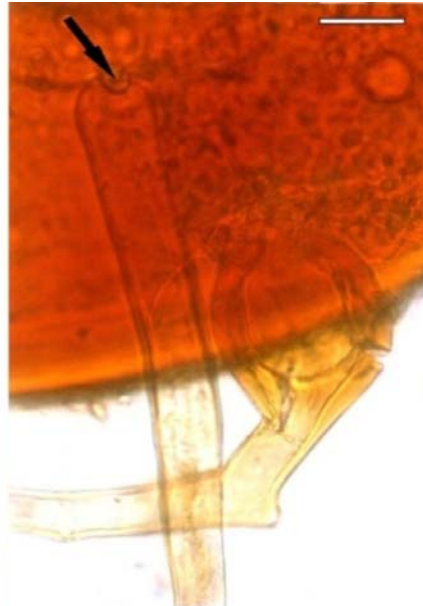


Fig. 12. Straight germ tube in *Gigaspora decipiens* (Bar = 25 μ m)

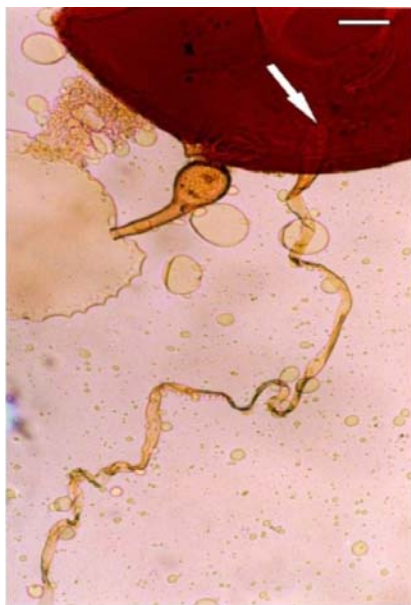


Fig. 13. Coiled germ tube in unidentified *Gigaspora* sp. (Bar = 100µm).



Fig. 14. Coiled germ tube in unidentified *Gigaspora* sp. (Bar = 50µm)

[* Note the presence of Germ pore (arrow) in Fig. 11-14]

Discussions

Now, the phylum *Glomeromycota* comprises about 200 described morpho-species that traditionally have been distinguished by features of the spore wall. WALKER (1983) established the concept of “murographs” to describe and compare the layered structure of the spore walls more easily. MORTON (1995) and STÜRMER & MORTON (1997) and STÜRMER & MORTON (1999) included considerations of the spore development to group these wall components hierarchically into complexes linked by ontogeny [REDECKER & RAAB, 2006]. Therefore in the present study various characteristics of spore especially the spore attachment of *Gigaspora* viz. bulbous suspensor and sporophore was studied in detail to support the species concept.

Till date, eight species exists under genus *Gigaspora* of the family *Gigasporaceae*. They are as follows: *G. albida* Schenck & Smith, *G. alboaurantiaca* Chou, *G. candida* Bhattacharjee, Mukerji, Tewarii & Skoropad, *G. decipiens* Hall & Abbott, *G. gigantea* (Nicol. & Gerd.) Gerd. & Trappe, *G. margarita* Becker & Hall, *G. ramisporophora* Spain, Sieverding & Schenck, *G. rosea* Nicol. & Schenck [OEHL & al. 2008]. The synonymy of *G. ramisporophora* with *G. margarita* [BENTIVENGA & MORTON, 1995] is not accepted based on molecular [DE SOUZA & al. 2004] and morphological [SPAIN & al. 1989] differences. *Gigaspora rosea* and *G. candida* are also treated as separate species. *Gigaspora tuberculata* Neeraj, Mukerji, Sharma & Varma earlier reported under genus *Gigaspora* [SCHENCK & PEREZ, 1990] was later reported as synonym of *Scutellospora persica* (Koske & Walker) Walker & Sanders [BENTIVENGA & MORTON, 1995].

It is reported that spore size and colour are stable distinct characters to support species concept in *Gigaspora* [BENTIVENGA & MORTON, 1995]. In contrast to this,

development of spore with two permanent layers is shared with all *Gigasporaceae* members. Also the variation of spores in *Gigaspora* is more limited than *Scutellospora* that exhibit wide range in colour and are often ornamented. This is due to strong genetic and developmental constraints which appear to limit the expression of variation in *Gigaspora* [BENTIVENGA & MORTON, 1995]. However, in the present study large variation was seen in the subtending hypha of *Gigaspora* species. Five different types of subtending hypha were recorded in *G. margarita*. The species recorded three different types of bulbous suspensor viz. clavate, subglobose and globose. The connecticle were of three types viz. funnel shaped, vase shaped and elongated while the sporophores were also of three types viz. straight, funnel shaped and curved. Lateral hyphal projection was present in subglobose bulbous suspensor. In *G. decipiens*, the bulbous suspensor was clavate and of two types, thin walled and thick walled. Here the sporophores were also of two types, straight and recurved. In unidentified species of *Gigaspora*, the bulbous suspensor was clavate with recurved sporophore.

Another keen observation recorded in the present study was that septum delimiting the bulbous suspensor from the sporophore was present immediately at the base of all clavate bulbous suspensors and even at the base of subglobose bulbous suspensor of *Gigaspora* species. However, at the base of all globose bulbous suspensors, connecticles were present followed by septum delimiting the sporophore from it. These connecticles varied in shape and size and were associated only with globose bulbous suspensors of *G. margarita*.

In *Gigaspora* species, a vital life history function is germination of spore. This germination of spore is always associated with the spore wall [BENTIVENGA & MORTON, 1995]. The present study upholds the view of several workers [MAIA & al. 1993; SWARD, 1978; SWARD, 1981] who reported that germination takes place through the formation of germ tube which always arises from inner papillate layer and pushes through the spore wall. However, the present study brought out the fact that its development is like that of bulbous suspensor which is attached to the spore through a pore and in case of germ tube the pore is designated as germ pore. No feature of this germ pore, described in the present study, distinguishes any of the *Gigaspora* species compared in this study. However, through the present study, it is confirmed the germ tube is always associated with germ pore. Further, in the present study, the type of germ tube varied within the species of *Gigaspora*. It was curved in *G. margarita*, straight in *G. decipiens* and coiled in unidentified *Gigaspora* species. Presence of germ pore was recorded in all the three species.

Conclusions

Glomeromycotan fungi are of great interest to ecologists because of its potential influence on ecosystem processes, its role in determining plant diversity in natural communities and the ability of the fungi to induce a wide variety of growth responses in coexisting plant species. Difficulties in identification, the inability to grow the fungi in pure culture, problems of taxonomic classification and a lack of basic information on the life histories of AM fungi hinder studies of the ecological significance of diversity of AM fungi. Nucleic acid based techniques have the potential to fill this gap in our knowledge by offering better means of identification and the opportunity to study links between the genetic diversity of AM fungi and functional and morphological diversity. The application of genus specific molecular markers has shown that different genera of AM fungi coexist in plant roots and that this is a common occurrence [SANDERS & al. 1996]. However the speciation concept still rests on the morpho-taxonomy of the spore. In *Gigaspora*, where there are relatively few species with small number of taxonomic characters, the present paper gives additional information on characters of taxonomic relevance.

New characters viz. bulbous suspensor, sporophore, germ tube, presence of septum and presence or absence connecticle are incorporated to differentiate *Gigaspora* members to species level. Even presence or absence of septum at the base of bulbous suspensor or the distance at which it is present from the base of bulbous suspensor is another distinguishing character. Further, the presence or absence of connecticle in *Gigaspora* species is newly introduced to carry out taxonomic studies. Additionally, the term germ pore used to designate the point of attachment of germ tube to the spore is also introduced for taxonomy of *Gigaspora* species. Further, my study contradicts the earlier reports that variations in *Gigaspora* are limited. My study brings out the facts that even the isolates of same species which was earlier distinguished on the basis of spore size and colour show large variations in the morphology of its subtending hypha and this aspect is newly studied in detail and documented in the present study.

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**COMPOSITION OF THE VOLATILE OIL EXTRACTED FROM
ABIES ALBA MILLER LEAVES PARASITIZED BY
MELAMPSORELLA CARYOPHYLLACEARUM (DC.) J. SCHRÖT.**

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MIRCEA CORNELIA², ȘPAC ADRIAN²

Abstract: Researches results highlights both qualitative and quantitative influences exercised by the parasitic species *Melampsorella caryophyllacearum* on the composition of the volatile oil extracted from *Abies alba* leaves, prelevated in 2010 from Oituz river basin (Bacău county). The isolation of volatile oils has been realized by hydrodistillation in Neo-Clavenger installation, followed by gas-chromatography coupled with mass spectrometry analysis. The increase of the monoterpenes concentration in the parasitized sample could be explained by the degradative action of the enzymes produced by the pathogenic species *Melampsorella caryophyllacearum* or by the incapacity of syntheses from these monoterpenes of some compounds presenting a more complex structure in the parasitized plant case.

Keywords: volatile oil, monoterpenes, sescviterpenes, *Abies alba*, *Melampsorella caryophyllacearum*

Introduction

The decline of *Abies alba* Mill. has been the subject of great concern in Central Europe and North America since the early 1970s [SKELLY & INNES, 1994]. Among the main proposed causes of fir decline were air pollutants, climatic and biotic factors. The use of dendro-ecological techniques has enabled researchers to date with annual resolution, and to quantify precisely the effects of fungal pathogens on radial growth [CHERUBINI & al. 2002].

The fungus *Melampsorella caryophyllacearum* (DC.) J. Schröt. (Fungi, Basidiomycota) also called fir broom rust, has been reported to cause serious damage on *Abies* species [NICOLOTTI & al. 1995; MANOLIU & al. 2009]. The fungus causes the production by the tree of witches' brooms, and hypertrophied ring growths on the trunk or branches resulting in spherical swellings [SOLLA & al. 2006]. Of greater concern, *M. caryophyllacearum* may contribute to a tree's death by weakening it such that wind breaks the tree at the site of the swelling.

The disease is common wherever firs grow, being present in North America [MERRILL Z & al. 1993], Europe [FRIGIMELICA & al. 2001], and Asia [ALEKSEEV & al. 1999].

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Material and methods

Identification and quantification of volatile oil [ȘTEFĂNESCU, 1988] have been realized using healthy and parasitized leaves samples from *Abies alba* (fir). Because the pathogenic fungus *Melampsorella caryophyllacearum* can not be cultivated on nutritive media in laboratory, the analyzed samples have been collected from trees growing in Oituz river basin (46°08,091' N; 26°30,985' E, 651 m alt.) and transported in freezers in the laboratory. The vegetal material vegetal has been dried and crumbled. The two samples have been encoded in this way: *Fr.S.* – healthy leaves sample and *Fr.B.* – parasitized leaves sample. Separated volatile oil has been analyzed by gas-chromatography coupled with mass spectrometry, using a Neo-Clavenger installation, (GC) Agilent Technologies gas chromatograph - type 6890N.

Method: 50 g of dry vegetal material crumbled in II sieve (*Farmacopeea Română*, Xth edition) have been treated with 500 ml distilled water and 30 ml glycerin. The glycerin added on the vegetal product has the role to favor hydratation and volatile oil extraction. After the introduction of the water in graduated tube of the device and in the separator, the samples have been distilled for 3 hours. After distillation, the separation of the volatile oil has been favored by adding of 1 ml xylene; this quantity will be dropped from the final volume of the volatile oil. The separated volatile oil has been inserted in a graduated tube where its volume has been identified and reported to 100 g vegetal product.

$$\text{ml volatile oil (\%)} = 100 V/a$$

where:

V – extracted volume of volatile oil, expressed in ml;

a – the mass of the used dry vegetal material, expressed in g.

Results and discussions

The achieved extraction capacity, expressed in ml volatile oil in 100g vegetal material, highlights a content of 2.76 in this type of compounds for *Fr.S.* sample and 0.37 for *Fr.B.* sample, where a content by approximate 7.5 times smaller is observed. The analyzed volatile oil is predominantly constituted by monoterpenes and sescviterpenes (Table 2, Fig. 1 and 2).

Tab. 1. The main compounds identified in volatile oil samples

t_R (min.)	Compound	Aria %	
		<i>Fr.S.</i>	<i>Fr.B.</i>
4.788	santene	3.74	1.94
5.429	tricyclene	1.64	0.78
5.602	α -pinene	6.46	13.97
5.887	camphene	6.94	5.62
6.329	β -pinene	10.18	15.51
6.407	myrcene	0.69	0.84
6.718	α -phellandrene	0.12	0.11
6.891	α -terpinene	0.08	0.06
7.013	p-cymene	0.07	0.10
7.116	limonene	9.48	10.06
7.160	sabinene	2.18	4.29
7.532	γ -terpinene	0.09	0.08

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7.965	α -terpinolene	1.50	0.57
8.034	p-cymenil	0.04	0.08
8.138	L-linalool	0.40	-
8.198	t-allocymene	0.26	-
8.389	mentha-1,4,8-triene	0.02	-
8.519	fenchol	0.09	0.05
8.623	α -campholenic aldehyde	0.42	0.26
8.978	camphor	0.08	0.11
9.107	exo-methyl-camphenilol	0.14	0.06
9.194	pinocarvone	0.02	0.14
9.229	isoborneol	0.03	-
9.298	α -phellandrene-8-ol	-	0.04
9.358	endoborneol	1.66	-
9.419	isopinocampone	0.05	0.05
9.454	terpinen-4-ol	0.12	0.07
9.557	cis-m-menth-8-ene	0.08	
9.670	α -terpineol	1.37	0.80
10.371	t- β -ocymene	0.20	-
10.544	piperitone	0.01	-
10.596	(E)-2-decenal	0.02	0.14
10.873	lavandulyl acetate	0.05	-
10.916	felandral	0.03	0.09
11.003	(-)-bornyl acetate	12.14	7.68
11.419	t,t-2,4-decadienal	-	0.06
11.548	1,3,5-tris(methylene)cycloheptane	0.08	-
11.812	(+)-m-mentha-1,8-diene	-	0.15
11.825	α -cubebene	0.14	0.08
11.981	α -longipinene	1.87	0.92
12.301	α -copaene	0.20	0.14
12.206	δ -3-carene	0.80	-
12.345	longicyclene	-	0.19
12.466	β -elemene	0.27	0.33
12.552	sativene	0.06	-
12.596	α -ylangene	0.03	0.24
12.691	(-)-isolekene	0.49	-
12.700	aromadendrene	-	0.17
12.838	isolongipholen	1.21	-
12.959	(-)- β -caryophyllene	6.05	8.35
13.003	α -cedrene	2.43	1.03
13.063	(-)-sinularene	-	0.08
13.106	α -guaiene	0.04	-
13.202	cis- β -bisabolen	0.03	-
13.271	t- β -farnesene	0.37	0.33
13.375	α -himachalene	0.64	0.25
13.444	α -humulene	2.69	4.59
13.609	cis-cariophyllene	1.31	0.50
13.660	γ -muurolene	-	0.31

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13.756	γ -himachalene	0.65	-
13.816	widdrene	1.34	-
13.877	β -selinene	3.13	-
13.989	α -selinene	-	1.12
14.050	β -himachalene	1.91	1.16
14.206	α -amorphene	1.24	0.69
14.266	δ -cadinene	2.54	1.81
14.396	allo-aromadendrene	-	0.18
14.509	aromadendrene VI	-	0.24
15.063	valencene	0.03	-
15.184	cariophyllen oxide	0.29	1.22
15.513	longiborneol	0.54	-
15.833	α -gurjunene	2.10	3.64
15.842	(-)-longipholene	-	2.22
15.963	β -paciulen	0.55	-
15.980	δ -cadinene	-	0.37
16.127	α -cadinol	0.85	-
16.327	t-muurolol	-	1.32
20.507	β -bisabolene	0.14	-

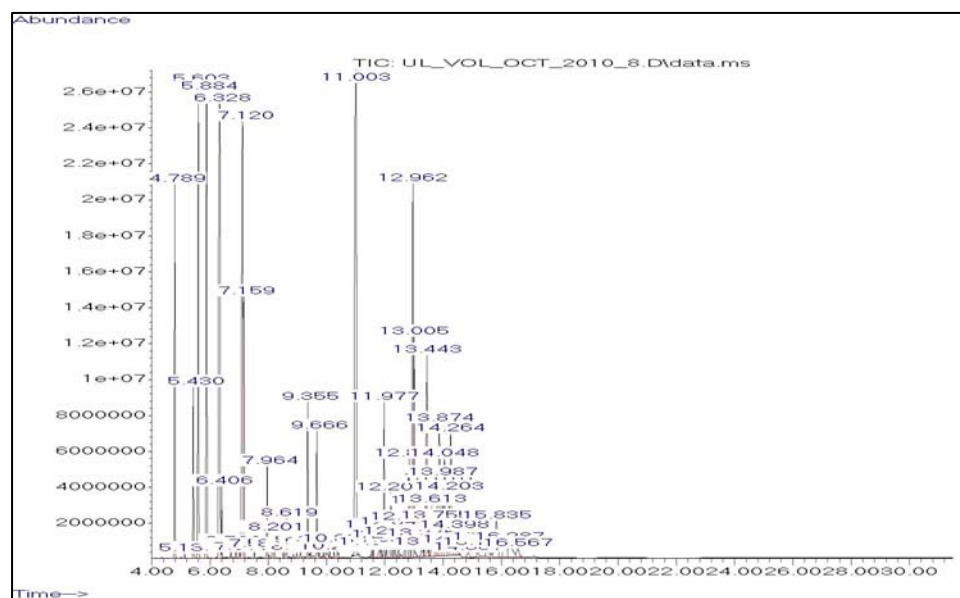


Fig. 1. Gas-chromatogram of the volatile oil – healthy leaves sample

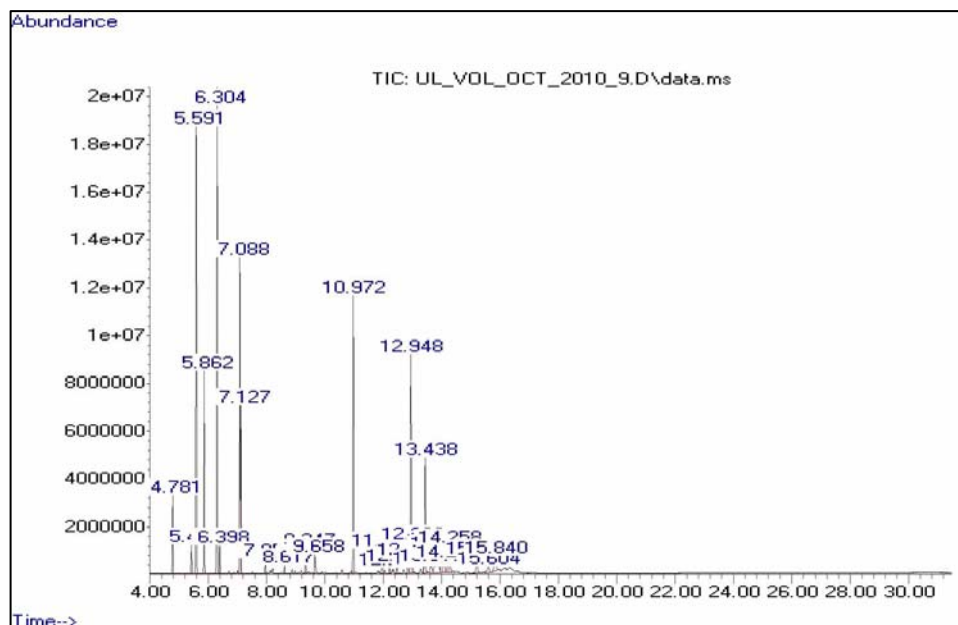


Fig. 2. Gas-chromatogram of the volatile oil – parasitized leaves sample

By comparison with *Fr.S.* sample, the *Fr.B.* sample is characterized by an increased level of monoterpenes (61.39% *Fr.S.*, 56.61% *Fr.B.*). The major monoterpene structures are hydrocarbons, the concentration of the oxygenated derivatives being increased in *Fr.S.* sample (16.61%) comparative with *Fr.B.* sample (9.35%), so the increasing of the monoterpenes content is realized through an increasing of the concentration in hydrocarbon structures. The sesquiterpenes concentration is the same in both two samples, a slightly increased value being registered yet in *Fr.S.* sample (33.94% comparative to 31.48%). Instead, the oxygenated sesquiterpenes presents an increased concentration in the parasitized sample (2.54% in *Fr.B.* sample comparing to 1.68% in *Fr.S.* sample). The reduction of the concentration in oxygenated compounds will determine a reduction of the therapeutic properties of the fir volatile oil de brad or the limitation of its use in aromatherapy.

Conclusions

The increase of the monoterpenes concentration in the parasitized sample could be explained by the degradative action of the enzymes produced by the pathogenic species *Melampsorella caryophyllacearum* or by the incapacity of syntheses from these monoterpenes of some compounds presenting a more complex structure in the parasitized plant case.

The major compounds characteristic and common in both samples are the next monoterpenes: santene, α - and β -pinene, camfene, limonene, sabinene, bornilacetate, and

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also some sesquiterpenic derivatives: β -caryophyllene, α -cedrene, α -humulene, β -himachalene, Δ -cadinene and α -gurjunene.

Modification of the content in volatile oil and of the quality of the volatile oil samples represents the consequence of the pathogenic process, which determines the impossibility of parasitized vegetal material use in order to obtain the volatile oil necessary in pharmacy, perfumes industry and aromatherapy.

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EVALUATION OF PERFORMANCE PARAMETERS FOR TRACE ELEMENTS ANALYSIS IN PERENNIAL PLANTS USING ICP-OES TECHNIQUE

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Abstract: The aim of this paper is to present the validation of inductively coupled plasma optical emission spectrometry (ICP-OES) method used for metals determination from several perennial plant samples. The suitability of two digestion procedures using wet digestion with mineral acids mixture on hot plate and microwave digestion was investigated to determine As, Cd, Cu, Fe, Mn, Pb and Zn in plants samples. The LOD of the seven analysed elements in solid samples varied between 0.20 μg g⁻¹ for Mn and 0.55 μg g⁻¹ for Pb. The found values for metals determined by ICP-OES in a vegetable certified reference material digested using the two procedures were compared with the certified values and good agreements between these values were obtained. The proposed method indicated satisfactory recovery, detection limits and standard deviations for trace metal determination in perennial plants samples.

Key words: ICP-OES, plant analysis, method validation, multielemental analysis

Introduction

For most of the analytical determinations from solid samples the sample digestion is required. The heavy metals like cadmium, copper, iron, manganese, lead and zinc in plants are determined after different digestion procedures including various mixtures of concentrated acids such as hydrofluoric acid (HF), hydrochloric acid (HCl), nitric acid (HNO₃), perchloric acid (HClO₄) and sulphuric acid (H₂SO₄). Different digestion equipment can be used: open beakers heated on hot plates, block digesters and digestion units placed in microwave ovens [MARGUI & al. 2005; GOMEZ & al. 2007].

The analytical techniques that can be used for metals determination from aqueous solutions obtained through plants digestion are mainly based on atomic spectrometry with mono-elemental detection, such as flame atomic absorption spectrometry (FAAS) [MENDIL, 2006], graphite furnace atomic absorption spectrometry (GF-AAS) [AYAR & al. 2009]. Inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) have the advantages of high samples throughput due to the multi-elemental determination and also, these methods have a wide working range [TORMEN & al. 2011].

Due to its advantages, ICP-OES has become one of the most used techniques for elemental determination, many studies being conducted to validate this method for metals analysis in a large variety of sample types. AYDIN (2008) has compared dry, wet and microwave digestion procedures for the determination of chemical elements in wool samples using ICP-OES technique, obtaining satisfactory recovery, detection limits and standard

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deviation for trace metal determination. BAKIRCIOGLU and co-workers [BAKIRCIOGLU & al. 2011] used ICP-OES technique for determination of some trace metals in cheese samples, packaged in plastic and tin containers, by ICP-OES after dry, wet and microwave digestion. ICP-OES and ICP-MS techniques were used for metals determination in vegetable seeds used in the production of biodiesel [CHAVES & al. 2010].

Dantas and co-workers [DANTAS & al. 2010] have measured the metals content in gum samples obtained from the deposits of internal combustion engines, using ICP-OES after microwave digestion. The heavy metal and trace element accumulation in edible tissues of farmed and wild rainbow trout was also studied by ICP-OES [FALLAH & al. 2011]. The ICP-OES technique was validated for the determination of trace elements in basil powder [GHANJAOUI & al. 2011] and for the quality control of herbal medicines [GOMEZ & al. 2007]. MARGUI & al. (2005) has made a comparative study between EDXRF and ICP-OES after microwave digestion for element determination in some plant specimens. ICP-OES was also used for metals determination in biological samples: animal tissues [MATOS & al. 2009], nuts and seeds [NAOZUKA & al. 2011] or in different food samples [NARDI & al. 2009]. This method was successfully applied for the measurement of metals in environment (water, soil and sediment samples) used in the assessment of environmental quality [FRENTIU & al. 2007; LEVEI & al. 2010; ZOBRIST & al. 2009; SENILA & al. 2011; SIMA & al. 2011].

For multivariate optimization of ICP-OES technique used for the determination of microelements in fruit juice, Santos Froes and co-workers [SANTOS FROES & al. 2009] have been used the exploratory analysis (Hierarchical Cluster Analysis, HCA, and Principal Component Analysis, PCA), which evaluated the plasma conditions (nebulization gas flow rate, applied power, and sample flow rate). In other study, fractional factorial design was used for the optimization of the digestion procedures followed using ICP-OES technique for multi-elemental determination in nuts [MOMEN & al. 2007].

The aim of this work is the development of an analytical method for the determination of elements (As, Cd, Cu, Fe, Mn, Pb and Zn) in perennial plants grown in mining polluted areas by dual view inductively coupled plasma optical emission spectrometry (ICP-OES) using two types of wet digestion methods.

Material and methods

A multi-elemental standard solution of 1000 mgL⁻¹ containing all analysed elements (As, Cd, Cu, Fe, Mn, Pb and Zn) supplied by Merck (Darmstadt, Germany) was used for calibration. HNO₃ 65% and H₂O₂ 30% from Merck (Darmstadt, Germany) analytical grade were used for samples digestion. Ultrapure water obtained by a Milli Q system (Millipore, France) was used for dilutions. A vegetable certified reference materials IAEA-359 Cabbage (Vienna, Austria) was used for the quality control of metals determination.

Determinations were carried out using a Perkin Elmer Model Optima 5300 DV spectrometer (Perkin Elmer, USA) ICP-OES equipped with a Ultrasonic Nebulizer CETAC U-6000AT+ (CETAC, USA) and an auto sampler AS 93-plus. Argon (purity higher than 99.995%) supplied by Linde Gas SRL (Cluj-Napoca, Romania) was used to sustain plasma and, as carrier gas. A closed-vessel microwave system Berghof MWS-3+ with temperature control mode, (Berghof, Germany) was used for wet digestion. All Teflon digestion vessels

were previously cleaned in a bath of 10% (v/v) nitric solution for 48 h to avoid cross-contamination.

Four specimens of perennial plants samples (*Agrostis*, *Agropyrum repens*, *Trifolium repens*, *Urtica dioica*), collected from a mining affected area of Baia Mare (Romania), were analysed in this study. Samples were dried in an oven at 40°C till constant weight was achieved. The dried samples were then grounded with a Mixer Grinder and sieved through a 100 microns mesh. All the samples were originally stored in closed plastics bags until analysis. The certified reference material were analysed in the same experimental conditions used for sample analyses in order to evaluate the accuracy of the method.

Wet digestion on hot plate

For the wet digestion a mixture of HNO₃/H₂O₂ was used in this study. For this procedure, the temperature was maintained at 120°C for 2 h during digestion of 1.0 g of plant sample with 16 mL of 6:2 HNO₃/H₂O₂ mixtures on the hot plate. After cooling, 10 mL of distilled water was added on the sample and mixed. The residue was filtered through filter paper and then the sample was diluted to 50 mL with distilled water. Metal contents of final solution were determined by ICP-OES.

Microwave digestion

Approximately 1.0 g of sample was digested with 6 mL of HNO₃ and 2 mL of H₂O₂ in microwave digestion system, according to the digestion program presented in Tab. 1. The resulting solutions were cooled and diluted to 50 mL with distilled water. The resulted solutions were analysed by ICP-OES.

ICP-OES determination

The operating conditions employed for ICP-OES determination were 1300W RF power, 15 L min⁻¹ plasma flow, 2.0 L min⁻¹ auxiliary flow, 0.8 L min⁻¹ nebulizer flow, 1.5 mL min⁻¹ sample uptake rate. Axial view was used for metals determination, while 2-point background correction and 3 replicates were used to measure the analytical signal. The emission intensities were obtained for the most sensitive lines free of spectral interference.

The calibration standards were prepared by diluting the stock multi-elemental standard solution (1000 mg L⁻¹) in 0.5% (v/v) nitric acid. The calibration curves for all the studied elements were in the range of 0.01 to 1.0 mg L⁻¹.

Results and discussion

Figures of merit

Method validation is an important requirement in the practice of chemical analysis and it is the process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with what the application requires. The estimation of the uncertainty associated to the analytical methods is necessary in order to establish the comparability of results, and it is an important parameter in reporting of analytical results.

The limit of detection (LOD) and limit of quantification (LOQ) of the method and also the main analytical characteristics of the calibration curves (slope and correlation coefficients) for the developed ICP-OES procedure are indicated in Tab 2. The instrumental detection and quantification limits were estimated by analysing ten blank solutions. The LOD is defined as three times the standard deviation of the ten measurements, while the LOQ are defined as ten times the standard deviation of the ten measurements. The LODs and LOQs were calculated for the original solid samples ($\mu\text{g g}^{-1}$) by taking into account the amount of sample digested and the final volume obtained by dilution. The LOD of the seven elements studied varied between $0.20 \mu\text{g g}^{-1}$ for Mn and $0.55 \mu\text{g g}^{-1}$ for Pb. These values are appropriate for the measurement with a good accuracy of maximum admitted limits of $1 \mu\text{g g}^{-1}$ for Cd, $2 \mu\text{g g}^{-1}$ for As and $10 \mu\text{g g}^{-1}$ for Pb, established by the European Directive 2002/32/EC on undesirable substances in animal feed. Recovery percentage values found for the analysis of spiked plants samples varied between 89% and 113%.

As no certified reference materials for perennial plants were available, the accuracy of the proposed method was evaluated by analysing a vegetable certified reference material IAEA-359 Cabbage (Vienna, Austria). The use of this material is appropriate, because the CRM was digested and diluted in the same way as the perennial plants. The results are shown in Tab 3. The obtained results by both digestion methods show good agreement for all analysed elements between found and certified values, according to the t-test for a 95% confidence level, and the method is thus considered accurate.

Repeatability was established from the average relative standard deviation (RSD) of three independent analyses at the real samples. Standard addition curves obtained from a plant sample spiked at different concentration levels from 50 to $500 \mu\text{g L}^{-1}$ were compared with external calibration lines established from multi-elemental standards in order to evaluate the matrix effect. The recovery values ranged between 86 and 119% of the spiked values. No matrix effect has been observed and therefore aqueous standards have been used for calibration.

Analytical results

Shoots of the four different perennial plants samples were digested using the wet digestion on hot plate and the microwave digestion. After digestion, 7 elements were determined by ICP OES: As, Cd, Cu, Fe, Mn, Pb and Zn, using external calibration with aqueous standard solutions. The obtained results are shown in Tab. 4, reported with 95 % confidence limit ($n = 3$).

According to a Student's t-test, there was no difference between the concentrations of all elements for wet digestion on hot plate and microwave digestion at a 95% confidence level. Generally, lower standard deviations were obtained using the microwave digestion method. The results obtained reveal that the proposed digestion methods and measurement technique (ICP-OES) can be successfully applied to different kind of plants analysis. The measured values for As, Cd and Pb were generally below the maximum admitted limits established by the European Directive 2002/32/EC on undesirable substances in animal feed, while for the others analysed elements there are not established maximum admitted limits.

Conclusions

The principal figures of merit for the determination of trace elements by ICP-OES from perennial plants were evaluated. Two methods of sample digestion: wet digestion on hot plate and microwave digestion were compared and no significant differences between the results obtained were observed. The obtained results using the two digestion methods show good agreement with the certified values of one analysed vegetable CRM, for all analysed elements. Standard addition curves obtained from a plant sample spiked at different concentration levels were compared with external calibration lines established from multi-elemental standards and no significant matrix effect has been observed. The results show that the proposed technique (ICP-OES) and also the two digestion methods are suitable for metals determination in perennial plants.

Acknowledgements

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Tab. 1. Operating conditions for the microwave digestion system

	Stage			
	1	2	3	4
Temperature / °C	160	120	100	100
Ramp time / min	5	1	1	1
Hold time / min	25	5	1	1
Power / %*	60	20	10	10

* 100 % power corresponds to 1400 W

Tab. 2. Analytical parameters for metals determination using ICP-OES

Element	Wavelength (nm)	LOD $\mu\text{g g}^{-1}$	LOQ $\mu\text{g g}^{-1}$	Slope	Correlation coefficient
As	188.979	0.30	0.90	13496	0.9996
Cd	228.802	0.25	0.75	108844	0.9998
Cu	327.393	0.30	0.90	245987	0.9995
Fe	238.204	0.35	1.05	203903	0.9991
Mn	257.610	0.20	0.60	1094626	0.9999
Pb	220.345	0.55	1.65	23734	0.9997
Zn	213.856	0.30	0.90	259230	0.9995

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Tab. 3. Analytical determination of metals in reference material. Values are expressed in $\mu\text{g g}^{-1}$ and reported as average \pm ts; n=3; 95% confidence level

Element	Certified content	Found content	
		<i>Hot plate digestion</i>	<i>Microwave digestion</i>
As	0.096 - 0.104	0.094 \pm 0.012	0.103 \pm 0.011
Cd	0.115 - 0.125	0.120 \pm 0.018	0.119 \pm 0.012
Cu	5.49 - 5.85	5.39 \pm 0.48	5.28 \pm 0.51
Fe	144.1 - 151.9	146 \pm 5.32	143 \pm 4.45
Mn	31.3 - 32.5	31.7 \pm 2.48	32.5 \pm 2.23
Zn	37.9 - 39.3	39.5 \pm 3.07	38.8 \pm 2.19

Tab. 4. Concentrations (average \pm ts; n=3; 95% confidence level) for perennial plants obtained by ICP OES (dry weight), expressed in $\mu\text{g g}^{-1}$

Sample/element	Digestion procedure	<i>Agrostis</i>	<i>Agropyrum repens</i>	<i>Trifolium repens</i>	<i>Urtica dioica</i>
As	HP*	0.55 \pm 0.06	0.46 \pm 0.08	0.35 \pm 0.06	0.47 \pm 0.07
	MW**	0.51 \pm 0.05	0.44 \pm 0.06	0.37 \pm 0.05	0.43 \pm 0.06
Cd	HP	0.89 \pm 0.09	0.69 \pm 0.07	0.55 \pm 0.06	0.96 \pm 0.12
	MW	0.93 \pm 0.08	0.66 \pm 0.06	0.54 \pm 0.07	0.93 \pm 0.10
Cu	HP	4.51 \pm 0.45	3.25 \pm 0.39	2.23 \pm 0.25	4.62 \pm 0.30
	MW	4.22 \pm 0.36	3.53 \pm 0.39	2.11 \pm 0.20	4.72 \pm 0.41
Fe	HP	79.2 \pm 4.6	65.1 \pm 4.8	41.4 \pm 3.5	72.9 \pm 5.5
	MW	75.5 \pm 3.8	64.2 \pm 2.2	42.9 \pm 2.1	70.1 \pm 4.0
Mn	HP	5.65 \pm 0.48	4.34 \pm 0.59	3.05 \pm 0.38	5.11 \pm 0.52
	MW	5.44 \pm 0.25	4.55 \pm 0.28	3.55 \pm 0.39	4.96 \pm 0.23
Pb	HP	3.51 \pm 0.22	2.08 \pm 0.33	5.18 \pm 0.42	6.41 \pm 0.58
	MW	3.09 \pm 0.25	2.21 \pm 0.24	4.96 \pm 0.34	6.77 \pm 0.36
Zn	HP	47.7 \pm 2.5	66.9 \pm 5.3	44.8 \pm 3.7	84.4 \pm 6.3
	MW	44.8 \pm 2.4	67.5 \pm 3.1	46.1 \pm 1.9	84.0 \pm 3.3

* Hot plate; **Microwave

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CONTRIBUTION TO THE MACROMYCETES BIODIVERSITY FROM BOLINTIN DEAL FOREST – GIURGIU, ROMANIA

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Abstract: The paper contains the results of macromycetes investigations within the Bolintin Deal Forest near the city of Bucharest. The research objective was to inventory the species for this area, establishing the present status of biodiversity of fungi. There were identified 59 species of macromycetes. All the identified species represent a new contribution due to the lack of mycological research in the given area. The study is part of a larger research regarding the diversity and distribution of macromycetes in Bucharest and its surroundings, Romania.

Keywords: fungal distribution and diversity, macromycetes, taxonomy, Bolintin Deal Forest, Giurgiu, Romania

Introduction

The Bolintin-Deal Forest, also known as Berceni Forest or Cetroceanca Forest is located in west of the Bucharest city, in-between the meadows of the rivers Ciorogârla (on the east side) and Sabar (on the west side). The forest should not be confused with Bolintin Forest or the Large Forest of Bolintin which is located more to the west and which is a SCI site according to the European Commission Habitats Directive (92/43/EEC). The geographic limits coordinates are East – Lat. 44°44'77" / Lon. 25°83'13", West – Lat. 44°44'33" / Lon. 25°86'49", North – Lat. 44°45'00" / Lon. 25°83'56" and South – Lat. 44°43'27" / Lon. 25°84'66" according to the WGS84 standard and is located in MK-02 according to the Universal Transverse Mercator (UTM) geographic coordinate system. The altitude is 102–117 meters.

From a geographical point of view the area is part of the Vlășia Plain, which is a climatic and hydro-geographically interference area belonging to the Valachian platform. The link-up of the atmospheric masses from the NE and W-SW reflects a characteristic vegetation and soil structure [POSEA & ȘTEFĂNESCU, 1983].

The climate is temperate with some slight immoderate changes. The excessive humidity area which would characterize the general location between two river meadows is slightly attenuated, as the forest is also neighbored on west by the village Bolintin Deal and on the east the village Ciorogârla. Overall the forest has, in spite of its location, slight xerophile characteristics, most of the small herbs found during early and mid summer having small vegetation period. The soils are typical brown and brown-auburn forest soils.

Vegetation is typical to the forest-stepic area and consists of trees (mainly *Quercus* genus) and small herbs (mainly from the Poaceae family).

The native flora consists especially of trees and bushes belonging to the following species: *Quercus pedunculiflora* L., *Quercus cerris* L., *Tilia platyphyllos* Scop., *Tilia*

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tomentosa Moench., *Ulmus* sp., *Carpinus betulus* L., *Cornus mas* L., *Crataegus monogyna* Jacq. On the southern side, there is a swampy area with *Salix* sp. and *Populus alba*. Also on each edge of the forest there are species of *Sambucus nigra* L. și *Prunus cerasifera* Ehrh. var. *cerasifera* (cultivated). The herbs consists of early species, which blossom before the trees can develop their crowns such as: *Corydalis cava* (L.) Schweigg. et Koerte, *Ficaria verna* Hudson, *Anemone ranunculoides* L., *Anemone nemorosa* L., *Scilla bifolia* L., *Viola hirta* L. etc. Also species of *Arum maculatum* L. and *Pulmonaria officinalis* L. are frequent.

Material and method

The mycological material was gathered during many field trips to the specified area in different seasons of the years 2009, 2010 and 2011. Some of the easiest identifications were made in the field and noted. Also in situ photographs have been taken using a Canon S3 IS camera. The material collected was brought to analysis in the laboratory. The examinations included macroscopic as well as microscopic aspects. The macroscopic consisted in the analysis of the color (cap, gills, spore-print, stalk), consistency, morphology, taste, odor, presence and characteristics of the latex and so on. For macroscopic analysis an Optika trinocular stereozoom microscope model SZM-GEM-2, eyepieces 10x and zoom head magnification 0.7x – 4.5x, equipped with an USB digital camera model Optikam Pro5, 5Mp have been used.

The microscopic features that were pursued are referring to the morphology of the spores and other structures (cystidia, cap cuticle etc.). The observations made were noted and used in the process of identification the species. Most of the material was dried and is in the author's possession.

During determination, on some taxons we have also analyzed chemical characteristics using ferrous sulphate (FeSO₄) – aqueous solution 10% and sulphovanilin (for *Russula* species), Melzer's reagent (aqueous solution of chloral hydrate, potassium iodide and iodine) – to test spores on white spored mushrooms – and potassium hydroxide – aqueous solution 10%.

For microscopic staining techniques several other chemicals were also used such as Congo red, aniline blue (Cotton blue 4B), Phloxine and Cresyl blue.

The mycological nomenclature used is taken after SĂLĂGEANU & SĂLĂGEANU (1985), ȘESAN & TĂNASE (2006), TĂNASE & ȘESAN (2006), TĂNASE et al. (2009) as well as some macromycetes books and monographs such as ROMAGNESI (1967, 1981), BON (1988a, b), BREITENBACH & KRÄNZLIN (1984, 1986, 1991), COURTECUISE & DUHEM (1994), BORGARINO & HURTADO (2001), ROUX (2006) and others. The international herbaria names are taken after HOLMGREN & al. (1990), the name of the authors, after KIRK & ANSELL (1992) and Systematics of the Fungi regnum after KIRK & al. (2001, 2008) and CANNON & KIRK (2007). The scientific names (current names) have been updated according to the *Index Fungorum* [KIRK, 2011]. The Tracheophytes nomenclature is taken after *Flora of Romania* [SĂVULESCU, 1952-1976].

Results and discussions

There were identified 59 species of macromycetes (49 genera) belonging to 3 classes of the regnum Fungi: 2 Myxomycetes (3%), 6 Ascomycetes (10%) and 51 Basidiomycetes (87%) (Fig. 1). All the taxons represent an absolute novelty for the studied area [ELIADE, 1965; BONTEA, 1985-1986; TĂNASE & POP, 2005].

Out of the taxons identified, from ecological point of view, there were identified 31 species (53%) of wood fungi and 28 species (47%) of soil fungi (Fig. 2); a number of 7 species (12%) form ectomycorrhizas, 9 (15%) are parasitic and 43 species (73%) are saprotrophs (Fig. 3).

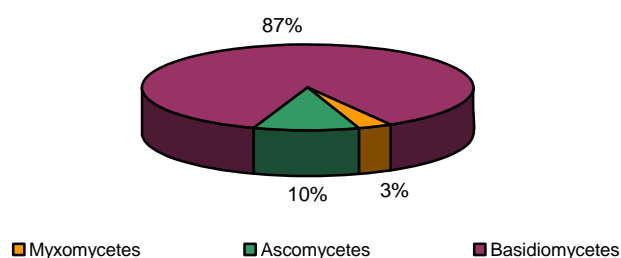


Fig. 1. Classes of macromycetes identified in the Bolintin Deal Forest

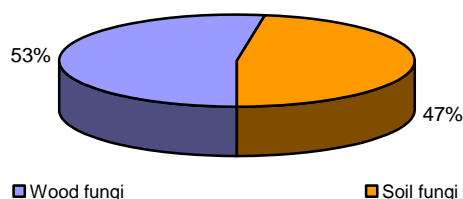


Fig. 2. Wood and soil fungi identified in the Bolintin Deal Forest

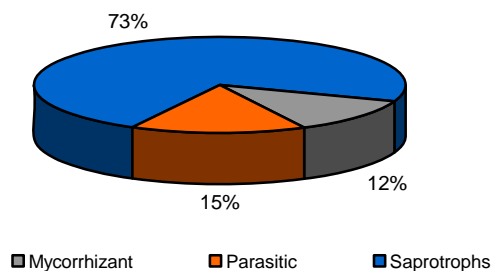


Fig. 3. Proportion of the fungi, after their nutritional characteristics, identified in the Bolintin Deal Forest

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List of species is presented in alphabetical order, with indication of location and date of first occurrence.

Myxogastrea

Arcyria denudata (L.) Wettst., ad solum - 21.05.2011, in *Carpino-Quercetum*, alt. 107 m.

Lycogala epidendrum (J. C. Buxb. ex L.) Fr., in ligno ramulos deciduos - 30.08.2009, in *Carpino-Quercetum*, alt. 107 m.

Ascomycota

Arachnopeziza aurelia (Pers.) Fuckel, ad ramulos deciduos - 20.06.2009, in *Tilio-Quercetum*, alt. 110 m.

Humaria hemisphaerica (F. H. Wigg.) Fuckel, ad solum - 30.08.2009, in *Quercetum*, alt. 110 m.

Hypoxyton fragiforme (Pers.) J. Kickx, ad truncus amputatus decorticatus- 30.08.2009, in *Carpino-Quercetum*, alt. 107 m.

Peziza vesiculosa Bull., ad solum - 21.05.2011, ad marginem *Quercetum*, alt. 115 m. (Fig. 4).

Tarzetta catinus (Holmsk.) Korf & J. K. Rogers, ad solum - 21.05.2011, in *Tilio-Quercetum*, alt. 110 m (Fig. 5).

Xylaria polymorpha (Pers.) Grev., in ligno trunci amputati - 30.08.2009, in *Quercetum* alt. 110 m.

Basidiomycota

Amanita rubescens Pers. var. *rubescens*, ad solum - 21.05.2011, in *Tilio-Quercetum*, alt. 110 m (Fig. 7).

Armillaria mellea (Vahl) P. Kumm. s.l., ad cortex truncus amputatus - 30.08.2009, in *Carpino-Quercetum*, alt. 107 m.

Auricularia auricula-judae (Bull.) Quél., ad cortex ramulos deciduos sambuci - 21.05.2011, ad marginem silvarum, alt. 115 m.

Auricularia mesenterica (Dicks.) Pers., ad truncus amputatus decorticatus - 30.08.2009, in *Tilio-Quercetum*, alt. 110 m.

Bjerkandera adusta (Willd.) P. Karst., ad cortex truncus amputatus - 20.06.2009, in *Quercetum*, alt. 117m.

Bolbitius titubans (Bull.) Fr. var. *titubans*, ad cortex putrido in solum - 21.05.2011, in *Tilio-Quercetum*, alt. 110 m.

Boletus badius (Fr.) Fr. ?, ad solum - 20.06.2009, in *Carpino-Quercetum*, alt. 107 m.

Calocera cornea (Batsch) Fr., ad ramulos deciduos - 21.05.2011, in *Quercetum*, alt. 115 m (Fig. 9).

Coprinopsis picacea (Bull.) Redhead, Vilgalys & Moncalvo, ad solum - 30.08.2009, in *Carpino-Quercetum*, alt. 107 m.

Coprinellus disseminatus (Pers.) J. E. Lange ['disseminata'], ad cortex putrido truncus amputatus - 30.08.2009, in *Quercetum*, alt. 110 m.

Cyathus striatus (Huds.) Willd., ad radices crataegi parti in solum - 13.08.2011, ad marginem silvarum, alt. 117 m.

Dacrymyces stillatus Nees, ad ramulos deciduos decorticatae - 30.08.2009, in *Tilio-Quercetum*, alt. 110 m.

Daedalea quercina (L.) Pers., ad cortex truncus amputatus - 20.06.2009, in *Quercetum*, alt. 117 m.

- Daedaleopsis confragosa* (Bolton) J. Schröt., ad ramulos deciduos - 13.08.2011, in *Tilio-Quercetum*, alt. 110 m.
- Fistulina hepatica* (Schaeff.) With., ad cortex quercinum - 13.08.2011, in *Quercetum*, alt. 117 m.
- Fomes fomentarius* (L.) J. J. Kickx, ad cortex tili - 30.08.2009, in *Tilio-Quercetum*, alt. 110 m.
- Galerina paludosa* (Fr.) Kühner, ad muscum super trunco amputato - 21.05.2011, in *Tilio-Quercetum*, alt. 110 m.
- Ganoderma applanatum* (Pers.) Pat., in ligno truncus amputatus - 20.06.2009, in *Carpino-Quercetum*, alt. 107 m.
- Ganoderma lucidum* (Curtis) P. Karst., in ligno truncus amputatus - 20.06.2009, in *Quercetum*, alt. 117 m.
- Gymnopus dryophilus* (Bull.) Murrill, ad solum - 20.06.2009, in *Carpino-Quercetum*, alt. 107 m.
- Gymnopus fusipes* (Bull.) Gray, ad solum - 30.08.2009, in *Quercetum*, alt. 115 m.
- Hymenochaete rubiginosa* (Dicks.) Lév., ad trunco amputato - 20.06.2009, in *Tilio-Quercetum*, alt. 110 m.
- Hyphodontia quercina* (Pers.) J. Erikss., ad ramulos deciduos - 20.06.2009, in *Carpino-Quercetum*, alt. 107 m.
- Hypholoma fasciculare* (Huds.) P. Kumm. var. *fasciculare*, ad cortex putrido - 20.06.2009, in *Quercetum*, alt. 110 m.
- Kuehneromyces lignicola* (Peck) Redhead, ad cortex truncus amputatus - 30.08.2009, in *Tilio-Quercetum*, alt. 110 m.
- Lactarius acerrimus* Britzelm., ad solum - 13.08.2011, in *Carpino-Quercetum*, alt. 107 m.
- Lenzites betulina* (L.) Fr., ad ramulos deciduos - 13.08.2011, in *Carpino-Quercetum*, alt. 107 m.
- Lepista nuda* (Bull.) Cooke, ad solum - 21.05.2011, in *Quercetum*, alt. 110 m.
- Leucopaxillus giganteus* (Sowerby) Singer, ad solum - 20.06.2009, ad marginem *Quercetum*, alt. 117 m.
- Lycoperdon pyriforme* Schaeff., ad solum - 20.06.2009, in *Tilio-Quercetum*, alt. 110 m.
- Macrolepiota procera* (Scop.) Singer var. *Procera*, ad solum - 30.08.2009, in *Quercetum*, alt. 117 m.
- Marasmius oreades* (Bolton) Fr., ad solum - 30.08.2009, ad marginem silvarum, alt. 117 m.
- Marasmius rotula* (Scop.) Fr., ad cortex putrido - 20.06.2009, in *Tilio-Quercetum*, alt. 110 m.
- Oudemansiella mucida* (Schrad.) Höhn., ad cortex truncus amputatus - 20.06.2009, in *Tilio-Quercetum*, alt. 110 m.
- Parasola plicatilis* (Curtis) Redhead, Vilgalys & Hopple, ad solum - 21.05.2011, ad marginem callis intram *Quercetum*, alt. 117 m.
- Phallus impudicus* L. var. *impudicus*, ad solum - 13.08.2011, in *Carpino-Quercetum*, alt. 107 m (Fig. 6).
- Phylloporia ribis* (Schumach.) Ryvarden, ad cortex crataegi - 20.06.2009, in *Tilio-Quercetum*, alt. 110 m.
- Pluteus cervinus* (Schaeff.) P. Kumm., ad cortex truncus amputatus - 30.08.2009, in *Tilio-Quercetum*, alt. 110 m.

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- Russula cyanoxantha* (Schaeff.) Fr., ad solum - 20.06.2009, in *Tilio-Quercetum*, alt. 110 m.
- Russula foetens* (Pers.) Pers., ad solum - 20.06.2009, in *Quercetum*, alt. 115 m.
- Russula heterophylla* (Fr.) Fr., ad solum - 13.08.2011, in *Tilio-Quercetum*, alt. 110 m.
- Russula persicina* Krombh., ad solum - 13.08.2011, in *Quercetum*, alt. 115 m (Fig. 8).
- Russula virescens* (Schaeff.) Fr., ad solum - 13.08.2011, in *Quercetum*, alt. 115 m.
- Schizophyllum commune* Fr., ad ramulos deciduos - 20.06.2009, in *Quercetum*, alt. 117 m.
- Scleroderma citrinum* Pers. var. *citrinum*, ad solum - 30.08.2009, in *Tilio-Quercetum* alt. 110 m.
- Scleroderma verrucosum* (Bull.) Pers., ad solum - 13.08.2011, in *Quercetum*, alt. 115 m.
- Stereum hirsutum* (Willd.) Pers., ad cortex truncus amputatus - 20.06.2009, in *Tilio-Quercetum*, alt. 110 m.
- Trametes hirsutum* (Wulfen) Lloyd, ad cortex truncus amputatus - 30.08.2009, in *Tilio-Quercetum*, alt. 110 m.
- Trametes versicolor* (L.) Lloyd, ad cortex truncus amputatus - 20.06.2009, in *Tilio-Quercetum*, alt. 110 m.
- Volvariella bombycina* (Schaeff.) Singer, ad cortex truncus amputatus - 20.06.2009, in *Carpino-Quercetum*, alt. 107 m.
- Xerula radicata* (Relhan) Dörfelt, ad trunco amputato putrido - 20.06.2009, in *Carpino-Quercetum*, alt. 107 m.

Conclusions

The study of macromycetes biodiversity in Bolintin-Deal Forest is the first one of this kind in this area, and since there are no bibliographic references, the whole study brings new mycological data regarding the surrounding forests of Bucharest that have been once part of the Vlăsia Plain.

Determination of 59 different taxa during 2009-2011 indicated a large biodiversity in an area that is virtually unknown.

Importance of the study resides in the new information of an area far too little investigated, keeping open a perspective of a more larger study of the forests near Bucharest with the purpose to know, protect and conserve their biodiversity.

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Fig. 4. *Peziza vesiculosa* Bull.

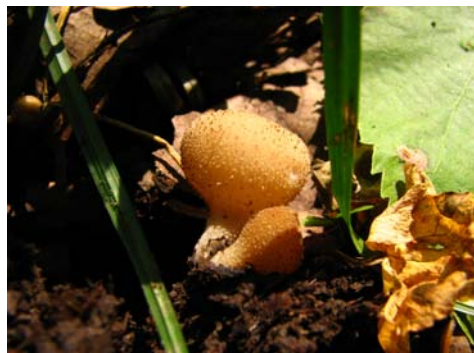


Fig. 5. *Tarzetta catinus* (Holmsk.) Korf & J.K. Rogers



Fig. 6. *Phallus impudicus* L. var. *impudicus*



Fig. 7. *Amanita rubescens* Pers. var. *rubescens*



Fig. 8. *Russula persicina* Krombh.



Fig. 9. *Calocera cornea* (Batsch) Fr.

GALIUM RUTHENICUM WILLD. IN FLORA OF ROMANIA

CIOCÂRLAN VASILE¹

Abstract: A newly identified species in the vascular flora of Romania, namely *Galium ruthenicum* Willd. is published now. This taxa has been identified in Tulcea county, Dobrudja province. It is grown on sunny, rocky places.
It is also mentioned the differences against *Galium verum* L., a morphologically close taxa in flora of Romania.

Keywords: *Galium ruthenicum*, features, ecology, distribution, Romania's flora

Introduction

The genus *Galium* L. has 145 species in *Flora Europaea* [EHRENDORFER & KRENDL, 1976]. In Romania, the same genus *Galium* L. include 27 species [PAUCĂ & NYÁRÁDY, 1961]. Later on, there has been added other 9 species [CIOCÂRLAN, 2009]. Thus, in Romania's flora there are 38 species in the genus of *Galium* L.

There is added an other species in this *Galium* L. genus in this paper.

Galium ruthenicum Willd., Sp. Pl. 1: 597 (1798) (*G. verum* L. subsp. *ruthenicum* (Willd.) P. Fourn.) is a morphologically close to a common species in flora of Romania, namely de *G. verum* L. These two species, *Galium ruthenicum* Willd. and *G. verum* L., are distinguished by several features presented further on, but especially by their ecology and distribution area.

Characteristics

The detailed description of *Galium ruthenicum* Willd. is made in *Flora of the USSR*, vol. XXIII [POBEDIMOVA, 1958]. The own characteristics of this species are like the next:

- leaves linear-filiforms, 7-8 in whorls (at *G. verum* the leaves are disposed as 8-12 in whorls), 25-30 mm long and 0.5 mm wide (at *G. verum* the leaves are of 0.5-1.5 (–2) mm wide);
- pedicels and especially the fruits have densely, harsh hairs (at *G. verum* the fruits are glabrous) (Fig. 1).

Distribution and ecology

From distribution and ecology points of view, these two taxa are fundamentally different. *Galium verum* is a species with a wide area of distribution, as it is distributed in the whole area of Eurasia. It is grown from the sea level to ca 1200 m.

Galium ruthenicum is a pontic species, growing in steppic areas, often in rocky places.

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In Europe, *Galium ruthenicum* is distributed in Russia of SE, Ukraine, R. of Moldova [STANKOV & TALIEV, 1949; MAIEVSKII, 1954; POBEDIMOVA, 1958; GEIDEMAN, 1954; ZEROV, 1965].

In România, *Galium ruthenicum* has been identified in Dobrudja, Tulcea county, on a stony hill, ca 2 Km North of Făgărașul Nou village.

Accompanying species:

Achillea leptophylla,
Allium guttatum,
Cleistogenes bulgarica,
Hieracium echioides subsp. *procerum*,
Moehringia grisebachii (on rocks, only)
Onobrychis arenaria,
Seseli pallassii,
Stachys angustifolia,
Veronica spicata subsp. *barrieleri*.

Voucher specimens were deposited in the Herbarium BUAG, sheet no. 23947.

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Fig. 1. *Galium ruthenicum* Willd. (original)

THE VARIABILITY OF *CEPHALARIA URALENSIS* (MURRAY) ROEM. ET SCHULT.

CIOCÂRLAN VASILE¹

Abstract: The variability of *Cephalaria uralensis* (Murray) Roem. et Schult., incl. subsp. *dobrogensis* Ciocârlan, subsp. nova, and an identification key, are presented in this paper.

Keywords: *Cephalaria uralensis*, variability, features, ecology, distribution, Romania's flora

Introduction

Cephalaria uralensis (Murray) Roem. et Schult. is a Pontic–Balkan species, distributed in Russia (C, W, E, Crimea), Bulgaria, Romania, Serbia, and, probable, in Greece [FERGUSON, 1976].

This species is distributed in Transylvania, Banat, Oltenia, Muntenia, Moldavia, Bucovina, and Dobrudja [OPREA, 2005].

As ecology, it is a xerophyllous species, growing on dry and sunny places, but also on rocky substrates.

Material and methods

Data on the variability of *Cephalaria uralensis* (Murray) Roem. et Schult. exist in flora of Serbia, only [DIKLIC, 1973], where are given two varieties, as: var. *uralensis* and var. *puberula* (Adamović) Diklić.

In *Flora of R. P. Române*, vol. VIII [PRODAN, 1961] it is given a form, namely f. *obtusilaciniata* Räv.

In *Conspectus Florae Romaniae* [BORZA, 1949] there is given an other forms, namely: f. *dentata* Schur, f. *intermedia* Schur, and f. *tenuisecta* Schur.

In *Flora of R. S. România*, vol. XIII [BELDIE & VACZY, 1976] there is given a subspecies, namely subsp. *multifida* (Roman) Roman et Beldie.

This last taxa, *Cephalaria uralensis* (Murray) Roem. et Schult. subsp. *multifida* (Roman) Roman et Beldie is worth to be noted, being an endemite in Romania's flora (neoendemite), registered in *Flora of R. S. România*, vol. XIII [BELDIE & VACZY, 1976]. Also, this taxa is included onto the *Romanian Red List* [OLTEAN & al. 1994].

Unfortunately, this taxa is missing in the Romanian Red Book [DIHORU & NEGREAN, 2009].

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Results and discussions

The author of this paper discovered a new taxa in the flora of Dobrudja (South-East Romania) is presented further on.

Cephalaria uralensis (Murray) Roem. et Schult. subsp. *dobrogensis* Ciocârlan, subsp. nova [BARANOV, 1971].

Perennial plant, with a stem of 30-60 cm in height; leaves opposed, penately-sectate, with 5-8 pairs of lanceolate segments, entirely, small, 0.8-1.2 cm long, decurrents; head of flowers of 0.8-1 cm in diameter; the outer calyx has pretty small and uniform teeth; the achenes are tetragonal, of 4.5-5 mm long, hairy (Fig. 2 – habitus and fruits).

The habitat: this subspecies is growing on dry, rocky places, between 100 and 400 m. s. l.

Spread: Alah-Bair hill (near Băltăgești village, Constanța county) and on the Pietrosul hill (near Agighiol village, Tulcea county).

Holotypus: in herbarium BUAG – University of Agricultural Sciences, Bucharest, conservatur also in herbarium I (University “Alexandru Ioan Cuza” of Iași, Romania) as isotypus.

The differential characteristics of this new taxa (subsp. *dobrogensis* Ciocârlan, subsp. nova) against the type subspecies (subsp. *uralensis*) and subsp. *multifida* (Roman et Beldie [ROMAN, 1971]), are given in the next dichotomic key of identification:

- 1a Basal leaves with 2-4 (-5) pairs of entire segments, the terminal one being larger. The outer calyx has 4 long teeth and other 4 intermediately and very short teeth.subsp. *uralensis* (Fig. 1 - habitus and fruits)
- 1b Basal leaves with more than 5 pairs of segments. The outer calyx with uniform and very small teeth 2
- 2a Stem of 30-60 cm height; basal leaves with 5-8 pairs of entire segments, small, 0.8-1 cm long; achenes of 4.5-5 mm long.subsp. *dobrogensis* (Fig. 2 - habitus and fruits)
- 2b Stem of 50-150 cm height; leaves with 8-12 (-14) pairs of linear segments, lobulately; achenes to 10 mm longsubsp. *multifida*

Obs.: *Cephalaria uralensis* subsp. *dobrogensis* and *C. uralensis* subsp. *multifida* are morphologically similar to *C. media* Litv., an endemic species distributed in Caucasus region [BOBROV, 1957].

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Fig. 1. *Cephalaria uralensis* subsp. *uralensis* – plant habitus and fruits (original)



Fig. 2. *Cephalaria uralensis* subsp. *dobrogensis* – plant habitus and fruits (original)



PILOSELLA HILL GENUS IN THE BESSARABIA'S FLORA

IONIȚA OLGA¹

Abstract: As a result of floristic and taxonomic investigations of the *Pilosella* genus within the flora of the Bessarabia have been established 11 species: *P. officinarum* F. Schultz et Sch. Bip., *P. aurantiaca* F. Schultz et Sch. Bip., *P. praealta* (Vill ex Gochn.) F. Schultz et Sch. Bip., *P. piloselloides* (Vill.) Sojak, *P. glaucescens* (Bess.) Sojak, *P. echioides* (Lumn.) F. Schultz et Sch. Bip., *P. caespitosa* (Dumort.) P. D. Sell et C. West, *P. cymosa* (L.) F. Schultz et Sch. Bip., *P. lactucela* (Wallr.) P. D. Sell et C. West, *P. rojowskii* (Rehm.) Schljak. & *P. flagellare* (Willd.) Arv.-Touv., the last three are new taxa recently detected for the flora in the study. After chorological analysis, has been concluded that 6 species are rare. Ecological and biomorfological characteristics of taxa have been established, the determination key of the *Pilosella*'s species has been drawn.

Key words: genus *Pilosella* Hill, Bessarabia's flora, taxonomy, bioecology, chorology

Introduction

Hieracium L. s. l. being one of the most polymorphic, complicated and bulky genera of magnoliophyta from the Holarctic flora, is considered nowadays by a large number of the authors as two separated genera *Hieracium* L. s. str. and *Pilosella* Hill (*Asteraceae* Dumort. family, *Hieraciinae* Dumort. subtribe, *Cichorieae* Lam et DC. tribe). A number of common morphological features are characteristic for both genera, which determine the exceptional diversity of the forms and complicate the determination of the taxa, different modes of reproduction - apomixes (required and optional), amphimixis etc. are inherent for them. Auto incompatibility is not compulsory and it is possible to form offspring from cleistogamy [TUPICINA, 2004].

Pilosella genus described by HILL (1756) soon after publication of the *Hieracium* genus (Linnaeus, 1753), has not been accepted as an independent generic taxonomic unit for a long time, but considered as a taxonomic unit with a status of subgenus - *Pilosella* [GRAY, 1821] or section - *Piloselloidea* [KOCH, 1837] within the *Hieracium* genus [TUPICINA, 2004]. Among the botanists from the 18-19 century only SCHULTZ & SCHULTZ-BIPONTINUS (1862) and ARVET-TOUVET (1880) have acknowledged the existence of *Pilosella* genus, as separate taxonomic units. The final delimitation of the *Hieracium* s. str. and *Pilosella* Hill. genera was done barely in the second half of the 20th century as a result of researches and the appearance of works of a number of the botanists as: SOJÁK (1971), SELL & WEST (1976), DOSTÁL (1984), ŠLÁKOV (1989) and others [TIHOMIROV, 2001].

The delimitative criteria are related to the structural features of the generative organs, in this case of the morphology of the fruit components, which usually manifest conservative properties more advanced unlike the vegetative organs and is practiced safer in separating activities of the taxonomic categories [IONIȚA & NEGRU, 2010].

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The most important characters that separate the *Pilosella* from the *Hieracium* genus are related to the achen structure, are essentially distinctive, that determine us to accept the separation of species previously assigned to the *Hieracium* genus from Bessarabia's flora.

The *Hieracium* L. s. str. genus has achenes from 2.5 to 5 mm length; ring shaped, apical with bristles in two series, but the *Pilosella* Hill genus - 1-2 (2.5) mm length, costate; apical, crenated coronule with bristles in one row.

Material and methods

During floristic investigations as a study material has served both *Hieracium* L. collections from Botanical Garden herbarium of ASM and that of the Department of Botany of the State University of Moldova and our own collections, made during the last years. The critical analysis of *Pilosella* species was performed by the classical comparative-morphological method [KOROVINA, 1996]. The material collected in the field was herbarized then determined in office conditions, using contemporary floristic literature [NYARADY, 1965; SELL & WEST, 1976; NEGRU, 2007; GHEIDEMAN, 1986; ŠLĀKOV, 1989; DOBROCEAEVA & al. 1999] and some basic guidance on the nomenclature and bioecology of infrageneric taxa [SELL & WEST, 1976; CEREPANOV, 1995; POPESCU & SANDA, 1998; CIOCĂRLAN, 2009]. General Map of Bessarabia was taken from: *Derev'ja i kustarniki Moldavii* [ANDREEV, 1957].

Results and discussions

After the deep consulting of the literature and thorough analysis of the herborized plants' collections of *Pilosella* the taxonomic composition, the biomorfological, ecological and corological features of the species, synonymy and detailed morphological description have been determined.

Genus *PILOSELLA* Hill

1756, Brit. Herb.: 441; P. D. Sell a. C. West, 1967, *Watsonia*, **6**, 5: 313. – *Hieracium* subgen. *Pilosella* Tausch, 1828, *Flora* (Regensb.), **11**, 1, Erg.-Bl.: 50. – *Hieracium* sect. *Piloselloidea* Koch, 1844, *Syn. Fl. Germ.*, ed. 2, 2: 509

L e c t o t y p e: *P. officinarum* F. Schultz et Sch. Bip.

There are thousands of species widespread in the extratropical regions of the Eurasia (excluding East Asia) and the North Africa [ŠLĀKOV, 1989].

The key to determining species

- 1a** Flowering stem, scapiform (basal rosette leaves only) with an anthodium. Leaves with dense stellate hairs on the underside *P. officinarum* F. Schultz et Sch. Bip.
- 1b** Flowering stem (without basal rosette leaves), with 1-4 cauline leaves and 1-3 bracts. Anthodiums - 2 or more. Leaves without or with few stellate hairs on the underside ... **2**
- 2a** Ligules deep-orange, turning purplish when dry ... *P. aurantiaca* F. Schultz et Sch. Bip.
- 2b** Ligules yellow **3**
- 3a** Anthodiums not more than 2-6 (8) **4**
- 3b** Anthodiums numerous, more than 10 **5**

- 4a Basal rosette glaucous, glabrous or with few eglandular hairs on the margin and median rib. Involucral bracts 5-9 mm *P. lactucella* (Wallr.) P. D. Sell et C. West
- 4b Basal rosette with simple eglandular hairs on both surfaces and with stellate hairs on underside. Involucral bracts 9-12 mm *P. flagellare* (Willd.) Arv.-Touv.
- 5a Stems and leaves glabrous or with rare simple eglandular hairs 6
- 5b Stems and leaves with numerous simple eglandular hairs or glandular 9
- 6a Stolons get out not only from basal rosette but also from the axils of the lower cauline leaves *P. rojowskii* (Rehm.) Schljak
- 6b Stolons get out only from basal rosette 7
- 7a Peduncles with dense stellate hairs *P. praealta* (Vill ex Gochn.) F. Schultz et Sch. Bip.
- 7b Peduncles without or with few stellate hairs 8
- 8a Involucral bracts and peduncles without or with few glandular hairs, simple hairs dispersed *P. piloselloides* (Vill.) Sojak
- 8b Involucral bracts and peduncles with glandular hairs, from dispersed till dense, without or with occasional simple hairs *P. glaucescens* (Bess.) Sojak
- 9a Plants with dense, simple rigid hairs, the cauline hairs appressed-ascending *P. echioides* (Lumn.) F. Schultz et Sch. Bip.
- 9b Plants with rigid hairs, very rare, the cauline patent 10
- 10a Stolons long *P. caespitosa* (Dumort.) P. D. Sell et C. West
- 10b Stolons absent or short 11
- 11a Cauline leaves or bracteant *P. cymosa* (L.) F. Schultz et Sch. Bip.
- 11b Cauline leaves or bracteant 5-20 *P. echioides* (Lumn.) F. Schultz et Sch. Bip.

S e c t i o n 1. **Echinina** (Naeg. et Peter) Schljak. comb. nova. – *Hieracium* sect. *Piloselloidea* subsect. *Echinina* Naeg. et Peter, 1885, Hier. Mitt.-Eur. 1: 117.

Stems and leaves hard-bristled, few or numerous cauline leaves. Basal leaves during flowering usually dry, rarely partially preserved. Ground stolons decumbent absent. Involucral bracts light green, abundant stellate hairy.

1. *P. echioides* (Lumn.) F. Schultz et Sch. Bip. 1862, Flora (Regensb.), 45: 431; Шляков, 1989, Фл. евр. части СССР, 8: 329. – *Hieracium echioides* Lumn. 1791, Fl. Poson. 1: 348; Юксип, 1960, Фл. СССР, 30: 418; Гейдеман, 1986, Опред. высш. раст. МССР: 582; Доброч., Котов, Прокуд., 1999, Опред. высш. раст. Укр.: 380; Negru, 2007, Det. pl. fl. R. Mold.: 270; Ciocârlan, 2009, Fl. Ilus. Rom.: 870. – *H. echioides* subsp. *echioides*; Zahn, 1923, in Engl. Pflanzenreich, 82: 1368; P. D. Sell et C. West, 1976, Fl. Europ. 4: 375, s. restr.

Hemicryptophyte, grows on dry herbaceous places, sunny hills, rocky coasts, sands, steppes. Eurasian element; xeromesophyllous, mesothermal, low acid-neutrophilous. Sporadically in Chilia, South Bugeac, North Bugeac, Gârneț, Codrii, Rezina, Râșcani and Briceni geobotanical districts. The species areal includes the Central Europe (the East), the East Europe (excluding the North), the Caucasus, Mediterranean region, Asia, Mongolia, Iran.

S e c t i o n 2. **Praealtina** (Gremli) Schljak. comb. nova. – *Hieracium* subgen. *Pilosella* grex *Praealtina* Gremli, 1878, Excurs.-Fl. Schweiz, ed. 5: 330 (sine dignitate). – *Hieracium* subgen. *Piloselloidea* sect. *Praealtina* (Gremli) G. Schneid. 1891, in Sagor. u. G. Schneid. Fl. Centralkarp. 2: 295; Zahn, 1923, in Engl. Pflanzenreich, 82: 1391.

Compact stem, without cavities, with solitary or rare setaceous hairs, till dispersed, the upper part slightly stellate-tomentose. Leaves blue-green, rough, usually,

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glabrous, only few thorny thurst on the margins, sometimes with stellate hairs along the midrib. Inflorescence corymbose, rarely umbellate, consisting of small anthodium. Involucral bracts lax adherent. Ligules yellow, without red stripes on outer part. Stigmas yellow.

2. *P. praealta* (Vill. ex Gochn.) F. Schultz et Sch. Bip. 1862, Flora (Regensb.), 45: 429; Шляков, 1989, Фл. евр. части СССР, 8: 332. – *Hieracium praealtum* Vill. ex Gochn. 1808, Tent. Pl. Cichor.: 17; Юксих, 1960, Фл. СССР, 30: 441. – *H. florentinum* All. subsp. *praealtum* (Vill. ex Gochn.) Zahn, 1923, in Engl. Pflanzenreich, 82: 1402. – *H. praealtum* subsp. *praealtum*; P. D. Sell a C. West, 1976, Fl. Europ. 4: 371, s. restr.

Hemicryptophyte identified on sunny meadows, glades and forest edges, steppe slopes (dry), field margins. European element (Mediterranean); xeromesophyllous, mesothermal, low acid-neutrophilous. Sporadically in the North Bugeac, Gârnet, Codrii, Bălți, Rezina, Râșcani, Briceni and Hotin geobotanical districts. The species areal includes the East and Central Europe, the Mediterranean region.

3. *P. piloselloides* (Vill.) Sojak, 1971, Preslia (Praha), 43, 2: 185. – *P. aggr. piloselloides* (Vill.) Sojak, Шляков, 1989, Фл. евр. части СССР, 8: 334. – *H. piloselloides* Vill. 1779, Prosp. Hist. Pl. Dauph.: 34; Гейдеман, 1986, Опред. высш. раст. МССР: 584; Доброч., Котов, Прокуд., 1999, Опред. высш. раст. Укр.: 380; Negru, 2007, Det. pl. fl. R. Mold.: 271; Ciocârlan, 2009, Fl. Ilus. Rom.: 870. – *H. florentinum* All. grex *florentinum* Naeg. et Peter, 1885, Hier. Mitt.-Eur. 1: 554, s. 1.; Zahn, 1923, in Engl. Pflanzenreich, 82: 1409. – *H. piloselloides* subsp. *piloselloides*; P. D. Sell a C. West, 1976, Fl. Europ. 4: 371.

Hemicryptophyte, in herbaceous and rocky places met. European element (Mediterranean); xeromesophyllous, mesothermal, low acid-neutrophilous. Sporadically in Gârnet, Codrii, Rezina, Bălți and Briceni geobotanical districts. The species areal includes the East, South and Central Europe, the Caucasus.

4. *P. rojowskii* (Rehm.) Schljak. comb. nova; Шляков, 1989, Фл. евр. части СССР, 8: 334. – *Hieracium magyaticum* Naeg. et Peter subsp. *rojowskii* Rehm. 1897, Verh. Zool. Bot. Ges. Wien, 47: 288. – *H. bauhinii* Bess. subsp. *rojowskii* (Rehm.) Zahn, 1923, in Engl. Pflanzenreich, 82:1417. – *H. praealtum* Vill. ex Gochnat subsp. *bauhinii* (Bess.) Petunnikov, Sell & West, 1976, Fl. Europ. v. 4, p. 372. – *H. rojowskii* (Rehm.) Юксих, 1960, Фл. СССР, 30: 452, s. restr. (sine auct. comb.). – *H. rojowskii* Rehm. Доброч., Котов, Прокуд., 1999, Опред. высш. раст. Укр.: 381.

Hemicryptophyte, grows on dry and herbaceous slopes, in rural areas, on wayside. Continental Eurasian element; xerophyllous, mesothermal, acid-neutrophilous. Rare taxa, collected from Codrii and Gârnet geobotanical districts (Fig. 1). The species areal includes the Caucasus, the East and Central Europe, the Mediterranean region.

5. *P. glaucescens* (Bess.) Sojak, 1971, Preslia (Praha), 43, 2: 185, s. restr.; Шляков, 1989, Фл. евр. части СССР, 8: 341. – *Hieracium glaucescens* Bess. 1809, Prim. Fl. Galic. 2: 149; Юксих, 1960, Фл. СССР, 30: 462; Гейдеман, 1986, Опред. высш. раст. МССР : 584; Доброч., Котов, Прокуд., 1999, Опред. высш. раст. Укр.: 381; Negru, 2007, Det. pl. fl. R. Mold.: 271. – *H. magyaticum* subsp. *magyaticum*; Naeg. et Peter, 1885, Hier. Mitt.-Eur. 1: 576. *H. bauhinii* Bess. subsp. *magyaticum* (Naeg. et Peter) Zahn, 1923, in Engl. Pflanzenreich, 82:1421. – *H. praealtum* Vill. ex Gochnat subsp. *thaumasium* (Peter) P. D. Sell, 1976, Fl. Europ. 4: 372.

Hemicryptophyte identified on meadows, dry slopes, forest edge, the edge of fields and roads. European (mediterranean) element; xerophyllous, mesothermal, acid-

neutrophilous. Rare spread in the Codrii district. Collected only from two localities of the Hâncești district: Sărata Galbenă and Bozieni villages (Fig. 1). The species areal includes the East and the Central Europe, the Mediterranean region (East), the Minor Asia.

S e c t i o n 3. Cymosina (Naeg. et Peter) Schljak. comb. nova. – *Hieracium* sect. *Piloselloidea* subsect. *Cymosina* Naeg. et Peter, 1885, Hier. Mitt.-Eur. 1: 116, 398. – *Hieracium* subgen. *Piloselloidea* sect. *Cymosina* G. Schneid. 1891, in Sagor. u. G. Schneid. Fl. Centralkarp. 2: 292; Zahn, 1923, in Engl. Pflanzenreich, 82: 1149, 1305 (cum auct. Naeg. et Peter, 1885).

Stem with numerous bristles, on lower part often upward, abundantly stellate pubescent. Leaves with simple hairs on both surfaces, with stellate hairs on both parts or only beneath. Cauline leaves 2-4 (7), with glandular hairs often covered on the top. Inflorescences umbellate or corymbose. Anthodiums numerous; outer involucral bracts appressed, with simple hairs and often with glandular hairs. Yellow flowers. Stigmas yellow. Supraterraneous stolons slender.

6. *P. cymosa* (L.) F. Schultz et Sch. Bip. 1862, Flora (Regensb.), 45: 429; Шляков, 1989, Фл. евр. части СССР, 8: 344. – *Hieracium cymosum* L. 1763, Sp. Pl., ed. 2: 1126, p. p.; Юксих, 1960, Фл. СССР, 30: 549, p. p.; Гейдеман, 1986, Определ. высш. раст. МССР: 584; Доброч., Котов, Прокуд., 1999, Определ. высш. раст. Укр.: 384; Negru, 2007, Det. pl. fl. R. Mold.: 270; Ciocârlan, 2009, Fl. Ilus. Rom.: 870. – *H. cymosum* subsp. *cymosum* Naeg. et Peter, 1885, Hier. Mitt.-Eur. 1: 401; Zahn, 1923, in Engl. Pflanzenreich, 82: 1309; P. D. Sell a C. West, 1976, Fl. Europ. 4: 372, s. restr.

Hemicryptophyte, vegetates on meadows, steppes slopes. Eurasian element; xeromesophyllous, mesothermal, low acid-neutrophilous. Found sporadically in the North Bugeac, Gârnet, Codrii, Bălți, Rezina, Râșcani, Briceni and Hotin districts. The species areal includes Scandinavia (South), the East and the Central Europe, the Mediterranean region (East).

S e c t i o n 4. Pratensina (Aschers. et Graebn.) Zahn, 1923, in Engl. Pflanzenreich, 82: 1148-1149, 1239 (cum auct. Aschers.). – *Hieracium* sect. *Piloselloidea* subsect. *Collinina* Naeg. et Peter, 1885, Hier. Mitt.-Eur. 1: 116.

Fistulose stem, (1) 2-3 (4) leaves, with distanced simple hairs, horizontally or patent, disperse stellate-tomentose. Leaves soft, thin, green or yellowish green, sometimes glaucescent, upper surface with few or without stellate hairs on the ribs, with simple hairs on the both surfaces. Peduncles stellate-tomentose. Involucres disperse stellate-tomentose, (5) 7-9 mm length, outer involucral bracts lax adherent. Stigmas dark, sometimes the same color as the ligules. Ground and underground stolons present.

7. *P. caespitosa* (Dumort.) P. D. Sell et C. West, 1967, Watsonia, 6, 5: 314, s. restr.; Шляков, 1989, Фл. евр. части СССР, 8: 349. – *H. caespitosum* Dumort. 1827, Fl. Belg.: 27; P. D. Sell a C. West, 1976, Fl. Europ. 4: 373; Гейдеман, 1986, Определ. высш. раст. МССР: 584; Negru, 2007, Det. pl. fl. R. Mold.: 270; Ciocârlan, 2009, Fl. Ilus. Rom.: 870. – *H. pratense* Tausch, Юксих, 1960, Фл. СССР, 30: 596. – *H. pratense* Tausch subsp. *pratense*; Zahn, 1923, in Engl. Pflanzenreich, 82: 1269.

Hemicryptophyte identified on the meadows and forest edges. Eurasian element; mesophyllous, mesothermal, acid-neutrophilous. Rare species in the Codrii, Bălți, Briceni and Hotin geobotanical districts (Fig. 1). The species areal includes West Siberia, New Zealand, the Caucasus (revealed by V. Nicolaev (1989) after one specimen) [TUPICINA, 2004].

8. *P. aurantiaca* (L.) F. Schultz et Sch. Bip. 1862, Flora (Regensb.), 45: 426; Шляков, 1989, Фл. евр. части СССР, 8: 351. – *Hieracium aurantiacum* L. 1753, Sp. Pl.: 801; Юксип, 1960, Фл. СССР, 30: 653, р. max. p.; Гейдеман, 1986, Опред. высш. раст. МССР: 584; Доброч., Котов, Прокуд., 1999, Опред. высш. раст. Укр.: 385; Negru, 2007, Det. pl. fl. R. Mold.: 270; Ciocârlan, 2009, Fl. Ilus. Rom.: 869. – *H. aurantiacum* subsp. *aurantiacum*; Zahn, 1923, in Engl. Pflanzenreich, 82 :1242; P. D. Sell a C. West, 1976, Fl. Europ. 4: 374, s. restr.

Hemicryptophyte, vegetates in the stand glades of oak with birch and oak with cherry. Eurasian element; mesophyllous, microthermal, low acid-neutrophilous. Rare taxa, spread only in the Briceni district (Fig. 1). The species areal includes the North and Central Europe, Balkans; adventive in North America.

Section 5. *Auriculina* (Fries) Schljak. comb. nova. – *Hieracium* subgen. *Pilosella* II. *Auriculina* Fries, 1862, Uppsala Univ. Årsskr. (Mat.-Nat. – Epicr. Gen. Hier.): 18. – *Hieracium* subgen. *Piloselloidea* sect. *Auriculina* (Fries) G. Schneid. 1891, in Sagor. u. G. Schneid. Fl. Centralkarp. 2: 284.

Stem 25 (50) cm, slender, usually with 1 leaf, with slender decumbent, glabrous or glabrescent stolons at the base, with distanced leaflets. Leaves glaucous, spatulate to linear-lanceolate, with few or without stellate hairs beneath, on the midrib. Inflorescence (1) 2-6 (8) anthodiums. Involucres 6-8 (9) cm; involucral bracts green or blackish, usually whitish marginate. Ligules yellow, without red stripes. Stigmas yellow.

Pilosella lactucela (Wallr.) P. D. Sell et C. West

9. *P. lactucela* (Wallr.) P. D. Sell et C. West, 1967, Watsonia, 6, 5: 314; Шляков, 1989, Фл. евр. части СССР, 8: 355. – *Hieracium lactucella* Wallr. 1822, Sched. Crit. 1: 408; P. D. Sell a C. West, 1976, Fl. Europ. 4: 369, s. restr.; Ciocârlan, 2009, Fl. Ilus. Rom.: 869. – *H. auricula* auct. non. L.: Lam. et DC. 1805, Fl. Fr., ed. 3, 4: 24; Юксип, 1960, Фл. СССР, 30: 670. – *H. auricula* subsp. *auricula* auct.: Naeg. et Peter, 1885, Hier. Mitt.-Eur. 1: 189; Zahn, 1923, in Engl. Pflanzenreich, 82: 1198.

Hemicryptophyte, grows on the meadows. European element; mesophyllous, amphotolerant, acid-neutrophilous. Rare species, registered in Briceni, Codrii and South Bugeac geobotanical districts (Fig. 1). The species areal includes Scandinavia (South), the East and South Europe, Atlantic Europe (East) and Mediterranean region (East).

Pilosella flagellare (Willd.) Arv.-Touv.

10. *P. flagellare* (Willd.) Arv.-Touv. 1873, Monogr. Pilos. Hier. Dauph.: 13. – *P. flagellaris* (Willd.) Arv.-Touv., Шляков, 1989, Фл. евр. части СССР, 8: 375. – *Hieracium flagellare* Willd., Zahn, 1923, in Engl. Pflanzenreich, 82: 1278, pro sp. coll. (= *H. pratense-pilosella*); Юксип, 1960, Фл. СССР, 30: 643; P. D. Sell a C. West, 1976, Fl. Europ. 4: 369, pro sp. coll. propr.; Ciocârlan, 2009, Fl. Ilus. Rom.: 869. – *H. flagellare* (Willd.) Naeg. et Peter, Доброч., Котов, Прокуд., 1999, Опред. высш. раст. Укр.: 385. – *H. flagellare* subsp. *petunnikovii* Peter, 1893, Nachr. Ges. Wiss. Götting. 2: 74. – *H. petunnikovii* (Zahn.) Юксип, 1960, Фл. СССР, 30: 638 (sine auct. comb.).

Hemicryptophyte, identified on meadows, forest edge and glades, dry slopes, sands. European element; xeromesophyllous, mesothermal, acid-neutrophilous. Rare spread in Briceni, Codrii and Râșcani districts (Fig. 1). The species areal includes Scandinavia (Finland), the East and the Central Europe.

Section 6. *Pilosella*. *Hieracium* subgen. *Pilosella* I. *Pilosellina* Fries, 1862, Uppsala Univ. Årsskr. (Mat.-Nat. – Epicr. Gen. Hier.): 10.

Steam scapiform with an anthodium. All leaves basal, with densely stellate hairs on the underside, sometimes on the top. Involucres with densely stellate hairs. Ligules yellow, those marginal with red stripes on outer face.

11. *P. officinarum* F. Schultz et Sch. Bip. 1862, Flora (Regensb.), 45: 421.; Шляков, 1989, Фл. евр. части СССР, 8: 358. – *Hieracium pilosella* L. 1753, Sp. Pl.: 800, p. p.; Zahn, 1923, in Engl. Pflanzenreich, 82: 1158; Юксип, 1960, Фл. СССР, 30: 692; P. D. Sell a C. West, 1976, Fl. Europ. 4: 368; Гейдеман, 1986, Определ. высш. раст. МССР: 582; Доброч., Котов, Прокуд., 1999, Определ. высш. раст. Укр.: 379; Negru, 2007, Det. pl. fl. R. Mold.: 270; Ciocârlan, 2009, Fl. Ilus. Rom.: 867. – *Pilosella communis* Arv.-Touv. 1873, Monogr. Hier. Pilos.: 13.

Hemicryptophyte, grows on herbaceous and sunny places. European element (Mediterranean); xeromesophyllous, amphotolerant, euryonic. Commune in Chilia, sporadically in the North Bugeac, Gârnet, Codrii, Bălți, Rezina, Râșcani, Briceni and Hotin districts. The species areal includes Scandinavia, the central and Atlantic Europe (except the North), the Mediterranean region, Minor Asia, Caucasus, adventive in the North America, New Zealand.

Conclusions

- The spontaneous flora of the Bessarabia includes 11 species of the *Pilosella*: *P. officinarum* F. Schultz et Sch. Bip., *P. aurantiaca* F. Schultz et Sch. Bip., *P. praealta* (Vill ex Gochn.) F. Schultz et Sch. Bip., *P. piloselloides* (Vill.) Sojak, *P. glaucescens* (Bess.) Sojak, *P. echioides* (Lumn.) F. Schultz et Sch. Bip., *P. caespitosa* (Dumort.) P. D. Sell et C. West, *P. cymosa* (L.) F. Schultz et Sch. Bip., *P. lactucela* (Wallr.) P. D. Sell et C. West, *P. rojowskii* (Rehm.) Schljak. and *P. flagellare* (Willd.) Arv.-Touv.
- Three new species for the flora in the study have been identified: *P. lactucela* (Wallr.) P. D. Sell et C. West, *P. rojowskii* (Rehm.) Schljak. and *P. flagellare* (Willd.) Arv. Touv.
- Of all highlighted taxa, 6 are rare: *P. aurantiaca* F. Schultz et Sch. Bip., *P. glaucescens* (Bess.) Sojak, *P. caespitosa* (Dumort.) P. D. Sell et C. West, *P. lactucela* (Wallr.) P. D. Sell et C. West, *P. rojowskii* (Rehm.) Schljak. and *P. flagellare* (Willd.) Arv. Touv. Numerous investigations and inventory in field are necessary further to make possible the indication of rare degree and endangered status of the mentioned taxa and to elaborate special measures for their conservation.

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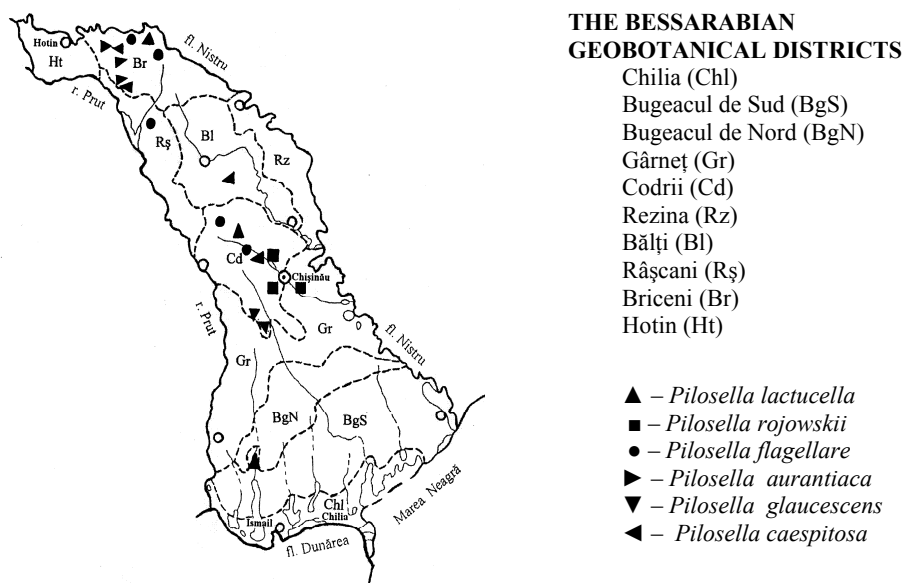


Fig. 1. The spread of rare species of *Pilosella* Hill on the Bessarabia's territory

TAXONOMICAL POSITION AND DISTRIBUTION OF *BUSCHIA LATERIFLORA* (DC.) OVCZ. (RANUNCULACEAE JUSS.) SPECIES IN THE BESSARABIA

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Abstract: Having the target of taxonomic concretization the *Ranunculus* L. and *Buschia* (DC.) Ovcz. genera from Bessarabia flora, the Herbarium specimens of Botanical Garden Academy of Sciences, and Moldova State University were investigated and analyzed. Research results attest the priority concept of *Ranunculus* L. genus and the presence of *Buschia lateriflora* (DC.) Ovcz. species in native flora. Revealing a new habitat for *Buschia lateriflora* species complete the species area within South-East Europe limits. Morphologic distinctive criteria of studied genus are given.

Key words: taxonomical position, distribution, *Buschia lateriflora* (DC.) Ovcz., Bessarabia

Introduction

It is known that from BERNARD DE JUSSIEU (1789) and up to present the *Ranunculaceae* family system is constantly exposed to taxonomic treatments and modifications. For its classification, this family, possessing pronounced and difficult heterobaty, is the subject of a comprehensive study concerning to all methods of modern taxonomy. However, up to date, a perfect system of classification of this family is absent.

According to A. Takhtajan's phylogenetic system [TAKHTAJAN, 1987], the *Ranunculaceae* family is divided into 6 subfamilies: *Coptidoideae* (phylogenetically, the most archaic and primitive), *Thalictroideae* (including *Isopyroideae*), *Anemonoideae*, *Ranunculoideae*, *Delphinioideae*, *Hellebroideae*. The *Ranunculoideae* subfamily, where is included the *Ranunculus* genus (including *Buschia*), numbers 21 genera.

Analyzing the data referring the *Ranunculus* L. genus for the monograph "Flora of Bessarabia", we deviated from the traditional classification system on above-mentioned genus, following the N. Tsvelev's opinion [TSVELEV, 2001] in assessing the systematic value of taxa at the level of genus. We are considering really and more adequate such treatment of the volume "in *sensu stricto*" and the delimitation within the *Ranunculoideae* subfamily of the *Ranunculus* L., *Ficaria* Guett., *Batrachium* (DC.) S.F. Gray, *Ceratocephala* Moench, *Buschia* Ovcz. genera, earlier and at present recognized by us, confirmed in the limits of the Bessarabian territory.

Material and methods

As biological material for investigations the Herbarium of Botanical Garden (Institute) and, Moldova State University exsiccates were served. The basic methodical recommendations [KOROVINA, 1986] were used, for performing the expedition and cameral studies. The taxa nomenclature at the level of family, genus and species was taken

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from the fundamental published works by [CEREPANOV, 1995; TUTIN & al. 1993-2006] and, the bioecological peculiarities of species are exposed [POPESCU & SANDA, 1998].

Results and discussions

The formal system of *Ranunculus* L. s. l. genus includes a great biodiversity of species (over 600) that distinguish themselves both by distinctive morphologic criteria and, geographic localization. The morphologic non-homogeneity of species of given genus induced the systematic investigators' to such attempts of dividing this genus into a number of generic taxa.

According to Ovczinikov's [OVCZINIKOV, 1940] opinion, scientist-taxonomist, Prantl's introduction (1876) in taxonomic composition of *Ranunculus* genus the *R. lateriflorus* DC. and *R. nodiflorus* L. species is considered unjustified. These species, based on comparative analysis of their morpho-structural peculiarities, could not be assigned to any sections of the genus *Ranunculus*. The distinctions being rather evident, the author, analyzing the *Ranunculaceae* family for the USSR Flora, includes these two taxons in the *Micranthus* Ovcz. subgenus (OVCZINIKOV, 1937). Later on, OVCZINIKOV (1940) combines both species into *Buschia* Ovcz. new genus.

Tab. 1. Morphological distinctive criteria of the *Ranunculus* and *Buschia* genera.

Genus/Criterion	<i>Buschia</i>	<i>Ranunculus</i>
Calyx structure	Perigon (petaloid, deciduous)	Perianth (bicalyculate)
Flower position	Sessile, axillary in the dichotomies stem	Flowers are not axillary, sessile
Honey-leaves	Reduced, elongated, spoon-shaped	Honey-leaves of another type (sessile)
Stem branched	Dichotomous – dichasial	Cauline ramification of another type
Differentiation of the inflorescence from the vegetative part	Lack of differentiation in the upper part to separate from the vegetative part of the inflorescence	Presence of inflorescence and of vegetative part

From the ecological point of view *Buschia* genus prefers the humid and swampy habitats (biotopes), sometimes halophilous; it is also identified in water surfaces forming a number of ecological modifications. The terrestrial forms are small (4-13 cm), branched from the base with short internodes; the aquatic forms (*Ranunculus lateriflorus* form *natans* Gluck) are high, achieving to 15-20 cm, subramose, with long internodes and narrow leaves. Among these forms there are crossing varieties.

The investigation of exsiccates, existent in the Herbarium of the Botanical Garden Academy of Sciences of Moldova concerning the *Ranunculus* genus permitted the revealing of an exsiccate, collected by Zelenetskij N. and identified initially as *Ranunculus nodiflorus* L. (the South of Bessarabia, Tatarbunar, on the alkaline soils) [ZELENETSKIJ, 1891]. Later on, V. Lipskij in 1892 reviewing Zelenetskij's herbarium, collected from the South of Bessarabia, determined this sample as *R. lateriflorus* DC. We confirm the correctness of this species identification. With the purpose of discovering the new places of growth of the

species *Buschia lateriflora* (DC) Ovcz. within Bessarabia's limits, new additional expedition studies are necessary.

Genus *Buschia* Ovcz.

1940, Bot. journal. 25, 4-5: 339. - *Ranunculus* L. subgen. *Micranthus* Ovcz. 1937, USSR Flora, 7: 474

Annual herbs, erect or ascending. Stems dichotomous branched. Basal alternately-leaved, complete, ovate-oblong, long-petiolate, those superior are opposed, short-petiolate, oblong-lanceolate, rarely dentate. Very small flowers, achieves to 2,0-3,0 mm in diameter, those superior are 2-3, sessile, disposed in the axil of the branches, with an opposite bracteate's foliole. Perigon petal-shaped; tepals 5(4), yellow, partially bi-symphetalous, deciduous. Honey-leaves 3-5, membranous, candicant, spoon-shaped, sometimes very reduced, nectariferous foveole at the base, covered with a semicircular squama (rudimentary leaf), free in the superior part. Stamens 4-7, anthers small, orbiculated. Apocarpous gynoecium, numbers 3-10 uniovulate carpels. Receptacle glabrous. Relative numerous fruit (6-25) dispose in globulous cephalodium, granular-tuberculated, beak slightly dilated at the base, a little elongated (*B. lateriflora*) or short (*B. nodiflora*).

Typus generis: *Buschia lateriflora* (DC.) Ovcz. (*Ranunculus lateriflorus* DC.).

Species type of the genus in the native flora of Bessarabia is identified.

The genus includes 2-4 species, sporadic spread in the South-East Europe, in Mediterranean region (including, Northern Africa), Caucasus, Crimea, the inferior stream of Volga river, the Northern part of the Middle Asia.

***Buschia lateriflora* (DC.) Ovcz.** 1940, Бот. журн. 25, 4-5: 339; Доброч. и др. 1999, Опред. высш. раст. Украины: 52; Цвелев, 2001, Фл. Вост. Европы, X: 159. – *Ranunculus lateriflorus* DC. 1817, Reg. Veg. Syst. Nat. 1: 251; Овч. 1937, Фл. СССР, 7: 474; Tutin, 1964, Fl. Europ. 1: 236; Tutin et Akeroyd, 1993, Fl. Europ., ed. 2, 1: 284. Ciocârlan, 2009, Fl. Ilustr. a Rom.: 169. – Fig. 1.

Plants are of 4-15 (25) cm, glabrous. Stems dichotomic ramified. Basal leaves ovate or oval elliptical, complete or dentate, long-petiolate, lamina of 12-20 (25) x 5-8 (10) mm. Leaves caulinary elongated-lanceolate, rarely dentate, and short-petiolate. Flowers of 2.4-3.0 mm in diameter, sessile or subsessile, axillary in the dichotomies of the stem, in the superior part 2-3. Sepals 5, petal-shaped, membranous, elongated-elliptical, yellowish, deciduous. Honey-leaves 3, rarely 2, reduced, elongated spoon-shaped. Androecium of 4-5 stamens, anthers suborbicular. Receptacle conic, glabrous. Achenes achieve 2.8-3.3 mm, ovate-pear-shaped, brown, granular-tuberculated on the margins, beak of 1.0-1.3 mm. 2n=16.

Annual terofite, blooming in April-May, vegetates on sandy-place, humid, alkaline soil, river meadows temporary flooded.

Element eurasiatic (mediterranean), mesophyte (hygrophyte) species, halophilous, mesotherm, prefers soils with neutral-alkaline reaction. The species area covers continental Eurasia and North Africa. In the flora of Bessarabia it vegetates in the southern part of the territory (the steppe by *Stipa* L. with *Festuca* L. districts, on the alkaline soils, southern Budgeac).

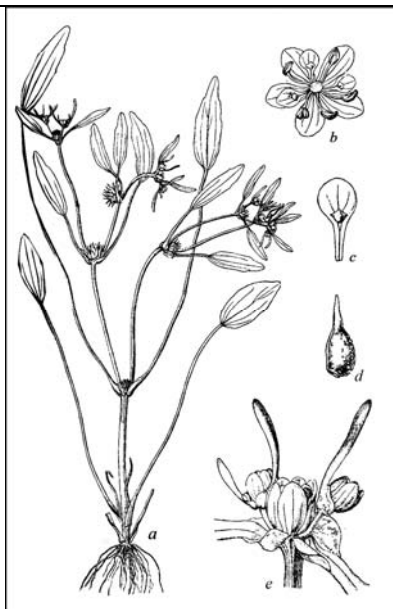


Fig. 1. *Buschia lateriflora* (DC) Ovcz. (after P. N. Ovczinikov).
a – general view; b – flower; c – nectary; d – achene; e – apical part of flowering shoots.

Conclusions

The study and the analysis of the herbarized material confirm the presence of the *Buschia lateriflora* (DC) Ovcz. species in the Bessarabia's flora.

The discovery of this habitat *Buschia lateriflora* completes the species area in the South-East Europe limits.

Buschia lateriflora represents a native floristic element (not adventive), spread rarely in the Bessarabia's limits and requires further research.

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NEW CONTRIBUTION TO THE STUDY OF ALIEN FLORA IN ROMANIA

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Abstract: In this paper, a number of seventeen alien plant species are presented, one of them being now for the first time reported in Romania (*Sedum sarmentosum* Bunge). Some species are mentioned for the first time in the flora of Moldavia (*Aster novae-angliae* L., *Cenchrus incertus* M. A. Curtis, *Chenopodium pumilio* R. Br., *Fraxinus americana* L., *Lindernia dubia* (L.) Pennell, *Petunia* × *atkinsiana* D. Don, *Solidago gigantea* Aiton, *Tagetes erecta* L.) or Transylvania (*Kochia sieversiana* (Pallas) C. A. Mey.), and some are reported from new localities (seven species). For each species, there are presented general data on the geographical origin, its distribution in Europe and worldwide, as well as its invasion history and current distribution in Romania. Some of these species manifest a remarkable spreading tendency, expanding their invasion area in Romania. Voucher specimens were deposited in the Herbarium of University of Agricultural Sciences and Veterinary Medicine Iași (IASI).

Keywords: alien plants, flora, new records, Romania

Introduction

According to ANASTASIU & NEGREAN (2005), the alien flora of Romania includes 435 species, of which 88.3% are neophytes and 11.7% are archaeophytes. Therefore, species of alien origin currently represent ca 13% of the total flora of the country, which was estimated by CIOCĂRLAN (2009) to 3335 species. In the last years there is a continuous enrichment of Romania's flora with new alien plant species [ANASTASIU & NEGREAN, 2008; OPREA & SÎRBU, 2010; SÎRBU & OPREA, 2011].

Some of these alien species can become invasive, threatening natural and agricultural ecosystems, causing damages to the economy and human health [PIMENTEL & al. 2000; McNEELY, 2001; WITTENBERG & COCK, 2001]. All signatories to the *Convention on Biological Diversity*, including Romania, are obliged to prevent the introduction of, control, or eradicate those alien species which threaten ecosystems, habitats or species [WITTENBERG, 2005]. Reporting those newly alien species arrived in Romania's flora, and monitoring the spreading of those previously reported, can be useful tools in establishing measures in order to eradicate them before become invasive and harmful.

In the present paper we report a new alien species for the flora of Romania, as well as other new or rare alien species for the flora of Moldavia and Transylvania, some of which have an invasive character.

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Materials and methods

All the species in this paper were recorded during our recent field works on alien plants, in the historical provinces Moldavia and Transylvania (Romania). The geographic coordinates were recorded using eTrex Legend HCx GPS system. Voucher specimens were deposited in the Herbarium of University of Agricultural Sciences and Veterinary Medicine Iași (IASI). Morphological characters of species were analyzed on the specimens collected from the field and compared with the data from relevant literature sources [TUTIN & al. 1964-1980, 1993; KUNJUN & OHBA, 2001; CIOCĂRLAN, 2009; OHBA, 2009]. The taxonomy and nomenclature of species follow *Flora Europaea* [TUTIN & al. 1964-1980, 1993], except *Sedum sarmentosum* Bunge [KUNJUN & OHBA, 2001; OHBA, 2009]. Terminology and definitions recommended by RICHARDSON & al. (2000) and PYŠEK & al. (2004) were used for the status of alien plants.

Results & discussions

During our recent field investigations, focused on alien plants in Moldavia and Transylvania (2010), we recorded a new alien plant species for the flora of Romania (*Sedum sarmentosum* Bunge), several new alien species for the flora of Moldavia (e. g. *Aster novae-angliae* L., *Cenchrus incertus* M. A. Curtis, *Chenopodium pumilio* R. Br., *Fraxinus americana* L., *Lindernia dubia* (L.) Pennell, *Petunia* × *atkinsiana* D. Don, *Solidago gigantea* Aiton, *Tagetes erecta* L.) or Transylvania (e. g. *Kochia sieversiana* (Pallas) C. A. Mey.), and other species identified in new localities, some of them with an invasive character (e. g. *Brachyactis ciliata* (Ledeb.) Ledeb., *Eleusine indica* (L.) Gaertn., *Euphorbia dentata* Michx., *Grindelia squarrosa* (Pursh) Dunal., *Impatiens parviflora* DC., *Sicyos angulatus* L.).

a) New records in the alien flora of Romania***Sedum sarmentosum* Bunge**

A species native to Eastern Asia (China) [KUNJUN & OHBA, 2001], cultivated for ornamental purposes and naturalized in Japan [MÜLLER & OKUDA, 1998], North America [OHBA, 2009], as well as in numerous European countries, such as: Spain [CASTROVIEJO, 1995], Montenegro [STEŠEVIĆ & al. 2008], Slovenia [JOGAN & al. 1995], Czech Republic [PYŠEK & al. 2002], Switzerland [WITTEMBERG, 2005], Belgium [VERLOOVE, 2006], Hungary (casual) [BALOGH & al. 2004], Croatia [ŠEGULJA & REGULA BEVILACQUA, 1994], Austria [FISCHER & al. 2008], Germany, Italy and Slovakia [MARHOLD, 2011].

In Romania, this species was recently found in Mediaș town (Sibiu county), on a platform of a concrete channel, along the Henri Coandă street (46°09'59.32"N; 24°21'37.50"E; leg. Eliáš P. jun., Oprea A., Sîrbu C., Ferus P., 2011 August 18) (Fig. 1). On that place, this species grow abundantly, forming mono-specific and compact clumps, presumably by vegetative reproduction.

We do not know the introduction date of this species into Romania. None of the floristic papers in Romania, either older [BAUMGARTEN, 1816; SCHUR, 1866; FUSS, 1866; SIMONKAI, 1886; KANITZ, 1879-1881; BRÂNDZĂ, 1879-1883; GRECESCU,

1898; PRODAN, 1939; BORZA, 1947], or more recent [RĂVĂRUȚ, in SĂVULESCU, 1956; BELDIE, 1977; OPREA, 2005; CIOCĂRLAN, 2009] does not mention it. Its morphological characters, based on the study of herbarium specimens, in agreement with the data from relevant literature sources [KUNJUN & OHBA, 2001; OHBA, 2009], are presented below.

S. sarmentosum is a perennial herb, glabrous, with stems creeping and ascending, branched, rooting at nodes, 10-25 cm; leaves 3-verticillate, sessile; blade pale yellowish-green, 10-25 × 4-6 mm, base abruptly narrowed, spurred, apex subacute; cyme corymbiform, bracts similar to leaves, smaller; flowers ± sessile, 5-merous; sepals lanceolate to oblong, 3.5-5 mm, green, apex acute to obtuse; petals yellow, lanceolate to oblong, 5-8 mm, apex long-mucronate; stamens 10, shorter than petals; carpels 5, distinct, oblong, 5-6 mm. Fruit polyfollicle.

S. sarmentosum is a polyploid species ($2n = ca\ 72$) [OHBA, 2009], blooming in May-June and fruiting in August [KUNJUN & OHBA, 2001; FISCHER & al. 2008]. The actively clonal reproduction allows it to maintain populations even when no seeds are formed (Croatia) [ŠEGULJA & REGULA BEVILACQUA, 1994]. Into the natural range, this species prefers rocky and shaded fields, up to the altitude of 1600 m [KUNJUN & OHBA, 2001]. In North America it is reported on dry rocks, between 0 and 500 m altitudes [OHBA, 2009]. In Croatia, it grows on sandy or rocky anthropogenic grounds and on old walls, where it can form almost pure stands [ŠEGULJA & REGULA BEVILACQUA, 1994]. In addition to its use as an ornamental plant, *S. sarmentosum* is indicated in folk medicine, e.g. in chronic viral hepatitis [HE & al. 1998], or as a vegetable [KUNJUN & OHBA, 2001].

b) New records in the alien flora of Moldavia

Aster novae-angliae L. (*Symphyotrichum novae-angliae* (L.) G. L. Nesom)

It is a species native to North America [FEHÉR, 2008], introduced in Europe as an ornamental plant, and naturalized in many regions [TAMAMSCHJAN, 1999/1959; YEO, in TUTIN & al. 1976]. In Romania, it is also cultivated in gardens [MORARIU & NYÁRÁDY, in SĂVULESCU, 1964], from where it sometimes escapes and spreads freely: Banat [ARVAT, 1977] and Muntenia [NEGREAN, 1972]. It was also found in Moldavia, in Iași city, on a vacant land, near the railway, ca 500 m, westward of the railway station (47°10'13.16"N; 27°33'36.60"E; leg. Sirbu C., 2010 October 12), where it grows into a phytocoenosis dominated by *Elymus repens*.

Cenchrus incertus M. A. Curtis

Species native to North and Central America [HITCHCOCK, 1950; SZIGETVÁRI, 2008], and naturalized in Southern, Central and Eastern Europe [CLAYTON, in TUTIN & al. 1980; SZIGETVÁRI, 2008; DAISIE, 2011]. In Romania it was relatively recent reported [CIOCĂRLAN & al. 1991], along the Black Sea littoral, in Vama Veche and subsequently in other localities from Dobruđja [CIOCĂRLAN, 2000; CIOCĂRLAN & al. 2004; OPREA, 2005]. In Moldavia, a small population of *C. incertus* has been identified at Galați railway station (45°26'38.09"N; 28°03'41.94"E; leg. Sirbu C., Oprea A., Eliăș P. jun., Ferus P., 2011 August 20). It seems to be now a species on the way of spreading in Romania.

Chenopodium pumilio R. Br.

This is a species originating in tropical regions, unintentionally introduced in Europe by importing wool from Australia [AELLEN, 1979, cited by CHYTRY, 1993]. In Romania, it was first mentioned by CHYTRY (1993) (leg. 1989) and COSTEA (1994), from the Danube Delta, on sandy river banks influenced by the human activities. In other areas it also grows on ruderal grounds, railway stations, and river banks [CHYTRY, 1993]. According to CHYTRY (1993), due to high capacity for dissemination and long viability of seeds, the species is expected to further spread in South-Eastern Europe. Indeed, we recently found it in other localities from the Danube Delta (e. g. Crişan, Sulina, Maliuc and Caraorman), but also in Southern part of Moldavia, on the left bank of the Danube river, in Galaţi town (between 45°25'06.11"N; 28°02'07.82"E and 45°26'11.75"N; 28°04'43.43"E; leg. Sîrbu C., Oprea A.; 2011 August 02), and at Cotul Pisicii (45°25'10.27"N; 28°11'17.09"E; leg. Sîrbu C., Oprea A., Eliáš P. jun., Ferus P.; 2011 August 20).

Fraxinus americana L.

This is one of the most common ash species in North America [GRIFFITH, 1991], introduced in Europe at 1724 [CSISZÁR & BARTHA, 2008], and now occasionally reported as sub-spontaneous tree in France, Bulgaria, Hungary and Lithuania [DAISIE, 2011]. In Romania it is cultivated as isolated trees in parks, along the streets and in forest plantations [DUMITRIU-TĂTĂRANU, 1960; MORARIU, in SĂVULESCU, 1961]. As a sub-spontaneous plant, this ash species was previously reported in Dobrudja, at Mamaia (on the Tăbăcărie lakesides) [FĂGĂRAŞ & al. 2008]. We also have identified this species, as sub-spontaneous, in Galaţi county, at Tirighina-Barboşi railway yard (45°24'07.13"N; 27°58'14.96"E; leg. Sîrbu C., Oprea A., 2011 August 02), Şendreni (near the road; 45°25'12.26"N; 27°53'48.45"E; leg. Sîrbu C., Oprea A., 2011 August 02) and Galaţi (near the railway station; 45°26'26.68"N; 28°04'00.85"E; leg. Sîrbu C., Oprea A., Eliáš P. jun., Ferus P., 2011 August 20).

Lindernia dubia (L.) Pennell

Species originating in North and South America, naturalized in a large part of South-Western Europe [WEBB & PHILCOX, IN TUTIN & al. 1972]. In Romania it was previously reported by CIOCĂRLAN & COSTEA (1994), on wet alluvia from the Danube Delta – Sacalin Island, towards the Sfântul Gheorghe distributary channel, and, subsequently, it was also mentioned from Chilia Veche and Periprava [CIOCĂRLAN, 1994, 2009]. We found this species along the Sulina distributary channel, at Mila 28 (West of Maliuc) (45°10'19.63"N; 29°02'56.96"E; leg. Sîrbu C., Oprea A., 2011 August 03), at Crişan (45°10'32.71"N; 29°23'06.52"E; leg. Oprea A., 2011 September 15), as well as in the city of Galaţi, on the left bank of Danube river (45°25'33.22"N; 28°02'55.32"E; leg. Sîrbu C., Oprea A., 2011 August 02).

Petunia × *atkinsiana* D. Don (= *P. axillaris* (Lam.) Britton, Sterns et Pogg. × *P. integrifolia* (Hook.) Schinz & Thell.; *P. hybrida* Vilm.)

Ornamental plant of hybrid origin, which was obtained from crosses between *P. axillaris* and *P. integrifolia*, in the second half of the nineteenth century [GUYOT, 1961]. Occasionally, it was reported as a refugee from the gardens in some countries from Central, Western and Southern Europe [DAISIE, 2011]. In Romania, it was introduced, probably,

towards the end of the nineteenth century, now being widely cultivated as an ornamental plant. It was occasionally reported as a plant escaped from gardens, in several localities from Transylvania [BORZA, 1959] and Dobruđja [HOREANU, 1975; FĂGĂRAȘ & al. 2008]. We also found it on vacant lands or roadsides, in many localities in Southern Moldavia (Galați county): Fârțânești (45°47'06.06"N; 27°58'54.76"E; leg. Sîrbu C., Oprea A., 2011 July 31), Tg. Bujor (45°52'27.05"N; 27°55'33.23"E; leg. Sîrbu C., Oprea A., 2011 July 31), Galați (45°24'46.41"N; 28°01'46.88"E; leg. Sîrbu C., Oprea A., 2011 August 02), Hanu Conachi (45°34'54.15"N; 28°35'42.14"E; leg. Sîrbu C., Oprea A., 2011 August 02), Costache Negri (45°42'02.03"N; 27°43'00.15"E; leg. Sîrbu C., Oprea A., 2011 August 02), Cudalbi (45°46'16.16"N; 27°41'11.46"E; leg. Sîrbu C., Oprea A., 2011 August 02), Pechea (45°37'20.24"N; 27°48'00.82"E; leg. Sîrbu C., Oprea A., 2011 August 02).

Solidago gigantea Aiton (*S. serotina* Aiton; *S. gigantea* subsp. *serotina* (Kuntze) McNeill)

Species native to North America (United States and Canada) [BRITTON & BROWN, 1970], from where it was introduced in Europe, as an ornamental plant, in 1758 (London) [JAKOBS & al. 2004; WITTENBERG, 2005; WEBER & JAKOBS, 2005]. Although the first naturalized populations in Europe were recorded shortly after its introduction, the plant has been spread throughout the continent mainly after the year of 1850 [WEBER & JAKOBS, 2005]. Nowadays, it is widespread in almost all european countries, between 42°N and 63°N [MCNEILL, in TUTIN & al. 1976; WEBER & JAKOBS, 2005]. In Romania, according to MORARIU & NYÁRÁDY, in SĂVULESCU (1964), *S. gigantea* was firstly published by SCHUR (1866), on the river meadows between Avrig and Bradu (Transylvania). This is, however, an erroneous information, because the species indicated by SCHUR (1866) is *S. canadensis*, and not *S. gigantea*. Therefore, probably, the first indication of this species in Romania remains that made by BORBAS (1886), cited by MORARIU & NYÁRÁDY, in SĂVULESCU (1964), which mentioned *S. gigantea* from Lipova (Arad county). In the last century the species has also been mentioned on the Danube river meadows and Danube Delta [PRODAN, 1935-1939, 1939], as well as from Transylvania, Maramureș, Banat and Oltenia [BORZA, 1947; MORARIU & NYÁRÁDY, in SĂVULESCU, 1964; ȘTEFUREAC & al. 1971; ROMAN, 1974; DIHORU & al. 1968-1970]. In Moldavia, it has been previously known only from gardens. As a sub-spontaneous plant, it was recently found in the following localities: Fundu Moldovei (on the left bank of the Moldova river; leg. Sîrbu C., 2006 July 25), between Pojorâta and Sadova (the left bank of the Moldavia river; 47°32'01.79"N; 25°29'32.36"E; leg. Sîrbu C., 2011 September 01) (Suceava county), as well as in Răducăneni (the left bank of the Bohotin river; 46°57'37.32"N; 27°56'34.69"E; leg. Sîrbu C., Oprea A., 2011 September 11) (Iași county). Currently, we can state that in Romania, this species is quite common (invasive) on the river meadows in Transylvania, Crișana, Maramureș, and Banat, but it is still rather rare in the other provinces of the country.

Tagetes erecta L.

Species native to Central America (Mexico), from where it was introduced into Central Europe, in the 1573 [NYÁRÁDY, in SĂVULESCU, 1964; GUYOT, 1961], for ornamental use. Today, it is reported as a casual alien plant from many european countries [GUYOT, 1961; ESSL & RABITSCH, 2002; MOSYAKIN & YAVORSKA, 2002;

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PYŠEK & al. 2002; BALOGH & al. 2004; VERLOOVE, 2006; DAISIE, 2011]. In Romania, it was listed as a garden plant, starting from the 19th century [SZABO, 1841; PORCIUS, 1885; BRÂNDZĂ, 1879-1883; SIMONKAI, 1886; GRECESCU, 1898]. According to DIACONESCU (1961) and ANASTASIU (1994), it is naturalized in Bucharest (in the Botanical Garden). We have found it as a sub-spontaneous plant (escaped from gardens) in Horlești-Rediu, on a ruderal place (leg. Sîrbu C., 2006 September 03) (Iași county), Fundu Moldovei (on the left bank of the Moldova river; leg. Sîrbu C., 2006 July 25) (Suceava county), as well as at Crișan (Danube Delta) (45°10'25.41"N; 29°23'33.67"E; leg. Oprea A., 2011 September 15).

c) New record in the alien flora of Transylvania

Kochia sieversiana (Pallas) C. A. Mey. (*Bassia sieversiana* (Pallas) W. A. Weber; *K. scoparia* var. *sieversiana* Graebn.; *K. densiflora* Turcz. in DC.; *K. scoparia* var. *densiflora* Moq. in DC.).

A species originating in Central Asia and Siberia [ILJIN, 1970/1936], first time reported in Romania, from Muntenia, by CIOCÂRLAN (1991). Subsequently, it was identified in Dobruđja [MITITELU & al. 1992; CIOCÂRLAN, 1994], and Moldavia [OPREA, 1997a, 1998; OPREA & al. 1997; SÎRBU & OPREA, 1998; COROI & COROI A.M., 1999; COROI, 2001; COROI A.M., 2001; OPREA, 2005], but its area of invasion in Romania is certainly wider and in a continuous expansion. It is quite similar to *K. scoparia* (of which it is distinguished by the numerous whitish hairs, located under flowers), reason why, in many cases, it may have been erroneously identified, as *K. scoparia*. Recently it was also found in Transylvania, in Sibiu city, on a ruderal ground, near the railway station (45°47'32.25"N; 24°10'18.90"E; leg. Eliáš P. jun., Ferus P., Oprea A., Sîrbu C., 2011 August 19).

d) Alien species found in new localities

Brachyactis ciliata (Ledeb.) Ledeb. (*Erigeron ciliatus* Ledeb.; *Symphotrichum ciliatum* (Ledeb.) G. L. Nesom)

It is an Asian species [BOTSCHANTZEV, 1999/1959], known as alien plant in Poland [BRÓŚ & PODGÓRSKA, 2005], R. of Moldova [DAISIE, 2011] and Romania. It was found in Eastern Romania (Moldavia), in the year 1967 [VIȚĂLARIU, 1971; POP & VIȚĂLARIU, 1971]. Subsequently, it has spread fairly quickly in this province, as well as in Muntenia and Dobruđja (including the Danube Delta) [OPREA, 2005]. In Transylvania, this plant was previously reported only from Cluj-Napoca [FILIPAȘ & CRISTEA, 2006]. We also found it at Gheorgheni (Harghita county) (on a ruderal ground, near the railway station; 46°43'10.68"N; 25°34'21.71"E; leg. Sîrbu C., Eliáš P. jun., Ferus P., Oprea A., 2011 August 18), as well as in Sibiu (Sibiu county), near the railway station (45°47'14.51"N; 24°10'46.95"E; leg. Sîrbu C., Oprea A., Eliáš P. jun., Ferus P., 2011 August 19).

Eleusine indica (L.) Gaertn.

Species originating in tropical and subtropical Asia [CIOCÂRLAN, 2009] or Africa [HILDEBRAND, 2008], now widespread throughout the world, mainly in regions with warmer climates [HITCHCOCK, 1950; BRITTON & BROWN, 1970; JÜRGENS,

1977; SALIMATH & al. 1995; CLAYTON & al. 2006]. It is reported as a naturalized alien plant in Southern Europe [HANSEN, in TUTIN & al. 1980] and as casual in the central and western regions [LE CLERCH, 1973; HANSEN, in TUTIN & al. 1980]. The first report of this species in Romania (as a sub-spontaneous plant) was from Iași, where it seems to have arrived accidentally (in 1957) with seeds of *Lolium perenne* bought from the market, and used for lawns in the surroundings of the Agronomical Institute [RĂVĂRUȚ & MITITELU, 1960]. Subsequently, *E. indica* has not been confirmed as a sub-spontaneous species in Iași. Instead, it was reported from Crișana (North-Western Romania) [NEGREAN & KARÁCSONYI, 1984], Dobrudja [COSTEA, 1996], as well as from Muntenia [NEGREAN & CONSTANTIN, 1999; OPREA & al. 2004]. Recently it has been found in Galați railway station (45°26'41.69"N; 28°03'40.48"E; leg. Sîrbu C., 2011 July 20), in the second locality in Moldavia. *E. indica* is seen in general as a common and harmful weed of crops in warmer regions of the world [HITCHCOCK, 1950; JÜRGENS, 1977]. In Romania, although it was mentioned only in a few localities so far, the fact that his presence was noted in so distant regions (Maramureș, Moldavia, Dobrudja, Muntenia) may be an alarm signal on its invasive capacity, particularly in that regions with high temperature and light resources.

***Euphorbia dentata* Michx.**

Species of North American origin, naturalized in Ukraine [MOSYAKIN & YAVORSKA, 2002], R. of Moldova [MÎRZA & ȘABANOVA, 1992], Belgium, Italy [DAISIE, 2011], as well as in Eastern Asia [MA & LIU, 2003; LEE & al. 2009]. In Romania, it was previously known from Socola-Iași (including var. *cuphosperma* Engelm.) [OPREA, 1997b] and from Buzău railway stations [SÎRBU, 2005]. To these, we add now two other localities in Southern Moldavia: Tecuci – Southern railway station (45°25'04.23"N; 27°25'23.42"E; leg. Sîrbu & Oprea 2011, 2011 August 01) and Movileni - railway yard (45°24'17.50"N; 27°57'26.80"E; leg. Sîrbu & Oprea, 2011 August 02).

***Grindelia squarrosa* (Pursh) Dunal. (*Donia squarrosa* Pursh)**

Species native to North America [BRITTON & BROWN, 1970], accidentally introduced to Europe in the first half of last century (Ukraine) [TAMAMSCHJAN, 1999/1959; PROTOPOPOVA & al. 2006]. It is now known as an invasive plant in Ukraine and R. of Moldova [MÎRZA & al. 1987; MOSYAKIN & YAVORSKA, 2002; PROTOPOPOVA & al. 2006], naturalized in Central and Eastern Russia [TAMAMSCHJAN, 1999/1959; HANSEN, in TUTIN & al. 1976], with a casual status in other european countries [GUDZINSKAS, 1997; KUKK, 1999; PYŠEK & al. 2002; REYNOLDS, 2002; GREUTER, 2006-2009]. In Romania, it was previously mentioned from Iași at Socola railway station [SÎRBU & OPREA, 1998] and Galați [SÎRBU & OPREA, 2008]. Recently, it was also found at Movileni railway yard (between 45°24'19.33"N; 27°57'13.27"E and 45°24'26.89"N; 27°56'36.58; leg. Sîrbu & Oprea, 2011 August 02), as well as at Tirighina-Barboși railway yard (45°24'20.50"N; 27°59'31.95; leg. Sîrbu & Oprea, 2011 August 02) (Galați county).

***Impatiens parviflora* DC.**

Species originating in the mountainous regions of central Asia [POBEDIMOVA, 1974/1949], invasive in Europe, except the Mediterranean region [MOORE, in TUTIN & al.

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1968; WITTENBERG, 2005]. In Romania, it was mentioned initially by PRODAN (1939), as an ornamental plant, sometimes escaped from gardens (without location). It is now quite widespread in Transylvania [DRĂGULESCU, 2003; CIOCĂRLAN, 2006], Crișana [POP & al. 1968; RESMERIȚĂ, 1970], Maramureș [RESMERIȚĂ & al. 1975-1987, cited by OPREA, 2005; OPREA & SÎRBU, 2006], Banat [GOGA, 1980; PĂTROESCU & al. 2007], and Moldavia [OPREA & al. 1997; SÎRBU & OPREA, 1998; DARABAN, 2007]. In this paper, we mention it from the following new localities: Borșa (Maramureș county) (leg. Sîrbu, 2006 August 23), the chalet Bâlea-Râu (leg. Sîrbu C., Oprea A., 2009 August 17), Cârțișoara at Glăjărie (leg. Sîrbu C., Oprea A., 2009 August 19) (Sibiu county), Brașov (45°40'27.10"N; 25°38'33.82"E; leg. Sîrbu C., Oprea A., Eliáš P. jun., Ferus P., 2011 August 19), Sibiu - railway station (Sibiu county) (45°47'41.41"N; 24°10'09.82"E; leg. Sîrbu C. Oprea A., Eliáš P. jun., Ferus P., 2011 August 18), Bogății Forest (Brașov county) (leg. Sîrbu C., Oprea A., 2009 August 19), Gheorgheni (46°42'59.89"N; 25°34'29.15"E; leg. Sîrbu C. Oprea A., Eliáš P. jun., Ferus P., 2011 August 18) (Harghita county), Burdujeni-Suceava (railway station) (47°40'12.79"N; 26°15'50.45"E; leg. Sîrbu C., 2011 June 15), between Câmpulung Moldovenesc and Pojorâta (47°32'04.64"N; 27°29'45.86"E; leg. Sîrbu C., 2011 September 01) (Suceava county).

Juncus dudleyi Wiegand (*J. tenuis* Willdenow var. *dudleyi* (Wiegand) F. J. Hermann; *J. tenuis* var. *uniflorus* Farwell)

Species native to North America [BRITTON & BROWN, 1970], reported as an alien plant in some countries of Western and Central Europe [SNOGERUP, in TUTIN & al. 1980; DAISIE, 2011], previously mentioned in Romania from the Făgăraș Mountains, in Brezicioara valley (Brașov county) [NEGREAN, 1987; CIOCĂRLAN, 2009]. It was also found at Borzont (46°40'52.26"N; 25°23'35.69"E; leg. Sîrbu C., Oprea A., Eliáš P. jun., Ferus P., 2011 August 18) (Harghita county).

***Sicyos angulatus* L.**

Species native to North America [BRITTON & BROWN, 1970], and naturalized in a large part of Europe [VASILCHENKO, 1972/1957; TUTIN, in TUTIN & al. 1968; PYŠEK & al. 2002; ESSL & RABITSCH, 2002; STEŠEVIĆ & al. 2008; VIVANT, 1983; SANZ ELORZA & al. 2001]. In Romania, it is known as an alien plant (sporadically) in all provinces of the country [BAUMGARTEN, 1816; HEUFFEL, 1858; COMAN, 1946; BORZA, 1947; PRODAN & NYÁRÁDY, in SĂVULESCU, 1964; CIOCĂRLAN, 2009; ANASTASIU, 2010]. In Moldavia (Eastern Romania) it was previously reported from Suceava county only (Northern Moldavia) [MITITELU & al. 1989]. We have also found it in Galați city (Southern Moldavia), on the banks of a stream that flows into the Danube river (45°24'55.86"N; 28°01'53.35"E; leg. Sîrbu C., Oprea A., Eliáš P. jun., Ferus P., 2011 August 20).

Conclusions

In this paper, a number of seventeen alien plant species are presented, one of them being mentioned for the first time in Romania's flora, eight species are new in Moldavia and one species is new in Transylvania. Other seven species are reported now from new localities.

Some of these species (e.g. *Brachyactis ciliata*, *Chenopodium pumilio*, *Eleusine indica*, *Euphorbia dentata*, *Grindelia squarrosa*, *Impatiens parviflora*, *Kochia sieversiana*, *Solidago gigantea*, *Sicyos angulatus*) have a remarkable spreading tendency, expanding their area of invasion in Romania. Others are still quite rare, but the capacity of all these alien species to reproduce without human help must be taken into account in order to prevent their further invasion.

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Fig. 1. *Sedum sarmentosum* Bunge, at Mediaș, Sibiu county

ECOLOGICAL ANALYSIS OF *DIPTEROCARPACEAE* OF NORTH ANDAMAN FOREST, INDIA

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Abstract: Dipterocarpaceae is one of the important timber families of Andaman Islands whose members were largely exploited for their timber in the past. The current study discusses in detail about the family Dipterocarpaceae of North Andaman forest with reference to its species composition, population structure and other ecological entities. Data was analyzed using various ecological and statistical methods. Dipterocarps were encountered in 97 plots, occupying 80% of the sampled area with 68 stems ha⁻¹ and basal area of 8.2 m² ha⁻¹. Dipterocarpaceae ranked 3rd with reference to stem density (11%) and 1st with respect to basal area (18%). The family showed five species viz., *Dipterocarpus alatus*, *D. costatus*, *D. gracilis*, *D. grandiflorus* and *Hopea odorata* compounded from two genera – *Dipterocarpus* and *Hopea*. Keeping in view of the species demographic structure as well as regeneration status, conservative measures are suggested along with certain research questions which need immediate attention in the fragile insular ecosystems of Andaman Islands.

Key words: Andaman, dipterocarps, dispersion, endemic, regeneration, South East Asia

Introduction

Dipterocarpaceae is one of the main timber families in the forests of Southeast Asia that forms a high proportion of the emergent and main canopy strata of the forest [MANOKARAN, 1996]. The members of this family, besides playing a vital role as potential timber species that form an important means of economy in the timber market [APPANAH, 1998; POORE, 1989] also act as source of other non-timber products for the livelihood of the forest dwellers [PANAYOTOU & ASHTON, 1992]. The species of Dipterocarps often locally referred as Gurjan, are extensively utilized for the extraction of resins. From the oleoresins of *Dipterocarpus alatus* and *Dipterocarpus grandiflorus*, Gurjan oil is produced which is used as medicine to treat various skin ailments and ulcers. The resins also have industrial application as varnish and anti-corrosive coatings. The hard solid resin, commonly called as rock dammar, derived from *Hopea* species is used for making boats and handicrafts [SHIVA & JANTAN, 1998].

With reference to South Asia the family is distributed in India, Andaman & Nicobar Islands (A&N), Nepal, Bangladesh and Srilanka [ASHTON, 1982]. A detailed review on systematic distribution and taxonomical classification of Dipterocarpaceae globally was elucidated by MAURY-LECHON & CURTET (1998) and for Indian sub-continent by KUNDU (2008). The family Dipterocarpaceae derived its name from one of its important genera *Dipterocarpus* and has 17 genera with more than 500 species [MAURY-LECHON & CURTET, 1998] out of which, 10 genera and 99 species are exclusively found in South Asia (FAO 1985). Within the Indian forest scenario, the family is diversified by 31 species with 16 endemic (14 to peninsular India, one in North East and one in Andaman Islands) from 5 genera [TEWARY & SARKAR, 1987].

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In the past and current scenario, forests are exploited beyond their limit, ultimately threatening the survival of the species. A successful management of recycling process provides continuous supply of goods and is true even with the plant resources. If the species are utilized proportionately without disturbing their ecological conditions and are allowed for regular natural regeneration process, they may sustain themselves to provide the un-intermittent supply of products. But due to lack of this awareness and illicit anthropogenic activities many species are facing risk of extinction. The same is the case with Dipterocarpaceae members of A&N which fall under one of the five phytogeographical regions that show wide distribution of the family [APPANAH, 1998].

The forests of A&N were virgin until the establishment of the penal colonies around 1857 and then exploitation for timber, predominantly of Padauk (*Pterocarpus*) and Gurjan (*Dipterocarpus*). Forests were logged for timber by adopting either clear felling system or selective felling system by the forest department depending on the necessity and suitability of the scheme [DEVRAJ, 2001]. Forests areas which were extracted have been regenerated naturally or artificially by proposing various forest working plans such as conversion working circle, protection working circle, minor forest produce circle etc., for sustainable management [BASU, 1990; DEVRAJ, 2001]. Apart from the logging actions of forest department, the forests of A&N were also exploited to major extent by the encroachment activities of Island settlers. The study of PRASAD & al. (2010) detailed various anthropogenic and natural driving factors that have affected the forest of North Andaman, threatening phytodiversity. The factors discussed are more or less similar in the other adjacent Islands of archipelago with profound contribution in the deterioration of forest ecosystem. Keeping in view of the importance of Dipterocarpaceae of A&N and the logging activities these Islands faced till recent past it is of prime importance to have a database with reference to their species composition and demographic structure. This is essential for setting up priorities for conservation of the species based upon their population structure and endemism. However such kind of information for these Islands is scanty and limited. In this context, adding to the already existing database, the current study attempts to describe the ecological attributes and spatial distribution of the family Dipterocarpaceae of A&N archipelago.

Forests of A&N have mixed assemblage of species composition, showing similarities with the flora of mainland India, Malayasia and Indonesia [SINGH & al. 2002]. Several floristic [BHARGAVA, 1958; THOTHATHRI, 1961, 1962; BALAKRISHNAN & NAIR, 1977; DAGAR, 1989; REDDY & al. 2008; REDDY & PRASAD, 2008] and few ecological studies [PADALIA & al. 2004; TRIPATHI & al. 2004; PRASAD & al. 2007a, 2009a; RASINGAM & PARTHASARATHY, 2009; RAJKUMAR & PARTHASARATHY, 2008] were carried out to detail the structure, biological richness and diversity patterns of forest of Andaman Islands. However the family level species studies are new to these Islands and so far such kind of study was carried out by PRASAD & al. (2008) on Euphorbiaceae of North Andaman. Though Euphorbiaceae is one among the important species rich families, usually the forest of Southeast Asia are referred as *Dipterocarpus* forest, because of their distinct distribution in most of the Southeast Asian forests [APPANAH, 1998]. The spatial pattern of Dipterocarpaceae within A&N is unique and the family is represented only in Andaman Islands and absent in Nicobar [MATHEW & al. 2009].

Objective of the study

In general, majority of the field inventories focus on deriving the species richness and diversity at regional or at forest community levels. However, this type of studies usually specifies the phytodiversity patterns across the study area. A detail understanding about the species richness, spatial distribution and population structure of a plant family will help in the generation of quantitative database about the demography of the species within the family, their current status and threat they face if proper conservative steps are not initiated. It also helps in assessing the loss of ecological services rendered by the species for forest ecological dynamics and livelihood of the people, once the species enter into the phase of extinction. Towards this direction, the current study discusses in detail about the family Dipterocarpaceae of North Andaman forest of A&N archipelago with reference to its species composition, population structure and other ecological entities along with its occurrence, dominance and existence (?) in other adjacent Islands. The study provides an essential database of Dipterocarpaceae species towards their conservation efforts and supports further research for the future investigators to work on lesser known Dipterocarpaceae of Andaman forests.

Study area

The present study was carried out in the North Andaman (NA) forest of A&N (Fig. 1) which is one among the 14 identified Biosphere Reserves of India [DEVRAJ, 2001]. NA constitutes one of the important major Islands of A&N and lies between 12°95' N and 92°86' E covering an area of 1458 km². All the Islands of NA were declared either as protected areas or as wild life sanctuaries towards conservation measures [HANDBOOK, 1983]. Topography is undulating having hills and narrow valleys with highest elevation of 732 m above mean sea level represented by Saddle Peak, which is the top point in the entire A&N. Typical tropical rain forest climate exists in these Islands due to continuous showers from both south-west and north-east monsoons and with least temperature variations. The soils belong to Serpentine series with top soil having high base status and less nutrient values supporting dense evergreen forests of *Dipterocarpus* and its associates [DEVRAJ, 2001].

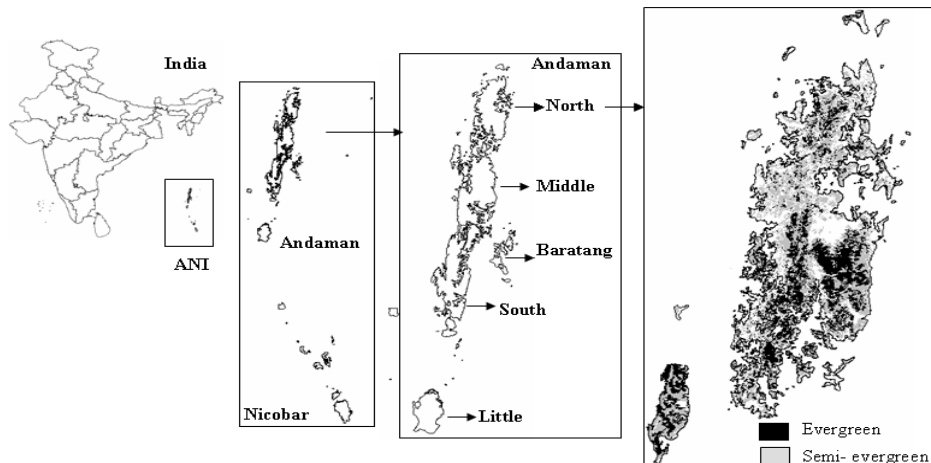


Fig. 1. Location map of the study area

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Though the topographic variations are minor with poor soil conditions these Islands seize an extraordinary vivid biodiversity and endemism. As per CHAMPION & SETH (1968) major hinterland vegetation types of study area include Andaman evergreen, Andaman Semi evergreen and Andaman Moist deciduous.

Materials and methods

The detailed vegetation map prepared using satellite data [PRASAD & al. 2007b] formed basis for the selection of plots (0.1 ha size) for field inventory in two predominant forest types *viz* evergreen (EG) and semi-evergreen (SEG) of the study area. About 120 plots (62 in EG, 58 in SEG) covering entire NA forest were surveyed during field inventory for phytosociological data collection. The size of each sample plot was 32 x 32 m for trees, 10 x 10 m for saplings (two opposite corners of the main plot) and 1 x 1 m for seedlings (all the four corners of the main plot). Within each plot all the trees having diameter at breast height (DBH) > 30 cms were measured, with simultaneous investigation on sapling and seedling data.

The data was analyzed to extract the structural and ecological aspects of Dipterocarpaceae using various phytosociological approaches by deriving frequency, density, basal area to compute Important Value Index [CURTIS & MCINTOSH, 1950]. Calculation of IVI facilitates in identifying the dominant and co-dominant species along with their association to form community within the study area. Girth class analysis was performed to view the contribution of stem density and basal area by various girth classes. Braun-Blanquet system (1932) was used to depict the constancy (presence of occurrence of species within the sampled plots) classes as; Rare constancy (0-20%), low (21-40%), intermediate (41-60%), moderately high (61-80%) and high (81-100%). This analysis helps in assessing the population status of the species.

To analyze the association between the species, the traditional method of chi-squared procedure [WAITE, 2000] was used. Since sample size is <200 (120 plots), Yate's correction was applied to improve the performance of the test as follows.

$$\chi^2 = n(ad - bc) / (a+b)(c+d)(a+c)(b+d)$$

Where n = number of plots sampled

a = plots showing presence of both the species

b = plots showing presence of first species and absence of second

c = plots showing absence of first species and presence of second

d = plots showing absence of both the species

However, this test was not performed for some species with small number of observations whose expected values are less than 5, even with Yate's correction. Alternatively Fisher's exact test (1954) was conducted for analyzing association among those species. The null hypothesis proposed for the both the tests is that the species are independent of each other.

The spatial distribution of species was derived using Index of Dispersion (ID) by calculating mean and variance of the species as follows (taken from WAITE, 2000).

$$ID = S^2 / m$$

where S^2 = the species variance

m = the species mean

Based on the ID values, distribution of species can be interpreted as random (ID = 1.0) clumped (ID >1.0) and regular (ID <1.0). Later chi-square test was applied to signify the departures in the values from unity. It is calculated as

$$\chi^2 = ID (N-1)$$

Where N = number of sample plots

Since N > 30 (120 plots) χ^2 was corrected using the following equation

$$d = \sqrt{2\chi^2} - \sqrt{2(N-1)} - 1$$

Where d is the correction factor and used to define the distribution as

$d \leq 1.96$: the null hypothesis accepted (random)

$d < -1.96$: regular

$d > 1.96$: clumped

Results

The survey yielded a total of 7392 individuals from 60 families, 134 genera and 192 species. Out of 120 sampled plots, Dipterocarps were encountered in 97 plots *i.e* 80% of the sampled area was occupied by the species. This observation is apt with the ASHTON'S (1982) remark, who stated that 80% of the abundant, emergent individuals in lowland forest of Southeast Asia are Dipterocarps. Dipterocarpaceae ranked 3rd after Myristicaceae and Sterculiaceae with reference to stem density (11%) and 1st with respect to basal area (18%). The results are similar to the study of MANOKARAN & al. (1990) in 50 ha plot of Pasoh reserve forest where Dipterocarps dominated the site with 9% stem density and 24% basal area. Dipterocarpaceae in NA forest with 68 stems ha⁻¹, covering basal area of 8.2 m² ha⁻¹ showed five species *viz.*, *Dipterocarpus alatus*, *D. costatus*, *D. gracilis*, *D. grandiflorus* and *Hopea odorata* compounded from two genera – *Dipterocarpus* and *Hopea*.

Among the two forest types sampled 76% of the Dipterocarps stem density (616) was recorded from EG. With reference to *D. alatus*, 9 out of the 10 individuals were represented in SEG while for *D. grandiflorus* 289 out of 295 were encountered in EG indicating the species ecological amplitude and preferential habitats. Values for stems and basal area ha⁻¹ were more for *D. grandiflorus*. Maximum DBH was recorded in *D. gracilis* while minimum average DBH was observed in *D. grandiflorus*. Though *D. alatus* represented with a population of 10 individuals it has showed high average DBH. The Braun-Blanquet constancy classification scaled *D. alatus* (3.3%) and *D. costatus* (6.7%) under rare, *D. grandiflorus* (27.5%) and *Hopea odorata* (20.8%) at low and *D. gracilis* (46.7%) on intermediate constancy.

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Tab. 1. Species parameters of Dipterocarpaceae

Species	<i>D. alatus</i>	<i>D. costatus</i>	<i>D. gracilis</i>	<i>D. grandiflorus</i>	<i>H. odorata</i>
Number of Plots in which species occurred	4	8	56	33	25
Stems recorded	10	43	417	295	46
Mean	0.08	0.36	3.48	2.46	0.38
Standard Deviation	0.54	1.86	7.19	5.52	0.90
Variance	0.74	1.36	2.68	2.35	0.95
Index of Dispersion	8.85	3.80	0.77	0.96	2.47
χ^2 correction factor	30.49	14.68	-1.85	-0.32	8.87
IVI	0.80	1.86	17.59	11.07	2.68
Stems ha ⁻¹	1	4	35	25	4
Basal area ha ⁻¹	0.2	0.4	4.4	2.6	0.5
Saplings ha ⁻¹ (%)	--	--	--	12.3	--
Seedlings ha ⁻¹ (%)	--	--	0.8	14.6	--
Max-DBH	302	301	452	404	300
Average-DBH	160	102	102	95	109

Understanding the distribution and dominance of the species is one of the important aspects of ecosystem research. Species, in general, tend to undergo intra-species and inter-species competitions for the deployment of available optimal resources in their niches. The one which is the successor of the struggle proves itself dominant by showing wide eco-regional distribution dominating the sites with their stem density, area occupancy, abundance, etc. So when one species is identified as dominant, it is also interesting to know about the species which are acting as competitors for the species. In other words, it is to make out the other co-dominant species that are associated with the dominant species and forms the distinct community. Based on the derived IVI value, *D. gracilis* (17.59) was found to be the dominant species both in Dipterocarpaceae as well as in the entire sampled area and forms a community with *Myristica glaucescens* and *Pterygota alata*. Though the other four species of Dipterocarpaceae, didn't dominate the study area they have their associated or neighbouring species based on IVI as follows: *D. grandiflorus* with *Artocarpus chaplasha* and *Celtis wightii*; *D. costatus* along *Mitragyna rotundifolia* and *Baccaurea sapida*; *D. alatus* with *Canarium manii* and *Antiaris toxicaria*; *H. odorata* under *Artocarpus lakoocha* and *Dillenia andamanica* community.

The χ^2 value obtained for the species *D. gracilis* – *D. grandiflorus*, *D. gracilis* - *H. odorata* and *D. grandiflorus* – *H. odorata* exceeded the values of $\chi^2 = 3.841$ ($p < 0.05$), $\chi^2 = 6.635$ ($p < 0.01$) and χ^2 ($p < 0.001$) with 1 degree of freedom respectively. Thus the null hypothesis of independence is rejected. Also, since the observed values are greater than the expected values there exists a positive association between the species (Tab. 2). Application of Fisher's exact test for the association of *D. alatus* and *D. costatus* with the

other species showed significantly independent nature at 95% confidence interval with few exceptions (** in Tab. 2).

Tab. 2. Species association and independent distribution analysis

Test	Chi-Square test			Fisher's Test
	Observed values	Expected Values	chi-values	P-values (95% CI)
<i>D. gracilis</i> – <i>D. grandiflorus</i>	33.0	15.4	49.11	--
<i>D. gracilis</i> – <i>H. odorata</i>	25.0	11.7	33.43	--
<i>D. grandiflorus</i> – <i>H. odorata</i>	25.0	6.9	78.72	--
<i>D. alatus</i> – <i>D. costatus</i>	--	--	--	0.22197*
<i>D. alatus</i> – <i>D. gracilis</i>	--	--	--	0.04471**
<i>D. alatus</i> – <i>D. grandiflorus</i>	--	--	--	0.00498**
<i>D. alatus</i> – <i>H. odorata</i>	--	--	--	0.45826*
<i>D. costatus</i> – <i>D. gracilis</i>	--	--	--	0.00169**
<i>D. costatus</i> – <i>D. grandiflorus</i>	--	--	--	0.00002**
<i>D. costatus</i> – <i>H. odorata</i>	--	--	--	0.0001**

CI - Confidence Interval, * Not significant, ** statistically significant

The study of population structure provides information about the growth patterns and regeneration status of the species. The current analysis of girth wise stem and basal area distribution shows varied results for all the five species. With reference to high stem density, *D. alatus* showed equal number in both 60-90 cm and >240 cm class, while *H. odorata* showed in 60-90 cm. In the remaining three species more or less a reverse J shaped pattern was observed with high stem density in lower girth classes and low in higher implying negative exponential relationship. Except in *D. gracilis* and *D. grandiflorus*, the girth class 210-240 cm was completely absent in other species and in *D. alatus* even there was no representation of 120-150 cm girth class. With respect to basal area, an increasing trend was observed with low girth classes contributing low basal area and high by higher classes, with some exceptions in girth classes by different species as evident from the Fig. 2. In general an ideal representation of the girth classes in terms of stem density and basal area was shown by *D. gracilis*. The analysis of seedling and sapling data showed poor regeneration trend for all the five species. Overall observation of sampled data showed very low percent of saplings and seedlings for *D. grandiflorus*, only seedlings for *D. gracilis* and neither for the remaining three species (Tab. 1).

The *d* correction factor calculated for the five species showed two values viz., >1.96 and < -1.96, rejecting the null hypothesis of random distribution ($d \leq 1.96$). Out of the five species, *D. alatus*, *D. costatus* and *Hopea odorata* showed clumped pattern ($d > 1.96$) following negative binomial distribution, while *D. gracilis* and *D. grandiflorus* followed regular distribution ($d < -1.96$) with positive binomial distribution (Tab. 1). The clumping pattern as observed in some of the species perhaps could be one of the reasons for their poor regeneration status.

All the five species encountered in the study area are labeled under different IUCN categories viz., *D. grandiflorus* and *D. gracilis* as critically endangered, *D. alatus* and *D. costatus* – endangered and *Hopea odorata* under vulnerable categories

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(www.iucnredlist.org). The result of the Braun-Blanquet approach also confirms the rarity and low density of the species particularly *D. alatus* (endemic) *D. costatus* and *Hopea odorata*. The low population density coupled with listing under IUCN categories puts these species at high risk of threat and deserve special ecological importance for protection and conservation.

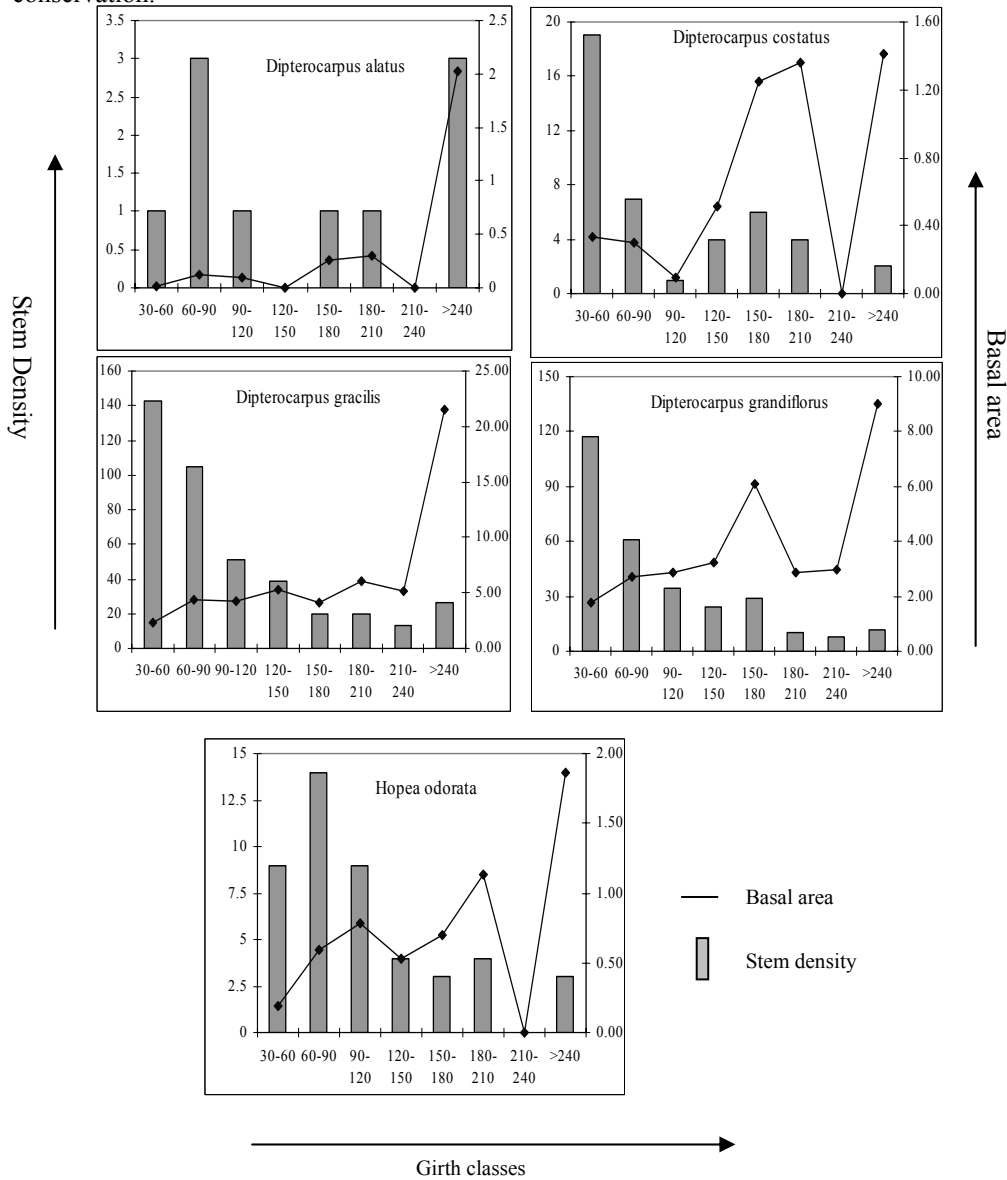


Fig. 2. Stem density and basal area distribution in different girth classes of *Dipterocarpaceae* species

Discussions

Dipterocarps are observed mostly on the low altitudinal zones [WHITMORE, 1988] and the number of individuals and species decreases with increasing altitude [DEVRAJ, 2001]. However in the current study, the altitude of sampled plots ranged between 10–350 m and doesn't show significant correlation between the species distribution and altitudinal levels. This distribution is similar to Peninsular Malaysia where the altitudinal zones of the family ranged between 0-300 m and the forest are usually referred as low-undulating Dipterocarp forest [SYMINGTON, 1943; WYATT-SMITH, 1963; APPANAH, 1998].

The constraint of accessibility restricted the researchers to explore these Islands widely in the past. In spite of this barrier, some of the workers carried out floristic studies and contributed fundamental information about the floristic elements of these Islands. In the recent past, researchers started working on the diversity patterns in different Islands of A&N archipelago and provided detailed account on the vegetation structure and richness patterns of various forest types existing in these Islands (Tab. 3). In all these studies Dipterocarpaceae was observed as one of the dominant families either in terms of stem density or basal area or any other phytosociological parameter.

To sum up, the study carried out under the project *Biodiversity characterization at landscape level in Andaman and Nicobar Islands* by Department of Space and Department of Science and Technology, India [HANDBOOK, 2003] enumerated nine Dipterocarpaceae species from the random survey of 539 plots (0.1 ha & 0.04 ha sizes) in all the major Islands of A&N. However the report [HANDBOOK, 2003] did not provide information about Island wise distribution of the species. Hence the other possible sources of literature were surveyed to detail Island wise distribution of Dipterocarps.

PADALIA & al. (2004) worked on Andaman Islands and observed Dipterocarps dominating the site with 18% (EG) to 15% (SEG) of stem density and *D. turbinatus* as second dominant species based on IVI. However the study cited only 3 species of Dipterocarpaceae (Tab. 3). The study carried out by RASINGAM & PARTHASARATHY (2009) in the Little Andaman recorded Dipterocarpaceae as 4th dominant family contributing 6.77% of the stem density. They have encountered 3 species from the survey of 8 ha plots laid in four different vegetation types. An interesting comparative observation of their study with the current one is with reference to *D. alatus*, an endemic species of the Island. The current study recorded only 10 individuals (1 stem ha⁻¹) in contrast to their observation of 103 (13 stems ha⁻¹). So far there is no detailed information on the Dipterocarps of South Andaman. A survey conducted by PANDEY & al. (2006) on home gardens and home forest gardens in South Andaman listed *D. grandiflorus* as one of the top storey species.

Tab. 3. Distribution of Dipterocarps in Andaman Islands

S.No	Species	A&N Handbook (2003)	Andaman Islands Padalia & al. (2004)	North Andaman Prasad (current)	Middle Andaman Rajkumar and Parthasarathy (2008)	Baratang Islands Chauhan (2004)	Little Andaman Rasingam and Parthasarathy (2009)	South Andaman Pandey & al. (2006)	
1	<i>Dipterocarpus alatus</i>	ψ		ψ			ψ		
2	<i>Dipterocarpus andamanicus</i>				ψ				
3	<i>Dipterocarpus costatus</i>	ψ		ψ	ψ				
4	<i>Dipterocarpus gracilis</i>	ψ	ψ	ψ			ψ	ψ	
5	<i>Dipterocarpus grandiflorus</i>	ψ		ψ			ψ		
6	<i>Dipterocarpus griffithii</i>	ψ			ψ	ψ			
7	<i>Dipterocarpus incanus</i>	ψ			ψ				
8	<i>Dipterocarpus kerrii</i>				ψ				
9	<i>Dipterocarpus turbinatus</i>	ψ	ψ			ψ			
10	<i>Hopea helferi</i>	ψ							
11	<i>Hopea odorata</i>	ψ		ψ					
12	<i>Dipterocarpus grandis</i>		ψ --?						
ψ – Present, ? – Doubtful record									

With respect to Middle Andaman, the study of RAJKUMAR & PARTHASARATHY (2008) on tree diversity using one ha plot each in two different locations of Andaman Giant EG forest, recorded a total of five Dipterocarpaceae species out of which two species viz., *D. kerrii* and *D. andamanicus* were not encountered in the previous and the current studies. They have also observed Dipterocarpaceae as dominant family in terms of stem density, basal area and biomass and listed *D. incanus* as abundant species. The research work of CHAUHAN (2004) in Baratang, a group of scattered Islands adjacent to Middle Andaman, listed three Dipterocarp species. One of the interesting observations (Tab. 3) with respect to Baratang and Middle Andaman is recording of *D. griffithii*. This species was not reported in other Islands of archipelago (except in HANDBOOK, 2003). This infers that the species is restricted to a group of Islands and since both are neighboring Islands, there is a possibility of occurrence of species in both the Islands. Results of the stratified random survey in EG forest of NA by PRASAD & al. (2007a) reported Dipterocarpaceae as dominant family based on the Family importance Value Index which is the sum of relative diversity, relative density and relative dominance.

From the above it is evident that these studies cumulatively provided the list of Dipterocarpaceae species that can be seen in Andaman Islands and also ranked it as one among the top families. They also conclude about the tracing of certain species which are having restricted distribution and require different sampling effort. For example, recording of *D. kerrii* and *D. andamanicus*, which were sampled only in Middle Andaman and none of the other surveys listed them. These lacunae can be attributed either to the sampling strategy adopted or site selected for study or can also be to the confined distribution of the species population.

Added to the above list of species, PADALIA & al. (2004) reported *D. grandis* in their work on phytosociological studies of Andaman Islands. But the literature survey on Dipterocarpaceae across the world did not yield such kind of species. Also the study [HANDBOOK, 2003] recorded *H. odorata* from mixed evergreen forest of Nicobar Islands, which is contradicting with the observations of SINGH & al. (2002) and MATHEW & al. (2010) who stated the absence of Dipterocarpaceae in Nicobar Islands. Both these information need to be further quantified and investigated. Apart from the species listed in the Tab. 3, DEVRAJ (2001) mentioned certain species of Dipterocarpaceae viz., *D. baudii*, *D. chartaceus*, *D. crinitus*, *D. dyeri*, *D. fagineus*, *D. hasseeltii*, *D. obtusifolius*, *D. oblongifolius*, *D. retusus*, *D. turbinatus*, *D. tuberculatus*, whose presence is doubtful in the Andaman Islands. However, the existence of some of these species like *D. turbinatus* [PADALIA & al. 2004; RAJKUMAR & PARTHASARATHY, 2008], *D. tuberculatus* [JHA & SARMA, 2008] *D. obtusifolius* (biotic.org) *D. baudii* and *D. dyeri* (apafri. org) were confirmed by some studies and elaborated investigations about their population structure needed to be worked out along with the status of other species from the above list which are not so far confirmed in A&N.

Hitherto *D. alatus* is considered to be the only endemic species of Dipterocarpaceae observed in the study area (Fig. 3) but SHIN & KYI (apafri. org, 2010) reported that *D. baudii* and *D. dyeri* are found only in Andaman and in that case they may also be considered as endemic too. Now the research question needed to be addressed is whether these species are really confined to these Islands? If these species are present in the Islands they should have been encountered in any of the floristic or ecological studies carried out till now. The possibility of their extinction couldn't be ruled out as forest of Andaman Islands are heavily exploited in the last four decades for valuable timber as well

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as a vast proportion of forest converted into settlement and agricultural purpose by the Island settlers [PRASAD & al. 2009b, 2010; Fig. 4]. Species which have narrow ecological amplitude doest not survive once their habitats are destroyed. It is also possible that these species may have very limited population in special pockets of Island vegetation in certain remote inaccessible location which has kept them isolated.



Fig. 3. *Dipterocarpus alatus* – An endemic and endangered species encountered in the study area



Fig. 4. *Dipterocarpus* species amidst agriculture field near the foothill of Saddle Peak National Park of North Andaman

One of the significant points from the above observations is with reference to the number of Dipterocarpaceae species. JACOB (1981) in his work on taxonomical distribution of Dipterocarpaceae cited presence of eight species of Dipterocarpaceae from Andaman Islands in contrast to the current study which showed 12 species compounded from the field studies and literature survey. Also he reported that the Dipterocarpaceae from Andaman Islands has only one endemic species contributing 12% of the endemicity while REDDY & al. (2004) enlisted two species *D. alatus* and *D. turbinatus* var. *andamanica*. So the issue to resolve is whether the increase in the species number as observed in the current study is real one or ambiguity in assigning the nomenclature to the species by various researchers. As mentioned by VASUDEVA RAO (2004) sometimes even within the same publication (local flora), the one and similar species is referred under two different names. He cited few species of Dipterocarpaceae from Andaman, which were misinterpreted as two different species eg., *D. griffithii* – *D. grandiflorus*; *D. turbinatus* – *D. gracilis*. Considering this statement, it has to be checked whether *D. turbinatus* var. *andamanica* and *D. andamanicus* are synonyms of single species or distinguishably two separate species?

Conclusions

Analysis of the current research and other relevant studies taken in the study area substantially supported Dipterocarpaceae as one the chief family contributing a good proportion of stem density and basal area to the vegetation of Andaman Islands. Depending

on the different sampling methods adopted, in different location of the Islands one or other species of Dipterocarpaceae dominated the site. A cumulative number of species belonging to the family were derived from the available literature, but still existence of certain species is doubtful, which needs further exploration. Towards this future, investigation should focus on detailed systematic family level studies, utilizing different sampling strategies in all the Islands of archipelago, to enumerate complete family species richness, their demographic status and uncertainty among the species citation. The study also showed poor regeneration status of the Dipterocarps. This is important to consider, especially for the endemic species *D. alatus*, in the NA whose adult population is also very low (Fig. 3). The low population of the species may be due to delayed flowering, poor / slower germination rate or unable to compete with the dominant species under closed canopy conditions or other unfavorable site conditions. The Andaman canopy lifting system developed for improving the regeneration patterns in Dipterocarps [CHENGAPPA, 1944] should be reconsidered with high priority to save the population of the species from entering into the status of threatened or extinct.

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EX SITU CONSERVATION OF *SAUSSUREA PORCII* DEGEN IN Y. FEDKOVYCH NATIONAL UNIVERSITY BOTANIC GARDEN

DEREVENKO TATIANA¹

Abstract: The International Agenda for Botanic Gardens in Conservation emphasizes on conservation of rare species *ex situ* as the main task and its aim is the creation of a reserve stock for the possibility of active recovery in nature. We have introduced in the culture and first have been studied rhythm of phenological development and flowering, depending on weather conditions, seed production and added guidelines for breeding the endangered Eastern Carpathians endemic species - *Saussurea porcii* Degen, which is listed in the Red Data Book of Ukraine and the European List of Globally Threatened Animals and Plants. We have created a field bank *S. porcii* – it is our contribution to the conservation of plant diversity.

Key words: *Saussurea porcii* Degen, *ex situ* conservation, Red Data Book of Ukraine, a field bank, the biology of developing

Introduction

The Global Strategy for Plant Conservation states that the conservation of living plants collections of endangered species of regional floras, especially endemics, as the most vulnerable is very important. [GLOBAL STRATEGY FOR PLANT CONSERVATION, 2002]. From the Carpathian region's 219 endemic taxons [ТАСЧКЕВИЧ, 2006] in the collections of CHNU Botanic Garden today are saved specimens of 11. *Saussurea porcii* Degen is the Eastern Carpathians endemic, which has the phylogenetic relationships of Siberia. In Ukraine it is distributed in: Chornogora mountain range (Polonyna Rogneska, ur. Primaratik), the upper Chorny and Bilyi Cheremosh (Mountain Gnetesa, polonyna Glystuvata, between polonynas Glystuvata and Preluky). In Romanian Carpathians it has been cited from the Maramureşului Mountains (at Borşa and Lanul Cercănel [ŞTEFUREAC, 1971], and in Rodna Mountains (on the Mountain of Corongiş, under the rocks called "Porjii", as well as on the opposite slope of it [ŞTEFUREAC, 1971; NYÁRÁDY, 1933]. This species is listed as disappearing into the Red Data Book of Ukraine [ДИДУХ, 2009], as critically endangered (CR) in Ukraine and as extinct (EX) in Romania – accordingly to the Red Data Book of Carpathians [ТАСЧКЕВИЧ, 2002], as endangered (EN), in the *European List of Globally Threatened Animals and Plants* (1991). Today are known only 6 populations of *S. porcii* in the Carpathian Mountains [БАГЛЕЙ & ДАНИЛИК, 2009], mostly on wetlands in the crooked green alder in the subalpine zone. It needs to establish reserves to protect it. Basically, it has a small populations numbering

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from 400 to 1000 individuals. The most numerous one has several thousand individuals and occupying an area of several tens of hectares on the polonyna Glystuvata (the Chyvchyn Mountains) (Fig. 1). Only this population from all has a high level vitality constituent individuals (Fig. 2) [БАГЛЕЙ, 2011].

Currently, only CHNU Botanic Gardens living collection present some specimens of *S. porcii*, unlike all the botanical gardens of Ukraine [ЛЕБЕДА, 2011].

Material and methods

Saussurea porcii Degen is a hemikryptophyte, herbaceous perennial plant, to 80 cm height, with sessile lanceolate leaves, forming wings along the stem and inflorescence corymbose. From polonyna Glystuvata locality the planting material has been introduced CHNU Botanic Gardens, in 2007. In the Botanic Garden a place in partial shade for planting *S. porcii*, and a high soil moisture provided by regular watering this population-locus was chosen (Fig. 3).

Monitoring the phenological stages was carried out by conventional methods, recommended by the Botanical Gardens of USSR Council [МЕТОДИКА ФЕНОЛОГИЧЕСКИХ НАБЛЮДЕНИЙ В БОТАНИЧЕСКИХ САДАХ СССР, 1979], and mathematical processing of phenological observations were carried out according to the method of the Central Botanical Garden [КРАТКОЕ ПОСОБИЕ ПО МАТЕМАТИЧЕСКОЙ ОБРАБОТКЕ ДАННЫХ ФЕНОЛОГИЧЕСКИХ НАБЛЮДЕНИЙ, 1972]. The study of seed production was carried out according to the guidelines by Т. О. РАБОТНОВ [РАБОТНОВ, 1992] and VAYNAGY [ВАЙНАГИЙ, 1974].

Results and discussions

The International Agenda for Botanic Gardens in Conservation emphasizes that conservation of rare species *ex situ* is the main task and its aim is the creation of a reserve stock for the possibility of active recovery in nature [МЕЖДУНАРОДНАЯ ПРОГРАММА БОТАНИЧЕСКИХ САДОВ ПО ОХРАНЕ РАСТЕНИЙ, 2000]. As for successful repatriation should be explored more fully the biological characteristics, which is possible only in *ex situ* conditions, we are studying the biology of developing and reproduction of *S. porcii* and it is a preparatory stage to the restoration in nature.

The results of the study of phenological rhythm of *S. porcii* in the collection of the CHNU Botanic Garden during 2007 – 2011 years are presented in Tab. 1. In the spring, *S. porcii* begins growing season in CHNU Botanic Garden in early April, when average daily air temperature did not fall below +5 °C, the bud of resumption elongates, shoots and leaves starts to grow. About a week after the stable transition of mean daily air temperatures over +10 °C an inflorescences are formed in the generative shoots, on average, this occurs early in the second decade of May, often coinciding with the transition of daily average temperatures over +15 °C. From the beginning of growing season before the flowering passes near 88 days (Table 2). It comes into flower in the last third of June – mid of July, until early of August. The earliest onset of phenophases had been observed in 2009 – the

first decade of June, when flowering time was 1.5 months, which is obviously connected with the early spring practically without a rain right up to June and the second wave of drought lasted from July to early October. The average duration of flowering *S. porcii* over the years of observation was 33 days. The shortest flowering period noted in 2010 was of 18 days, due to the fact that the average air temperature during the flowering time was approximately 3 °C above normal and reached 23.2 ± 0.7 °C. Inflorescences of *S. porcii* (Fig. 4) have a diameter from 2.5 to 7.5 cm, and a height from 4.5 to 11 cm; on average the inflorescences are composed of 30 botryoidal florets (from 15 to 60), each predominantly having 9 disk florets (rarely 7 or 10). In the florescence of disc florets clearly are observed 2 phases, namely: staminale and carpellary one. We determined the duration of each phases in sunny weather and at average air temperatures about 20 °C, starting by staminale phase, which lasts about 24 hours; after drying the stamens begins carpellary phase and lasts about 24 hours, too. Thus, about 20 hours lasts phase of the budding and florescence of disk florets actually lasts about 2 days.

We have observed there are infected plants by aphids in a phase of active growth of the inflorescence before flowering, which resulted in shrinkage of the tops of generative shoots and, accordingly, to a lack of florescence in 50% of generative shoots in the culture.

Seeds of *S. porcii* begin to ripen from mid August and by the end of it, dissemination is finish. Seeds are dark brown in color, having an oblong shape, slightly flattened at the sides, length from 0.72 to 0.62 cm and a diameter from 0.11 to 0.15 cm. Weight of 1000 seeds is about 1.400 Kg.

As an important indicator of the life of rare plants *ex situ* is their ability of generative reproduction. We investigated the seed production (SP) of generative shoots (a number of mature seed). *In situ* depending on the vitality of population SP ranges from 153.8 ± 2.01 to 280.2 ± 3.75 [БАГЛЕЙ, 2009]. In the CHNU Botanic Garden this number is fluctuating between 10 and 135, with an average of 55.4 ± 7.8 . Study of *ex situ* seed production coefficient (SPC) have found that mature seeds are formed only at 1/3 (32.4%) from all number of seed germs in each generative shoots of *S. porcii*. Well known is that the number of seed germs have a genetically fixed value and therefore varies considerably, with lower limits than the number mature seeds, which depends on a large number of different factors of exogenous and endogenous origin. The second level is repeated it again - the number of flowers in generative shoots (in the inflorescence) varies far less than the number of mature fruits. Both figures are in varying degrees depending on the specific conditions of growth of individuals of a particular species. In our case, such difference between seed productivity in nature (*in situ*) and in culture (*ex situ*) can be explained by the fact that the culture reduce the florescence period (only part of the flowers makes seeds, and the rest remains in various bud stages). Partly, it is a consequence of not perfect condition for cultivation of *S. porcii*, and partly - the result of the impact of weather conditions, and a certain lack of pollinators. In any case, it's an interesting question, and it requires additional and parallel studies, both in nature and cultivation.

In June and July at the base of the shoots, virginal and generative individuals are formed, from 3 to 7 resumptions buds. The growing season lasts an average about 200 days,

and finishes with the start of frost in late October – early November. Maximum seed germination was 83% in the autumn, sowing in boxes with a soil mix of deoxidized peat, leaf soil and sand. More than 70% of the seedlings in their second year become the generative phase of development.

Conclusions

We have introduced in the culture the endangered Eastern Carpathians endemic species of *Saussurea porcii* Degen. In accordance with our program of restore the nature populations of endangered plant species, we have created a field bank of *S. porcii*. To restore the disturbed populations should only correctly collect seeds and sow it, and through a growing season will have a representative genetic planting material. And that is important – without the high financial costs. Implementation of this program in the CHNU Botanic Garden is our contribution to the conservation of plant diversity and compliance with international obligations of Ukraine in the Global Strategy for Plant Conservation.

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Tab. 1. *Saussurea porcii* Degen rhythm of development in the collection of CHNU Botanic Garden

Year/ statistics	Beginning of grows	Formation of flower stalk	Efflorescence	Mass flowering	End of flowering	Beginning of ripening	Full ripening of seeds	End of vegetation	Duration of vegetation
2007	-	27. V	24. VI	5. VII	13. VIII	9. VIII	21. VIII	3. XII	-
2008	11. IV	19. V	30. VI	7. VII	26. VII	10. VIII	27. VIII	22. X	194
2009	1. IV	21. IV	2. VI	23. VI	15. VII	7. VIII	28. VIII	27. X	209
2010	4. IV	5. V	11. VII	15. VII	28. VII	15. VIII	30. VIII	18. X	197
2011	30. III	21. V	16. VII	19. VII	10. VIII	13. VIII	31. VIII	25. X	209
M±2m	3. IV±5,7	12. V±13,1	27. VI±15,4	6. VII±9,0	31. VII±10,5	11. VIII±2,8	28. VIII±3,7	31. X±16,6	202,3±7,9
V	6.1	11.0	9.6	5.3	5.5	1.4	1.7	6.1	3.9

Tab. 2. Climatic data during the *Saussurea porcii* Degen flowering in the collection of CHNU Botanic Garden

Year/ statistics	Number of days before flowering	Flowering period			T °C of air during the flowering			The number of days with precipitation	Σ precipitation (mm)
		Efflorescence	End of flowering	Duration	Max	Min	M ± m		
2007	-	24. VI	13. VIII	51	36.9	10.0	20.8±0.5	15	86.1
2008	81	30. VI	26. VII	27	32.3	9.9	19.5±0.5	20	185.8
2009	63	2. VI	15. VII	44	31.7	8.2	19.4±0.5	22	103.8
2010	99	11. VII	28. VII	18	32.6	12.9	23.2±0.7	7	53.7
2011	109	16. VII	10. VIII	26	33.1	10.3	20.4±0.5	17	123.6
M±m	88 ± 10.2			33.2 ± 6,1	33.3 ± 0.9	10.3 ± 0.8	20.7 ± 0.7	16.2 ± 2.6	110.6 ± 22.0
M±2m	23.1	27. VI ± 15,4	31. VII ± 10.5						
V		9.6	5.5	41.4	6.2	16.5	7.5	35.8	44.5



Fig. 1. The population of *Saussurea porcii* Degen on the polonyna Glystuvata
(foto by Vacyl Budjac)



Fig. 2. *Saussurea porcii* Degen *in situ*
(foto by Vacyl Budjac)



Fig. 3. *Saussurea porcii* Degen *ex situ* (in CHNU Botanic Garden)



Fig. 4. The inflorescens of *Saussurea porcii* Degen *in situ*
(foto by Vacyl Budjac)

**POLYGONATUM VERTICILLATUM (LINN.) ALL. AND
POLYGONATUM CIRRHIFOLIUM (WALL.) ROYLE: TWO
THREATENED VITAL HEALERS FROM ASTHAVERGA
NURTURED BY GARHWAL HIMALAYA, INDIA**

BISHT POONAM¹, PRASAD PRATTI¹, NAUTIYAL BHAGWATI PRASAD²

Abstract: The biodiversity of Garhwal Himalaya supports a large number of medicinal plants used in various ailments as a drug. *Polygonatum verticillatum* and *Polygonatum cirrhifolium*, the healers from 'Asthaverga' of 'Ayurveda', are reported from Garhwal Himalaya, but due to overexploitation are encompassed in threatened category. The present study is a documentation of these plants to facilitate the conservation of these crude drugs in their natural habitat and to domesticate them. The study also provides information regarding the resident's outlook, living in surrounding area of these species, towards these species.

Key words: conservation, medicinal, *Polygonatum verticillatum*, *Polygonatum cirrhifolium*

Introduction

India possesses the world's richest medicinal plant heritage and traditional and local knowledge and Himalaya is one of the mega biodiversity regions of the world [HEYWOOD, 2000]. The Indian Himalayan region (IHR) supports over 1748 (32.2% of India) plant species of known medicinal value [SAMANT, 1998]. The Garhwal Himalaya has been a centre of spiritual knowledge, religiosity and pilgrimage from ancient times and it is also rich in biodiversity. *Polygonatum verticillatum* and *Polygonatum cirrhifolium* are the two medicinal enticers from this goblet of biodiversity and key ingredients of 'Ashtaverga' of 'Ayurveda'.

Material and methods

The present manuscript was prepared by extensive literature survey of documented directories and a field survey was also conducted to verify the documentations.

The study was carried out in two districts of Uttarakhand viz. Pauri and Rudraprayag. Rudraprayag district covering an area of about 2439 sq. km lies between latitude 30°19' and 30°49' North and longitude 78°49' and 79°21'13" East. The climate varies from sub-tropical monsoon type (mild inter, hot summer) to tropical upland type (mild winter, dry winter, short warm summer). The soils are natural, dynamic, heterogeneous, non-renewable resource, which support plant and animal life [ANONYMOUS, 2009]. Pauri encompasses an area of 5230 sq. km and situated between

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Latitude 29°45' to 30°15' North and 78°24' to 79°23' Longitude East. The region has a sub-temperate to temperate climate, which remains pleasant throughout the year. The soils are derived from rocks and terraces present are silt to clayey loam and are very fertile [ANONYMOUS, 2011].

Local people were interviewed randomly concerning the local uses of the plants under study. During the interviews local name of the plants, parts used and formulations were asked (see Appendix). These participants were then divided into three groups according to the age groups and gender viz. category I (male 25-50 years), category II (male 51-90 years) and category III (female 25-90 years).

Result and discussion

Privileged Garhwal Himalaya

The hills of Uttarakhand, standing almost centrally in the long sweep of Himalaya, are also known as the 'Garh-Kum' region [KANDARI & GUSAIN, 2001]. Garhwal Himalaya lies between 77°33'5" to 80°6' E longitude and 29°31'9" to 31°26'5" N latitude [NAND & KUMAR, 1989]. Thus the Garhwal region enjoys a wide range of altitudes extending from about 325 m in the *Bhabar* tract to the height of about 7,817 m forming the Nanda Devi peak of the Greater Himalaya or Himadri [KANDARI & GUSAIN, 2001]. The Garhwal Himalayas due to its distinct meteorological, geographic, geological and ecological patterns is rich in bio-resources as well as diverse flora and fauna [GAIROLA & BISWAS, 2008]. The high altitude regions of Uttarakhand Himalayas can be divided into three main climatic zones viz. alpine, temperate and sub-tropical [SHAH & JIAN, 1988]. The alpine zone which ranges between 2500 to 4000 m is rich in wild medicinal plants like *Angelica glauca*, *A. archangelica*, *Dactylorhiza hatagirea*, *Carum carvi*, *Picrorhiza kurroa*, *Aconitum heterophyllum*, *Nardostachys jatamansi*, *Saussurea lapa*, *Podophyllum hexandrum*, *Rheum emodi*, *R. moorcroftianum*, *Aconitum balfourii*, *Swertia* spp. etc. The temperate zone commencing from 1000 to 2700 m elevation is rich in many orchids of medicinal importance, *Rhododendron arboreum*, *Corylus jackmontii*, *Hippophae rhamnoides*, *Polygonatum verticillatum*, *P. cirrhifolium*, *Hypericum oblongifolium*, *Arisaema intermedium*, *Hedychium spicatum* etc. The sub-tropical zone is the region existing in the valleys of the temperate zone. They are not characterized by as rich a variety of medicinal plants but they also account for the bio diversity of the state [ANONYMOUS, 2002]. The medicinal plant diversity available in tropical belt mainly incorporates: *Embelica officinalis*, *Terminalia chebula*, *T. bellirica*, *Cinnamomum tamala*, *Zanthoxylum alatum*, *Berberis* ssp., *Rubus ellipticus*, *Gloriosa superba*, *Withania somnifera*, *Rauwolfia serpentina*, *Aloe vera* etc. [KANDARI & GUSAIN, 2001].

Verve of "Ayurveda"

The literal meaning of "Ayurveda" is (Ayur = life and veda = knowledge) the way or science of life. Ayurveda (1000-500 BC) originated from our ancient literature – "Atherva-veda", the knowledge of which was documented in 'Charak-Samhita' (1000 BC) and 'Sushruta-Samhita' and are considered to be the authentic books. "Ayurveda" may be said to be a treasure house of knowledge about medicinal plants. All of the plants which are used for their medicinal properties have been thoroughly evaluated and classified for thousand of years. It is an ancient philosophy based on a deep understanding of eternal truths about the human body, mind and spirit. Unlike orthodox medicine, it is not based on

the frequently changing findings of specific research projects, but rather on permanent, wise, eternal principals of living [GODAGAMA & HODGKINSON, 1997].

"Ayurveda" is bestowed with "Asthverga" a group of eight plants used as tonic which promotes body heat, dries up serious fluids, carminative and antitussive, and are useful in vitiated conditions of pitta and vata, agalactia, seminal weakness, internal and external haemorrhages, cough, bronchitis, burning sensation and general debility. These eight plants belongs to two families, 'Liliaceae' comprising mahameda (*P. verticillatum*), meda (*P. cirrhifolium*), kakoli (*Roscoea alpina/purpurea*), ksheerakakoli (*Lilium pollyphyllum*), and 'Orchidaceae' comprising jeevak (*Malaxis acuminata*), rishibhak (*M. muscifera*), riddhi (*Habenaria edgeworthii*), vridhhi (*H. intermedia*) [VARIER, 1995].

Introduction to the plants

Polygonatum is a genus of erect or decumbent perennial herbs belonging to family Liliaceae and distributed in the temperate regions of the northern hemisphere. Thick fleshy creeping sympodial rhizomes characterize the genus. According to MILLER (1754) the generic name of *Polygonatum* is derived from the character of the rhizome which resembles much as yovi, a Knee, because it has many little Knees. LINNAEUS (1753) listed three species of *Polygonatum* under the genus *Convallaria*, namely, *C. verticillata*, *C. polygonatum* and *C. multiflora* in his book 'Species Plantarum'. These were treated under the generic name *Polygonatum* by ALLIONI (1785). In the natural system of classification of BENTHAM & HOOKER (1862-1883) family liliaceae was classified in the series Coronarieae.

The systematic position of *Polygonatum* according to phylogenetic system of classification of HUTCHINSON (1973) was:

Phylum: Angiospermae
 Subphylum: Monocotyledons
 Divison: Corolliferae
 Order: Liliales
 Family: Liliaceae
 Genus: *Polygonatum*

Polygonatum is represented by 57 species in the world concentrated in Himalayas [OHARA & al. 2007]. Out of the species occurring in IHR two are imperative ingredients of Asthverga.

Polygonatum verticillatum (Linn.) All. syn. *Convallaria verticillata* (Linn.), is known as whorled Solomon's seal in English and locally known as mitha dudhia [NAUTIYAL & NAUTIYAL, 2004] and Kantula [GAUR, 1999]. The species is recognized as 'mahameda' in Ayurveda and in Sanskrit as Tridanti, Devamani and Vasuchhidra (Fig. 1). It is an erect tall herb, 60-120 cm high. Leaves are whorled, sessile, 10-20 cm long, linear or lanceolate, acute or rarely tip carinate, glaucous beneath, occasionally ciliolate on margins and veins. Flowers are white, pinkish white or pale green, in whorled racemes, rarely lilac. The flowering and fruiting takes place in the month of June to October. This species is found in the temperate Himalayas at altitudes of 1800-3900 m amsl. From Garhwal Himalaya *P. verticillatum* was reported from Bhuna, Dunagiri and Niti by NAITHANI (1984), Binsar by GAUR (1999), Tungnath, Rudranath, Valley of Flowers and Dayara by VASHISTHA (2006).

Polygonatum cirrhifolium (Wall.) Royle syn. *Convallaria cirrhifolia* Wall. another member of Asthverga recognized as King's Solomon's seal in English, locally as Khakan

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[GAUR, 1999], 'meda' in Ayurveda, Dhara, Manichhidra and Svalpaparni in Sanskrit (Fig. 2). It is also a tall erect, perennial herb, 60-120 cm high with whorled (3-6) sessile, linear leaves having tendril like tips. Flowers white, green purplish or pink on short stocks and the fruit is round blue-black berry found in the temperate Himalayas at the altitudes of 1200-4200 m. Rhizomes are thick and fleshy. In Garhwal, NAITHANI (1984) reported it from Gulabkoti, Sitapur and Sutul while Gaur (1999) from Khirsu.



Fig. 1. *P. verticillatum*: Leaf pattern



Fig. 2. *P. cirrhifolium*: Leaf pattern

About 'mahameda' and 'meda' it was documented in "Abhinav niguntu" that 'meda' initiate from the same place from where 'mahameda' originates, simply implying that both *Polygonatum verticillatum* and *P. cirrhifolium* grows together. The term 'meda' used in both the species symbolize the 'mucilage' present inside the rhizomes of these plant species. Several workers explore these two species of 'Asthverga' either together or individually [VARIER, 1995; SINGH, 2006; HUSSAIN & HORE, 2008]. SZYBKA-HRYNKIEWICZ & JANECKO (2004) studied the effect of plant growth regulators and steroidal hormone on a quantity of diosgenin in callus tissue of *P. verticillatum*. In another study they examined the effect of coconut water (CW), plant growth regulators, and steroidal hormones on callus of *P. verticillatum*. Despite underground parts recently above ground aerial parts of *P. verticillatum* are also tested for insecticidal and leishmanicidal properties [SAEED & al. 2010].

Polygonatum is a complex genus with a wide range of chromosome counts ($2n = 16, 18, 20, 21, 22, 24, 26, 27, 28, 30, 31, 36, 38, 40, 42, 46, 59, 56, 60, 62, 64, 66, 84, 86-91, 90$) [LATOO & al. 2005]. In India, chromosome numbers reported for *P. cirrhifolium* are $2n = 38$ (Shimla), 26 (North Sikkim), 28 (Northeast Sikkim) and 56 (China hills, Western Burma) [KUMAR, 1959b, 1959c, 1960, 1964-1965]. LATOO & al. (2005) established a new chromosome number in *P. cirrhifolium* i.e. $2n = 32$. The most common chromosome number for *P. verticillatum* is $n = 28$. Although, one tetraploid strain with $2n = 60$ chromosomes and two hexaploid strains with $2n = 90$ chromosomes have been encountered [THERMAN, 1953].

Curative assets of the plants

Collectively, meda and mahameda are used as tonic and promotes body heat, dries up serious fluids, carminative and antitussive. Both species are used against loss of vigor, pain in the kidney and hips, swelling and fullness in the abdominal region, accumulation of fluids in bone joints, skin eruptions and cough [NAUTIYAL & NAUTIYAL, 2004].

Individually, *Polygonatum verticillatum* is eaten raw or cooked, the powder is given for gastric complaints, and the paste applied to wounds [NAUTIYAL & al. 1998; GAUR, 1999]. The rhizome is valued as salep, a strength giving food. The plants possess diuretic properties and the rhizome of this species is eaten as food in the Kurram valley [ANONYMOUS, 1969]. It contains digitalis glucoside, saponosides A, B, C and D, lysine, serine, aspartic acid and threonine [ANONYMOUS, 1969]. SOOD & al. (2005) mentioned rhizomes contain diosgenin.

Similarly, *P. cirrhifolium* is reported to be used as a tonic and vulnerary. A root infusion with milk is used as an aphrodisiac and blood purifier for tumors and piles. According to report of ANONYMOUS (2003), it is useful in burning sensation, skin diseases wounds, ulcers, tuberculosis, fever, cough, bronchitis and general debility. Investigations in China have reported hypoglycemic, hypotensive, antibacterial and antifungal effects of *P. cirrhifolium* [SINGH, 2006]. Rhizomes contain starch, protein, pectin and asparagin [NAUTIYAL & NAUTIYAL, 2004]. It was also reported to be used in major ayurvedic formulations like Asoka Ghrta, Sivagutika, Amrtaprasa Ghrta, Dasam, ularista, Dhanvantara Taila, Brhatmasa Taila, Mahanarayana Taila, Vasacandanadi Taila.

In the survey it was found that people included in category I were less aware about the plant and its uses while the category II was a combination of people who recognize the plant or were familiar about the uses of plants or the parts used for making formulations. The participants included in category III were least aware about the plant and its uses. Although, how to prepare formulations was unknown by most of the participants.

The vaidyas of both of the districts however recognize the habitat, uses, parts of plant used and how to prepare various formulations. These vaidyas were the local healers which cure the people from diseases. In olden times they were the only people to cure the inhabitants later medicinal facilities were provided by the Government, so now doctors were there for treatment of the inhabitants. But these vaidyas still heal the residents in far flung areas. Since earlier only vaidyas were aware about the distribution and methods of preparation therefore they limit the knowledge to themselves for the sake of their profession and to protect the plants from overexploitation by the villagers. Only the son or student who takeover the profession after the existing vaidyas was given the information regarding the distribution, identification, plant and part used, formulations and doses.

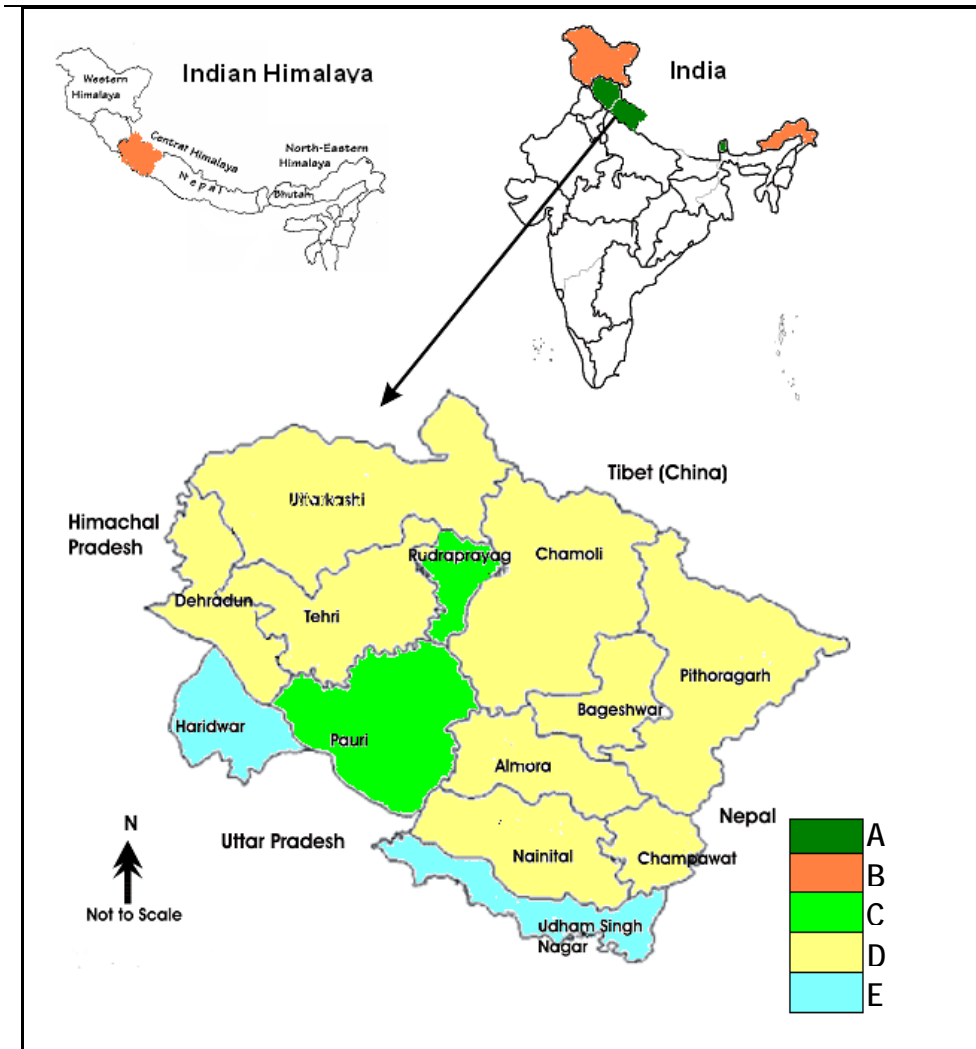
Threat of Extinction

It is accountable that Garhwal Himalaya is enriched by both the *Polygonatum* species which are potential future drugs and can be a milestone in the drift from allopathy to herbal health care system. *P. verticillatum* was found vulnerable in Uttarakhand, Himanchal Pradesh, Jammu & Kashmir and Arunachal Pradesh while *P. cirrhifolium* is endangered in Himanchal Pradesh and vulnerable in Uttarakhand (Fig. 3) [VED & al. 2003]. This status is assigned by IUCN through FRLHT (Foundation for Revitalization of Local Health Traditions) which utilized the CAMP (Conservation Assessment and Management Prioritization) process to undertake rapid assessment of prioritized medicinal plant species of conservation concern in different states/regions of India. Through this evaluation taxa have been assigned Red List status of “threatened” category i.e. critically endangered, endangered and vulnerable. The reason behind there threatened status is their exploitation for their medicinal value. In addition habitat specificity, narrow range of distribution, land-use disturbances, introduction of non-natives, habitat alteration, climatic changes, heavy livestock grazing, explosion of human density, fragmentation and degradation of plant density, population bottleneck and genetic drift [KALA & al. 2006; KALA, 2007] are the potential causes of rarity in medicinal plant species. The women of these areas carry all the activities of domestication of cattle. They collect the food and fodder from the nearby forests and due to lack of identification cut the *Polygonatum* species along with the fodder grasses. This is therefore one of the reasons of threatened status of these plant species.

Conclusions

The present study concludes that the distribution and ethnobotanical uses documented were correct but the knowledge was limited to only local healers and inhabitants were unknown towards the identification of plant species studied. This is although beneficial for protecting the plant from overexploitation and thereby illegal trade but this unfamiliarity is also posing threat for the existence of these species. Therefore immediate steps were needed for educating local residents about the plants in vigilance of Government so that plants can be protected from both overexploitation and negligence.

The study also emphasize that these two plant are suffering from negligence of people both common man and researchers and to revitalize these two magnificent species. Conservation initiatives are urgently required. The study also suggests that despite the so much work done there is still a dearth of research to prove the potential of the natural Himalayan habitats in terms of medicinal plant production.



A: Distribution of *Polygonatum verticillatum* and *P. cirrhifolium* in India; B: Distribution of *P. verticillatum* in India; C: Districts of Uttarakhand where present study was undertaken; D: Hill areas of Uttarakhand; E: Plain areas of Uttarakhand.

Fig. 3. Map of Uttarakhand showing the study areas in Garhwal Himalaya.

Appendix

Name of the participant

Age of the participant

What is the local name of the plant used?

For which diseases do you use the plant?

Which parts of the plant do you use?

How do you prepare the plant for use?

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ECOLOGICAL AND AESTHETIC ROLE OF SPONTANEOUS FLORA IN URBAN SUSTAINABLE LANDSCAPES DEVELOPMENT

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Abstract: The aim of this scientific paper is to promote sustainable methods with beneficial effects on the environment, with aesthetic effect on urban and rural landscapes. This paper highlights and promotes ecological and aesthetic role of spontaneous flora in sustainable landscape planning. This method in which spontaneous flora is an important environmental factor is minimal valued in terms of landscape, even less implemented in Romania. By covering current bibliographic references, by analysing contemporary urban landscape in Cluj-Napoca, Romania, in the present study, are exposed principles, benefits, constraints and legitimate questions about sustainable landscapes by introducing spontaneous flora or, more simply, through its conservation, practical examples of successful integration in the contemporary landscape of ruderal landscapes. Conclusions from this study refer specifically to the role of spontaneous landscapes in urban ecology, to the management of these landscapes and exposure of minimum guidelines so that this method has a decent start in Romania.

Key words: spontaneous flora, ruderal, landscaping, ecology, conservation

Introduction

Given the negative changes that occur in the natural environment due, in particular, to noxious anthropogenic factors, the main purpose of this paper is to highlight the ecological and aesthetic values of spontaneous vegetation and use/conservation of this type of vegetation in sustainable landscape.

In this paper is treated particularly the spontaneous flora from urban and ruderal landscapes that can be successfully valued to greening cities. In most urban areas, a cosmopolitan range of wild plants provide important ecological services, services which, in light of the expected impact of climate change could become increasingly important in the future. Peter Del Tredici asserts that the management of spontaneous vegetation in urban areas to increase its ecological and social values is a sustainable strategy rather than an attempt to restore historical ecosystems existing before the establishment of current cities [DEL TREDICI, 2010].

European ecologists have been analysing the historical development and spatial distribution of spontaneous urban vegetation over several decades [CHOCHOLOUŠKOVA & PYŠEK, 2003; PYŠEK & al. 2004; SUKOPP, 2002] and recently began documenting the coverage in urban ecosystems using GIS technology [HERBST & HERBST, 2006; RINK, 2009]. Peter Del Tredici make a comparison between European and North American ecologists showing that the last ones have been slow to adopt urban ecology and began to

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focus seriously on the subject since the 1990's [ALBERTI & al. 2003; GRIMM & al. 2000; ZIPPERER & al. 1997; DEL TREDICI, 2010].

Applying the comparison, we see that in Romania the interest in the spontaneous landscape environmental values is recent and is not yet well defined.

This paper supports the idea that spontaneous urban vegetation can effectively achieve many of the environmental objectives of traditional restoring with minimum financial investment and a greater chance of long-term success [CHOI, 2004; SAGOFF, 2005]. The information presented urges ecologists, landscape architects, and other professionals interested, taking into account, without prejudice, all biological resources and to recognize that cities spontaneous flora has the ability to make significant contributions to urban ecological functionality.

Material and methods

The paper is based on the study of scientific literature existing in Romania and internationally, and analysis and identification of Cluj-Napoca spontaneous landscape for application of sustainable methods in landscape planning.

Using these research methods, the paper highlights the characteristics of the urban environment, urban spontaneous vegetation, its management, and examples of successful integration of ruderal urban landscape in Germany and the United States of America, and presents elements that differentiate new designed landscapes that used spontaneous flora from spontaneous landscapes preserved and managed for aesthetics and urban greening.

Results and discussions

From a strictly functional perspective, most vegetated urban lands can be classified into one of the three broad categories: remnant native landscapes, managed horticultural landscapes, and abandoned ruderal landscapes [KOWARIK, 2005; KÜHN, 2006; WHITNEY, 1985; ZIPPERER & al. 1997]. These landscape types can be distinguished analysing: 1) their past land-use history; 2) the types of vegetation they contain; 3) the characteristics of their soils; 4) the levels of maintenance they require in order to preserve their integrity. All three types of landscapes are found in urban areas of Cluj-Napoca (Fig. 1).

The least studied of these types is the abandoned ruderal landscape represented by marginal or degraded urban land that receives little or no maintenance, dominated by spontaneous vegetation - a mix of species that grow and reproduce without human care or intent.

In Cluj-Napoca this type of landscape, ruderal, is common and we can clearly associate with the margins of transportation infrastructure, abandoned or vacant residential, commercial, and industrial property, and the interstitial spaces that separate one land-use function from another (Fig. 2, 3). While ruderal landscapes are often referred to as "wastelands" the progress of urban ecology place in a new light this neglected resource. Perhaps the most obvious aspect of the distinctive urban environments is the ubiquitous physical disturbance associated with the construction and/or maintenance of their infrastructure.

Being a city with relatively well developed economy, in Cluj-Napoca, a significant part of the factories are always in a process of dismantling and reconstruction, which tends to generate a constantly shifting mosaic composed of opportunistic plant associations dominated by disturbance-tolerant, early successional species. An edifying example of

tolerance and adaptability to extreme conditions offered by the urban areas is the way that a fern manages to survive on the wall of a building in Cluj-Napoca (Fig. 4, 5). The images were captured in 2009 and 2011, demonstrating the resilience and adaptation of this species in adverse conditions. After analysing the urban landscape of Cluj-Napoca we find that in neighbourhoods with the highest property value - spontaneous vegetation is found in relatively small quantities, while in the poorer neighbourhoods and industrial areas this type of vegetation abounds (Fig. 6). The origin and global dispersal of the spontaneous vegetation that dominates abandoned urban land is both a cultural and a biological phenomenon [KOWARIK, 2003; KOWARIK & LANGER, 2005; MACK & ERNEBERG, 2002]. This vegetation is represented by a cosmopolitan range of species, as follows: 1) native to the area; 2) formerly or currently cultivated for agricultural or horticultural purposes; 3) unintentionally introduced, disturbance adapted weeds.

An exhaustive literature review of the vegetation of 54 cities in Central Europe indicates a “remarkable concentration of aliens in urban areas” [PYŠEK, 1998]. In recent years, a number of European researchers have gone so far as to propose that certain inner-city areas with relatively old patches of spontaneous vegetation be actively conserved because of the role they play in generating and maintaining urban biodiversity [KOWARIK, 2005; MURATET & al. 2007; RINK, 2009].

PETER DEL TREDICI (2010) shows that the spontaneous vegetation of North American cities has not been studied as extensively as that of European cities. CLEMANTS & MOORE (2003) found that the non-native species richness of urban areas in the U.S. is probably more influenced by historical and socio-economic factors than by climate or latitude. Part of the spontaneous flora that dominates American urban landscapes is due to successive waves of immigrants, which along with their traditional cultures have introduced associated weeds [MACK, 2000; MACK, 2003; MACK & ERNEBERG, 2002]. Also, Del Tredici notes that in contrast to the large number of European plants introduced in North America, few species native to this region were able to penetrate and be naturalized in Europe [WITTIG, 2004; DEL TREDICI, 2010]. The asymmetry of the biological exchange between the two continents is partly a reflection of the lopsided nature of the cultural exchange between the two continents and partly a result of the fact that Europe, for reasons relating to both cultural and evolutionary history, seems to be unusually rich in disturbance-adapted herbaceous species [WEBER, 1997].

The idea that self-sustaining, historically accurate plant associations can be restored to urban areas is an idea with little credibility in light of the facts that 1) the density of the human population and the infrastructure necessary to support it have led to the removal of the original vegetation; 2) the abiotic growing conditions of urban areas are completely different from what they were originally; and 3) the large numbers of non-native species that have naturalized in cities provide intense competition for the native species that grew there prior to urbanization. [DEL TREDICI, 2010]. Ecosystem services provided by the urban spontaneous vegetation include: temperature reduction; food and/or habitat for wildlife; erosion control on slopes and disturbed ground; stream and river bank stabilization; excess nutrient absorption in wetlands (mainly nitrogen and phosphorus); soil building on degraded land; improved air and water quality; sound reduction; phytoremediation of contaminated soil [POREÇBSKA & OSTROWSKA, 1999]; and carbon sequestration. At the functional level, spontaneous urban vegetation can be considered sustainable in the sense that it is performing a wide range of quantifiable ecosystem services on marginal land with a minimal input of maintenance resources [DEL TREDICI, 2010; RINK, 2009].

While it is relatively easy to enumerate the ecological value of spontaneous vegetation, it is considerably more difficult to quantify its social and aesthetic value [KÖRNER, 2005]. Many of the people who live in cities tend to interpret the presence of spontaneous urban vegetation in their neighbourhood as a visible manifestation of dereliction and neglect, even though they may view the same plants growing in a suburban or rural context as “wildflowers” (Fig. 7). Unfortunately, the social and aesthetic values of spontaneous, ecologically functional urban landscapes often leave something to be desired. This raises the question of whether or not there is a way to harmonize the ecological functionality of spontaneous urban vegetation with people's desire to live in a safe and beautiful environment.

Perhaps the most famous example of the successful integration of spontaneous vegetation into a designed landscape is Peter Latz's Landschaftspark in Duisburg-Nord, in the Ruhr area of Germany, which transformed the contaminated ruins of an abandoned steel mill into a dynamic, heavily visited cultural center (Fig. 8). Another worthy of being followed example, is the High Line Park in New York (Fig. 9). It is noted in recent years, an encouraging evolution, but not sufficient, of these types of sustainable landscapes, in which the spontaneous flora is an important factor, being preserved and ruderal and post-industrial urban landscapes are rehabilitated with minimum interventions in behalf of an ecological environment.

Conclusions

Environmental education in Romania, both of specialists and the general public, improves visible and continuously. Given the conditions - economic, political, social - from Romania we expect that concrete actions are needed for the increased interest on this issue will not remain at the “trend” scenario, projects, experiences and forecasts level but also to be applied in order to obtain tangible and extensive results of this type of sustainable landscape.

We recommend therefore that all those involved to overcome the divisive arguments on the relative value of native species versus exotic species, and to focus on the ecological functionality of spontaneous urban vegetation.

The task we face is not how to eliminate these plants, but rather how to manage them to increase their ecological, social, and aesthetic values. In the short term, after the scientific literature review and spontaneous landscape of Cluj-Napoca brief analysis, emerges a clear but not optimistic conclusion: due to simultaneous or separate action of several factors (economic, perceptual, political, social environmental, climatic, etc.) spontaneous vegetation is unlikely to play a significant role in future development of urban ecosystems in our country.

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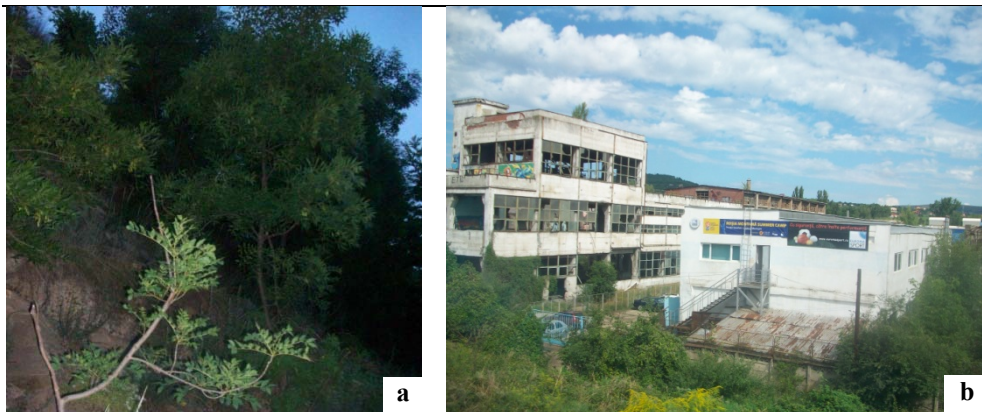


Fig. 1. Remnant native landscape (a), abandoned ruderal landscape (b) and managed horticultural landscape (c) – also identified in Cluj-Napoca



Fig. 2. Ruderale landscape – margins of transportation infrastructure – Cluj-Napoca



Fig. 3. Ruderale landscape – abandoned industrial property (a), interstitial spaces (b) Cluj-Napoca



Fig. 4. Fern development on the Cluj-Napoca building wall, May, 2009



Fig. 5. Fern development on the Cluj-Napoca building wall, August, 2011



Fig. 6. Cluj-Napoca – spontaneous vegetation in zone with different values: (a) industrial areas and (b) in the poorer neighbourhoods, Cluj-Napoca



Fig. 7. Spontaneous vegetation in rural landscape and in urban landscape – different perceptions - (a) positive perception in rural landscape; (b) negative perception in urban landscape



Fig. 8. Landschaftspark-Duisburg
Source: <http://www.landschaftspark.de/architektur-natur>



Fig. 9. High Line Park – New York
Source: <http://flolo.blogspot.com/2010/09/life-on-high-line.html>

GLOBAL WARMING: IMPLICATIONS AND ANTICIPATORY ADAPTIVE MEASURES

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Abstract: Our earth is warming up. There is no denying to this fact that the gradual heating up of our globe has a tremendous effect on the climate. It in turn has affected the biotic factors that make up our biosphere, eventually directing the course of our socio-economic development. Some workers are, however, optimistic about this natural phenomenon. Various ways have been suggested to mitigate the effects of global warming, but the damage already done cannot be revoked. Hence, the thing that we are left with is to go for anticipatory adaptive measures so as to tone down the intensity of future implications of global warming.

Key words: global warming, anticipatory adaptive measures, climate change, biophysical impacts

Introduction

There is a sudden uproar all-round the world about the increasing global temperature. Atmospheric CO₂ is accelerating upward from decade to decade. In the past decade, the average annual rate of increase was 1.91 parts per million (ppm). This rate of increase is more than double what it was during the first decade of CO₂ instrument measurements at the Mauna Loa Observatory. The concentration of CO₂ had gone from 350 ppm in 1950 to 385 ppm in 2009 [ESRL, 2009]. Statistically, the scientists all round the globe agree on appoint that our earth is definitely warming up. The change on earth is defined as “a change in the climate which is attributed directly or indirectly to human activities that alter the composition of the global atmosphere and which are in addition to natural climate variability observed over comparable time period” [UNFCCC, 1997]. In this definition human has been accounted as the sole reason for the changes in the climate, which is opposable if we take into account the solar activities.

Global warming cannot be simply and wholly attributed to the increase in the green house gases (GHGs) due to human-activities. Solar activities and related aspects have to be given due consideration, at the same time. Since the solar activities are by the far, under-estimated and the activities of the human are more evident, we seem to direct all our attention and resources towards the “anthropogenic causes” of global warming. In this paper we have tried to sum up the impacts of the global warming and tried to take a holistic approach and discussed. The factors contributing largely to global warming have been dealt with in details to give a fair picture of the global scenario.

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Material and methods

This paper is a compilation of a large number of works done on global warming both by Indian as well as foreign researchers. The paper has been prepared with a simple, yet necessary perspective of summing up the effects of climate change due to global warming on vegetation and other living organisms and the ultimate effect on the socio-economic aspect. In due course, it is our belief that certain misconception and under-estimation of certain facts can be overcome. All the factors that appear to be contributing to global warming have been discussed with examples from different parts of the world to give a crystal clear picture.

Results and discussion

1. Biophysical Impacts (Tab. 1)

1.1. Climatic and Geological aspects

Temperature has evidently increased all throughout the globe. There has been an increase in global temperature over the years [IPCC, 2007] report. With the increase in global temperature, the temperature of the sea water has risen, decreasing the solubility of carbon-dioxide on its surface which ultimately increases partial pressure of CO₂. Consequently, uptake of CO₂ by ocean water has reduced, thereby increasing greenhouse effect. RAWAT & RAWAT (1998) and NEGI & al. (2003) observed that monsoon rains have declined over the past seven decades, whereas, the local rains have substantially increased, while studying rainfall pattern in Doon valley. Also, precipitation is likely to increase in the mid and the high latitudinally placed regions in the northern hemisphere [LEGGETT, 1990]. With the increase in the evaporation the amount of water-vapour in the atmosphere is bound to increase which will consequently increase the humidity, affecting the overall precipitation regime. Clouds account for the cooling effect in the Earth's atmosphere. But if more clouds are formed at the higher altitudes (which are cooler), they will emit less radiation, supplementing to greenhouse effect and thereby increasing the temperature [RAMANATHAN & al. 1989]. Also it has been suggested by CHARLSON & al. [1987] that emission of di-methyl sulphide (DMS) by marine planktons, which serve as condensation nuclei, might act as global thermostat. This will increase in cloudiness and lead to cooling of the ocean surface. But this might also affect the activity of the plankton, thereby closing the loop.

According to the IPCC (2001), the pattern of wind is likely to change. The inter-decadal Pacific Oscillation and the Pacific Decadal Oscillation bring about variability in the climate of Pacific Basin. Likewise, North Atlantic Oscillation (NAO) accounts for the westerly over the Atlantic and the extra-tropical Eurasia, which are likely to become strong. A similar Antarctic Oscillation affects the westerly in the southern hemisphere which has shown an increase in the strength over the past 15 years. There would be a shutdown in the thermohaline and thermocline circulation.

The increase in the percentage of the atmospheric will cause an increased amount of CO₂ to dissolve in to the ocean. This leads to the formation of carbonic acid that lowers the pH of the ocean [UNEP, 2002]. In India, the Gangotri glacier has been receding at an average rate of 19m per year over the last 65 years [SHANKER & SRIVASTAVA, 1999]. Hence, melting of ice-caps and receding of glaciers will lead to increase in the level of the sea and submerge the low-lying coastal regions, consequently.

Climatic models have shown that drought is likely to increase from 5% to 50% by the year 2050 A.D. The hardest hit regions would be north Africa, south Africa, western Arabia, south east Asia, Mexico, Central America, south west USA, and the Mediterranean belt [IPCC, 1990]. The frequency and intensity of drought have been observed to increase in the recent decades. Also, part of the Amazon rain forest will turn into desert by 2050. Another concern is the possibility of a positive feedback loop, i.e., global warming can cause further warming in a vicious circle. Melting ice-caps appears to be causing the release of large amount of CO₂ and methane from decaying vegetation trapped beneath. It also could lead to increased heat absorption as ice reflects more because of its higher albedo than land and water.

1. 2. Edaphic factors

The decomposition of the soil depends upon the temperature of the soil, the presence/population of effective soil micro-flora and fauna, the type of litter (lignin: N ratio, carbon content etc.), availability of soil moisture in adequate amount, species composition and structure of plant community. It is an established fact that the colder biomes are much more sensitive and responsive towards global warming than the warmer ones. As a whole, litter decomposition in colder biomes is likely to increase, along with a steady influx of CO₂ into the atmosphere. The Q₁₀ value, which is taken to be 2 for biological processes, is found to be 3-4 in the arctic regions [ROBINSON & al. 1983]. The decomposition rate has been found to be more in the colder and wetter sites of higher altitudes than the warmer and drier sites of the lower altitudes [MURPHY & al. 1998]. IPCC (1990) states that it is not possible to predict reliably either the geographical distribution of changes in soil-water or the net effect of these changes on carbon fluxes and storage in different ecosystems': with the increase in the temperature, summer monsoon rainfall has increased over the Indo-Gangetic plains between 1829 and 1999, whereas, it has been estimated that the Indian sub-continent is to experience a decline of 5-25% in the winter rainfall [BHARDWAJ & PANWAR, 2003], which would have a profound effect on the soil moisture status.

Climate change impact on water quality is the result of precipitation above the infiltration capacity of the soil or the exceedance of the water holding capacity of the soil volume causing drainage of water through soil-profile. Increase in precipitation amount or frequency of storm even with decreased total amounts in each storm can have serious implications for surface run-off, drainage and water quality [HATFIELD & PRUEGER, 2004]. Nitrogen and Phosphorus cannot be applied in the soil when the probability of rainfall immediately after application is high [EGHBALL & al. 2002].

1.3. Biological factors

Species diversity will decline. Forests will be rapidly destroyed without their restoration or replacement. Species will be lost as climate and habitat migrate out from under them. Also the specific ecotypes- specific combination of genes accumulated for each locale by selection through many generations – will also be lost. Forests will be substituted by savannahs, shrub lands and grasslands.

If we study the adaptation pattern in the world of fauna and flora we get to see the following:

Swamp Adaptations in plants

Stilt roots for support in swampy areas, breathing roots or pneumatophores in mangrove species (either seasonal or permanent) might be seen to fulfill the oxygen requirement of roots [CHANDRASHEKARA & SREEJITH, 2003]. This is in particular to the areas where the water table has increased and hence plants and trees exhibit such type of swamp adaptations.

Phenological Adaptations

Climate change has led to an advance in phenology in many species. Synchrony in phenology between different species within a food chain may be disrupted if an increase in temperature affects the phenology of the different species differently, as is the case in the winter moth egg hatch–oak bud burst system. *Operophtera brumata* L. (winter moth) egg hatch date has advanced more than *Quercus robur* L. (pedunculate oak) bud burst date over the past two decades. Disrupted synchrony will lead to selection, and a response in phenology to this selection may lead to species genetically adapting to their changing environment [MARGRIET & al. 2007]. BROWN (2003) reported the early flowering of meadow foxtail and coxfoot 7-10 days earlier in 2002 than in 2001.

Seed dispersal

Different palaeo-ecological records predict that a 2.5 °C warming equates to an altitudinal displacement of more than 400 m and a latitudinal displacement in northern Europe by more than 300 Km [HUNTLEY, 1991] which allows them to adapt to the long distance seed-dispersal mechanism.

Other adaptations

According to GRIME (1979), we should expect the competitive species that dominate many ecosystems to be replaced by more ruderal species which might show various adaptations, like shortening of life-span, greater commitment of resources to propagule production and exploiting disturbed situations. The reproductive phenology of the plants is likely to change like inbreeding depression, ovule abortion, non-viable pollen production, etc. [KHANDURI & al. 2003] Also the dates of cultivation are likely to change by \pm one month in the warmer world. It is observed that if moisture is available, an increase in the temperature commonly increases rate of respiration by 10-35% or more per 1°C rise in temperature. In the warmer world, both the temperature and the moisture is higher which would increase the respiration rates of the living organisms, consequently reducing both net primary production and net ecosystem production [WOODWELL & WHITTAKER, 1968].

There are two schools of thought regarding the effect of global warming on the rate of photosynthesis. One says that increased level of CO₂ in the atmosphere with higher precipitation and temperature will increase photosynthetic rates in plants upto a certain limit. This will increase the net productivity or yield. For example, KELLOMAKI & al. (1997) reported that a combination of temperature increase of 0.4 °C per decade, 10% increase in annual sum of precipitation and 33 μ mol per decade increase in atmospheric CO₂ will increase timber yield by 30% in one rotation. Another group of scientists say that the warming in continental centers will increase the arid zones globally at the expense of currently forested areas which will lower the net production [WOODWELL & WHITTAKER, 1968].

The polar bear (*Ursus maritimus*), also known as the Great White Bear, Ice Bear, and Nanook, is the largest of the world's bear species. Polar bears live only in the Arctic and are completely dependent upon the sea ice for survival. Scientists have already recorded thinner bears, lower female reproductive rates, and reduced juvenile survival in

the Western Hudson Bay, polar bear population in Canada, which is at the southern edge of the species' range and the first to suffer impacts from global warming. This mighty hunter now faces likely extinction by the end of this century because its sea ice habitat is literally melting away due to global warming [Centre for Biological Diversity, 2005]. *Atalopedes campestris* (skipper butterfly), a cold-intolerant species, has expanded its territory and now has a range edge between Yakima and Ellensburg as the winters have become warmer in the northern California [CROZIER, 2004].

Tab. 1. Impacts of global warming on the biophysical aspects

PARAMETERS		POSSIBLE CHANGES
Climate and Geological	Temperature	Increase
	Precipitation	Increase in local rains, decrease in monsoon rains in the tropics. Increase in the mid and high altitudes of the northern hemisphere
	Humidity	Increase
	Cloudiness	At high altitude it would be more, leading to positive GHE, in sea-surface it would show negative GHE
	Atmospheric and Oceanic circulation	Wind direction to change, westerly to become stronger, in both north and south hemisphere, shut down of thermohaline and thermocline circulation
	Ice-cap and Glaciers	Melting at steady pace, increase in sea level
	Ocean Acidification	Acidity will increase
	Drought and Desertification	5-50% increase by 2050 A.D. in the lower latitudinally placed regions in the north hemisphere
	Hurricanes, Storms and tsunamis	Will increase with time
Compounding Effects	Further warming in vicious circle	
Edaphic factors	Litter Decomposition rate	Likely to increase in the colder biomes; in the tropical regions, it is likely to increase unto a certain limit and then decrease
	Soil Fertility	Decrease with increased run-off and high rate of erosion
	Soil Moisture Content	Will increase in higher altitude and decrease in lower altitude (depending upon soil type)
Biological factors	Species composition	Change according to Location, Climatic and Edaphic conditions: a. extinction
		b. change in migratory habits and migration to suitable sites
		c. habitat fragmentation
	Adaptations in plant communities	
	a. Phenological Adaptation	Stilt roots
	b. Seed Dispersal Mechanism	Plants likely to adapt themselves to long distance seed-dispersal mechanism.
	Other Adaptations	Early blooming, fruiting, shortening of life-span, etc.
	Respiration Rate	Increase
Photosynthesis Rate	Increase upto a certain level and then decrease	

2. Socio-economic Aspects (Tab. 2)

Worldwide, hundreds of millions of people would be displaced by the inundation of low-lying coastal plains, deltas, and islands in this very century if efforts to reduce the GHGs are not successful (Tab. 3). The situation will be grave due to spread of aridity and eventually impoverishment [LEGGETT, 1990].

Taking into account the composite effect of climate, geology and edaphic factors, the agricultural production is likely to be highly affected. The summers of 1988 A.D. was recorded to be unusually hot the drought that followed caused a loss of 30% in the grain production in North America [IPCC, 1990].

In many cases the amount of food available to the population at large may not be greatly reduced, but certain sectors of the population may be significantly affected in terms of entitlement to food, either by reduced income or lack of resources by subsistence agriculture [STRZEPEK & SMITH, 1995]. Cities, Hydro-power projects, irrigated agriculture, shipping, the various uses of waterways, fish and fisheries, the transport, dilution and treatment of sewage, and the circulation of coastal and oceanic water are all dependent upon the flow of fresh water from the land. As water resources go on diminishing, there is an increased risk of conflicts amongst and within Nations [IPCC, 1990]. With the increase in global warming; there will be a simultaneous increase in natural disasters. This, combined with the inter- and intra-national conflicts will cause extreme psychological disturbances to the survivors and the people involved in the conflicts [CHAMBERLIN, 1980].

Financial institutions, including the two largest insurance companies, Munich Re and Swiss Re warned that ‘the increasing frequency of severe climatic events, coupled with social trends could cost almost US\$150 billion in the coming decades’ [UNEP, 2002]. Production of non-wood produce from forests will decrease due to insufficient accumulated winter chilling, thereby affecting the economy of the tribals severely in India [TEWARI, 1994].

During summers the need of the energy will heightened and in winter it is speculated to go down, affecting production of energy accordingly, if low and mid latitudes of northern hemisphere would become warmer [ROSENBERG & CROSSON, 1991].

Climate change will directly or indirectly affect the human health in various ways. The distribution of a range of diseases currently confine largely to the tropics, viz., malaria, trypanosomiasis, kalaazar, filariasis, and various worm-infestations are correlated to the temperature and could be affected by climate change as vectors of these pathogens would have an extended habitat range. Distribution of other non-parasitic communicable diseases yellow fever, dengue, plague and dysentery is also related to temperature [GILLETT, 1981]. Development and multiplication of various parasites within their hosts depend on the mean ambient temperature [GARNHAM, 1964].

Tab. 2. Impacts of global warming on the socio-economic status of human beings

PARAMETERS	POSSIBLE CHANGE
Human Settlement and Society	Millions of people to become homeless due to inundation of low-lying coastal regions
Agriculture and Food Production	Production potential of mid and higher latitudes will increase and that of the lower latitudes will decrease in the northern hemisphere
Food Entitlement	Per capita will decrease
Conflicts between Nations	Will rise over sharing resources, particularly water
Mental health	Will deteriorate with increasing natural disasters and conflicts

Tab. 3. Land and population at risk due to 1 meter sea level rise

Country	Land at risk		Population at risk	
	Sq. Km	%	Sq. Km	%
Bangladesh	25000	17.5	13	11.0
Egypt	4200-5250	12-15	6.0	10.7
Senegal	6042-6073	3.1	0.1-0.2	1.4-2.3
Malayasia	7000	2.1	NA	NA
Nigeria	18398-18803	2.0	3.2	3.6
China	125000	1.3	72.0	6.5
Venezuela	5686-5730	0.6	0.06	0.3
Uruguay	94	<0.1	0.01	0.4
Argentina	>3430-3492	>0.1	NA	NA
TOTAL	194852-196498	-	94.4-94.5	-

(Source: NICHOLLS & LEATHERMAN, 1995)

Adaptations

There are two types of responses to climate change and its consequences: Mitigation and Adaptation. Mitigation means retarding or limiting or eradicating the 'causes' completely; whereas, adaptation means acclimatization to the 'effects', rather than the causes. Broadly adaptations are of 2 types – 'Autonomous' i.e., biophysical adaptation to climate change by living organisms and 'Fostered' i.e., adaptations driven by policies [PARRY & CARTER, 1998]. Again fostered adaptations are of 2 types- 'Reactive' (policies taken as response to the changes) and 'Anticipatory' (policies taken in advance to anticipated climate change) [SMITH & al. 1996].

Amongst the said steps, anticipatory adaptations policies should be given priority as they give maximum assurance in minimizing potential negative impacts of climate change advance.

Why should we go for adaptations?

Mitigation and adaptation are complementary responses and both are needed to offset the effects of climate change on earth. But mitigating measures show many problems and have less feasibility during implementation, particularly in cases directly effecting economic development as adaptations deals with the impacts rather than the causes, it is more realistic. Again, anticipatory adaptations are preferred in case of uncertain and irreversible impacts, dependent on climate whose rate of change is very rapid, or threaten human health and safety and which may cause risk if we are to follow short term approaches depending upon sudden climate extremes (Tab. 4a). Anticipatory policies are to be examined before and after implementation to assess net benefit, flexibility, urgency, further research and equity and political feasibility (Tab. 4b).

Anticipatory measures are to be preferred over reactive measures because reactive measures cannot offset the negative consequences of climate change, once they occur, thereby affecting both biophysical and socio-economic aspects. Besides these (Tab. 4c), anticipatory adaptations provide more time to living beings as well as the society to adapt themselves to the changing climate.

GLOBAL WARMING: IMPLICATIONS AND ANTICIPATORY ADAPTIVE MEASURES

Tab. 4a. Issues Involved in Determining the Need for Anticipatory Adaptation

Issues and Concerns	Characteristics
Uncertainty	Timing magnitude, direction of climatic change, impacts and their results are uncertain [SMIT, 1993]
Irreversibility	Certain impacts cause long term damages that require anticipatory adaptation
Effective life of policy	Long term decisions made today may need to be reduced down or toned down in future
Importance of climatic trend	Recent knowledge of storms or droughts should be taken into consideration to modify the long term anticipatory steps [GLANZ, 1988]
Rate of climate change	Reactive adaptation to a rapidly changing climate will be more difficult than to a gradually changing climate
Health and safety	Anticipatory steps can be said to be effective only if it does not harm or affect the health and condition of the ecosystem as a whole and jeopardize the safety of its components

(Source: SMITH & al. 1998)

Tab. 4b. Criteria involved in Designing and Implementing Anticipatory Policies

Criteria	Properties/ Structures
Benefit/ Cost Analysis	Discounted benefits of adaptation measures should substantially exceed the discounted cost to get the highest priority. A newly designed policy may not be appropriate if it does not take into account the special needs, e.g., threat to spp. extinction
Urgency	If the rate of change of climate is rapid rather than gradual, then the anticipatory policies should be implemented quickly to draw maximum benefit from it
Flexibility	It should be flexible enough to enable a resource to adapt itself successfully to a wide range of future climatic pattern, since the direction and the magnitude of climate change is uncertain
Use of Planning	Small adaptation policies with marginal action on pre-determined policies should be used so as to enhance the reacting ability of a resource system to climate change, within a low cost range
Mitigation Effect	It should be designed in such a way that it has a dual effect, i.e., these should help to mitigate climate-change impact to some extent, simultaneously with adaptation to climate change
Research	Appropriate research should be incorporated in the designing of the policy, though in case of urgency, there is little scope as it takes years to develop a model based on sustained climatic effects on various factors
Equity and Values	Every sector of a society should be treated and considered equally during policy designing, irrespective of their economic status. Policies should conform to the needs and wants of the human within the limitation of resources

(Source: STRZEPEK & SMITH, 1995)

Tab. 4c. Possible Anticipatory Adaptation Policies in Different Sectors

Sectors	Parameters	Possible anticipatory adaptation policies
Coastal Zone Management	Wetland Preservation	Healthy Wetlands are to be preserved so as to store the water of excessive precipitation
	Integrated Development	Identification of land areas to be affected by climate change and compilation of all data in an integrated form
	Improved Coastal Model	New and improved model to be made based on the prior evaluation of responses
	Land Use Planning	Perseverance of landscape, plans for shoreline development
Water Resources	Conservation	Drip irrigation, sprinkler irrigation, etc. are to be mass introduced by the government in agriculture
		Recycling of sewage water
	Market Allocation	Market based allocation of water
	Pollution Control	Assurance of quality and safety limits of water
	River Basin Planning	Sediment sluicing, maintenance of proper height of dam, length of canal, etc.
		International demarcations and compromise on common water resources
Environmental concerns and socio-economic factors are to be given due consideration		
Drought Contingency Planning	Conservation of wetlands, building of reservoirs, ensuring efficient water use, prediction of drought years, etc.	
Human health	Weather/ Health Watch/ Warning Systems	Prediction of possible outbreaks of diseases related to weather change
	Public health Improvement	Integrated pest management steps to combat large scale spread of contagious diseases
	Improvement of Surveillance System	Compilation and computation of future outbreaks based on previously collected relevant data
Ecosystem	Biodiversity	Ecological diversity and balance as well as the diverse gene pool are to be maintained
		Preservation of endangered spp. ex situ, preservation of their natural habitat, artificial regeneration
		Afforestation, reforestation and plantation (natural CO ₂ sink)
Agriculture	Protection and Enhancement of Migration Corridor	Corridors and buffer-zones around the reserved areas should be protected
	Watershed Protection	Reforestation of areas in watershed to prevent bank erosion, siltation and soil loss
	Irrigation Efficiency	Improved irrigation methods are to be adopted to decrease water consumption, after computing cost benefit analysis
	Development of New Crop-Types	Development of better heat and drought tolerant crops

(Source: SMITH & TRIPAK, 1989)

Conclusion

Summing up what we have compiled in this work, we can conclude that it is a fact that climatic change due to global warming cannot be denied and already substantial losses have been faced with by the earth and its inhabitants. But again there remains extensive scope for further research and experimentations into the causes of global warming. Though certain impacts are positive, most of them that we see and can estimate are negative and pose to be threat to our very civilization on earth. Hence arises, the need to mitigate the same and adopt preventive measures. But, once the damage has been done, it becomes difficult to restore the situation. Therefore, it is advisable to adopt measures that are in anticipation to the changing climate, based on previous records and data of changes on earth. Anticipatory adaptive measures provide time and scope for better acclimatization and are more practical and feasible with respect to implementation.

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PROFESSOR PhD TOADER CHIFU AT THE 75TH ANNIVERSARY



Professor PhD Toader CHIFU was born in Târnăuca, Jud. Dorohoi (nowadays the locality is in Ukraine Republic), on February 27, 1936.

He started the primary school courses in 1941 in his origin village Crihănești - Târnăuca, but because of the war he finished these studies in 1946 at the Șendriceni Applications School. The Secondary School has been realized in Pomârla (exception the 7th year followed in Bucecea). Also in Pomârla he followed the courses of the Technical Agronomical School (graduated in 1954). After the graduate of the secondary school he worked as agronomist technician (1954 - 1956).

In 1956 he became a student in the Natural Sciences – Geography Faculty within “Alexandru Ioan Cuza” University from Iași (graduated in 1960).

After he graduated the academic studies, he held the assistant function (1960 - 1965) in the Botany Department of the Biology - Geography - Geology Faculty and since 1965 until 1968 he worked as a botanist in the Botanic Garden from Iași. Since 1968 he worked as a Scientifically Researcher in the Geobotany Laboratory within Iași Filial of the Romanian Academy, and since 1976 worked as a Scientifically Researcher in the Biological Research Center from Iași, where he was the Chief of the Terrestrial Ecology collective. As recognition of the value of his research he was invited to keep courses of lectures and research stages in the National Agronomical Institute from Alger (1976-1980) in Systematic Botany, Phytogeography, Vegetal Ecology, General and applied biocoenotics domains. In 1981 - 1989 periods he gave lectures of Systematic Botany, Tropical and Subtropical Flora, Vegetal taxonomy, Photosynthesis and productivity of ecosystems and Environment Protection Fundamentals in the Faculty of Biology. In 1990 - 1992 he hold the Senior Researcher function in the Biological Research Institute from Iași, and in 1992 - 1995 period he hold the Senior Lecturer function within the Vegetal Biology Department of the Faculty of Biology from “Alexandru Ioan Cuza” University Iași. Starting with 1995 year he is a Professor at the same institution.

He holds a PhD diploma in Phytosociology - Mycology since 1971 at “Alexandru Ioan Cuza” University from Iași. Currently he is Consulting Professor and a PhD coordinator within the Faculty of Biology Iași.

The research activity of Professor Chifu, as reflected by over than 150 published papers and 7 books covers five main directions: mycology and mycocoenology, systematic botany, phytosociology, structure and productivity of ecosystems and nature conservation. In all of these areas he obtained significant results as:

- identification of 700 macromycetes taxa from Moldova and Dobrogea (approximately 50% from all taxa known in Romania);

- discovery of 20 new macromycetes taxa for Romania;
- contributions to the chorology of 2.300 cormophytes taxa (1 new for Romania) and over 300 coenotaxa (47 new for science: *Cembreto - Piceetum abietis* Chifu et al. 1984; *Medicagini - Festucetum valessiaca anthoxanthetosum* Chifu et Ștefan 1978 etc.);

During field and laboratory research activities professor Chifu collected and identified a rich floristic material and initiated a herbarium including vascular plants at the Biological Research Institute. Also he contributed to the enrichment of the herbarium collections from the Biology Faculty Iași (approximate 500 mycological herbarium sheets and 400 vascular plants sheets, inclusive species from Algeria).

He realized documentation trainings in Hungary (1974) and Switzerland (1991). Also, he participated to numerous international scientific meetings: Excursion International de Phytosociologie en Suisse, Geneva (1991); International Excursion of Phytosociology in Apuseni Mountains (1993); Colloque “Végétation et sols de montagnes”, Grenoble - France (1996); II-ème Congrès de la Fédération Internationale de Phytosociologie, Bailleul - France (1997); XXVIII-ème Colloque Phytosociologique, Camerino - Italy (1998) etc. He participated at numerous scientific and didactic excursions in Atlas Mountains (Algeria and Morocco), Sahara Dessert (Algeria), Swiss Alps and French Alps, French Central Massif, North Sea Shore (France), Pyrenees Mountains (Andorra and France), Apennines Mountains (Italy) etc.

He has, also, received multiple grants for research – 50 research grants as responsible or coordinator. Professor Chifu is a distinguished member of the scientific board for journals like: Romanian Journal of Biology – Plant Biology (ex *Révue Roumaine de Biologie, série Biologie végétale*); *Analele Științifice ale Universității “Alexandru Ioan Cuza” Iași, s.II.a Biologie vegetală*; *Cercetări Agronomice din Moldova*.

Professor Chifu is a founder member of the Romanian Phytosociological Society, Romanian Mycological Society and Gh. Lupascu Foundation for Science and Culture. He is also member in the Ecology Society from Romania, Amicale Internationale de Phytosociologie Society (Bailleul – France) and Association pour l'étude de la végétation (Uppsala - Sweden).

This is the didactic and scientific activity realized in his life by Professor Toader Chifu. Beyond all these important realizations, Professor Toader Chifu is a man with a high moral and professional conduit. All his achievements became possible due to his tenacity, perseverance and passion for botany and also due to his family understanding and support.

The 75th anniversary represents a jubilee moment both for professor Chifu, for his family and also for the entire academic community. On the behalf of all the colleagues from the Botanic Garden “Anastasiu Fătu” of Iași, we wish Professor Toader Chifu a long life, good health and all the best for many years to come.

Happy Anniversary!

Constantin MARDARI, Cătălin TĂNASE

BOOK REVIEW

Tatiana Eugenia Şesan & Cătălin Tănase, *Phytopathogenic ascomycetes*, 2011, Bucureşti University Publishing House

Phytopathogenic Ascomycetes volume represents a new contribution to the updating of knowledge on this group of fungi, important both theoretically, but especially from the practical perspective, especially targeting those (asco) taxa that are pathogens on crops but also on the spontaneous species. This paper is addressing to all those who are training and specializing in Phytopathology and Plants Protection domains.

Thus, the book is a continuation of the author's project to update the knowledge of plant pathology, after the publication in 2007 of the book *Anamorphic phytopathogenic fungi*, published by the same team in the prestigious Publishing House of University from Bucharest.

Ascomycetes taxonomy, published so far by the same authors [TĂNASE & ŞESAN, 2006; ŞESAN & TĂNASE, 2006] based on the *Dictionary of the Fungi*, IXth edition (2001), has been updated in this volume after the new edition of the *Dictionary of the Fungi* Xth edition [KIRK & al. 2008] and after the book *Fungal Families in the world* [CANNON & KIRK, 2007], published as an annex to the first dictionary. We mention that, besides the recognized difficulty of the taxonomy of fungi, in this area have recently been recorded many new results, modern, on which different authors have contributed to some changes, clarifications, evaluations and reevaluations that have made imperative the updating of the knowledge in this field, actualizations of which the authors of this paper wanted to align. In the future, the authors have also in preparation a volume dedicated to phytopathogenic basidiomycetes (2012) in order to complete the actualization of the most important taxonomic groups of fungi that cause damage to plants.

The book is divided into eight chapters, starting with general data, fundamental data on the macromycetes diversity and on the variability of this group, on the organization and taxonomy of phytopathogenic ascomycetes. For each phytopathogenic species are presented the relevant symptoms on the host plants, the life cycle, but also elements necessary to prevent and combat the attack. Given the applicability of research results in plant protection domain, tables have been prepared with the synthetic products plant effective in the protection against every disease, especially useful for practitioners.

Protection measures, prevention measures and control measures have been developed, for the diseases produced in crops, forestry, vine cultures, insisting on the un-polluting ones and on the biological control of plant diseases means. The paper includes unconventional elements of biological control of plant diseases, summarized from the current literature, but especially supported by the results obtained over a long career and experience of the authors in the field, confirmed by several works published in Romanian and international journals.

The volume includes 111 figures and 32 tables prepared by the authors and 6 color plates with original photographs. The figures illustrates very well the scientific content, the wealth of information contained in the book and allow more accurate and more intuitive understanding of the knowledge presented.

A glossary of scientific terms completes the book, to better explain the concepts, to understand, learn and use of these terms in the interpretation and evaluation of the achieved results.

We recommend this book especially to biologists students (from the departments of Biology, Ecology and Environmental Protection and Biochemistry), but also to those of the Faculty of Agriculture, Horticulture, Forestry, and amateurs who want to know the main novelty about phytopathogenic ascomycetes and how diseases in plant crops can be eradicated.

Prof. PhD. eng. Eugen ULEA
"Ion Ionescu de la Brad" USAMV, Iaşi

JOURNAL OF PLANT DEVELOPMENT GUIDE TO AUTHORS

Types of contributions: Original research papers, as well as short communications. Review articles will be published following invitation or by the suggestion of authors. "Journal of Plant Development" also publishes book reviews, as well as conference reports.

Submission of a paper implies that it has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that its publication is approved by all authors, and that, if accepted, will not be published elsewhere in the same form, in English or in any other language, without the written consent of the publisher.

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Titles would be written with bold, capital letters, 12 points, centered.

Names and Christian names of the authors would be written with capital letters, 10 points, centered. The names would not be abbreviated; each author name would be accompanied by a complete address, as a footnote on the first page.

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Key Words: few words, the most important ones, after someone could discover your paper on the internet engines.

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The scientific names of taxa would be italicized.

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MEHREGAN I. & KADEREIT J. W. 2008. Taxonomic revision of *Cousinia* sect. *Cynaroideae* (Asteraceae, Cardueae). *Willdenowia*. **38**(2): 293-362.

References for books:

BOȘCAIU N. 1971. *Flora și Vegetația Munților Țarcu, Godeanu și Cernei*. București: Edit. Acad. Române, 494 pp.

HILLIER J. & COOMBES A. 2004. *The Hillier Manual of Trees & Shrubs*. Newton Abbot, Devon, England: David & Charles, 512 pp.

Serials:

JALAS J. SUOMINEN J. LAMPINEN R. & KURTTO A. (eds). 1999. *Atlas Florae Europaeae. Distribution of vascular plants in Europe*. Vol. **12**. *Resedaceae to Platanaceae*. Helsinki: Committee for Mapping the Flora of Europe and Societas Biologica Fennica Vanamo. Maps 2928-3270, 250 pp., ill (maps), ISBN 951-9108.

TUTIN T. G., BURGESS N. A., CHATER A. O., EDMONDSON J. R., HEYWOOD V. H., MOORE D. M., VALENTINE D. H., WALTERS S. M. & WEBB D. A. (eds, assist. by J. R. AKEROYD & M. E. NEWTON; appendices ed. by R. R. MILL). 1996. *Flora Europaea*. 2nd ed., 1993, reprinted 1996. Vol. **1**. *Psilotaceae to Platanaceae*. Cambridge: Cambridge University Press, xlvii, 581 pp., illus. ISBN 0-521-41007-X (HB).

Chapters in books:

†TUTIN T. G. 1996. *Helleborus* L. Pp. 249-251. In: †T. G. TUTIN et al. (eds). *Flora Europaea*. 2nd ed., 1993, reprinted 1996. Vol. **1**. *Psilotaceae to Platanaceae*. Cambridge: Cambridge University Press, xlvii, 581 pp., illus. ISBN 0-521-41007-X (HB).

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