ACADEMY OF SCIENCES OF MOLDOVA
BOTANICAL GARDEN (INSTITUTE)

JOURNAL OF BOTANY

VOL. VII **NR. 1(10)**

Chisinau, 2015

FOUNDER OF THE "JOURNAL OF BOTANY": BOTANICAL GARDEN (INSTITUTE) OF THE ASM

According to the decision of Supreme Council for Sciences and Technological Development of ASM and National Council for Accreditation and Attestation, nr. 288 of 28.11.2013 on the approval of the assessment and Classification of scientific journals, "Journal of Botany" was granted the status of scientific publication of "B" Category.

Edition supported by the Supreme Council for Sciences and Technological Development of ASM

Botanical Garden (Institute)
MD – 2002, Padurii str. 18, Chisinau, Republic of Moldova
tel./fax.: (+373 22) 52-35-89; 55-04-43
www. gradinabotanica.asm.md
e-mail: cancelarie.gb@asm.md

CONTENTS

I. Structural and functional diversity of plant organisms

II. Conservation of biological diversity

III. Introduction of plants and sustainable use of plant resources

IV. Landscape architecture

I. STRUCTURAL AND FUNCTIONAL DIVERSITY OF PLANT ORGANISMS

EFFECTS OF NATURAL GROWTH REGULATOR *REGLALG* ON THE *RHODIOLA ROSEA* L. CALLUS GROWTH RATE

Calugaru-Spataru T.

Institute of Genetics, Plant Physiology & Protection, Academy of Sciences of Moldova, 20 Padurii str., MD-2002, Chisinau, Republic of Moldova

Abstract. The application of the natural growth regulator (NGR) Reglalg to nutritive medium of *Rhodiola rosea* L. callus cultivation in vitro was investigated. The highest callus biomass was recorded in the experiences with dilutions of Reglalg(R) with nutritive medium in ratio 1/1000. At the same time, it was established that Reglalg cannot substitute such growth regulators as 6-benzylaminopurine (BA) and α-naphthylacetic acid (NAA).

INTRODUCTION

New substances with physiological active properties are discovered permanently. These compounds, called plant growth regulators (PGR), are classified along with five known categories of classical phytohormones (auxins, cytokinins, gibberellins, abscisic acid and ethylene). Among PGR can be remarked polyamines, some oligosaccharides, jasmonic acid, sterols, brassinosteroids, salicylic acid and its derivatives (Gaspar, 1996).

Since 2000, a series of investigations were initiated in the Centre of Advanced Biological Technologies, Institute of Genetics, Plant Physiology and Protection, Academy of Sciences of Moldova, which aimed to study the influence of NGR *Reglalg* on some physiological processes in plants. It has been revealed that *Reglalg* is a natural growth regulator which at low doses stimulates the growth and development of seedlings (Dascaliuc et al., 2007). At the same time, NGR *Reglalg* at high doses exerted an inhibition on physiological processes, developed in vegetal cells. Also, it was established, that application of *Reglalg* conducted to an acquisition by plants of the systemic resistance against phytopathogenic (viral, bacterial or fungal) agents (Dascaliuc A., Voineac, 2006, 2012). The NGR *Reglalg* is supposed to be an important PGR involved in vegetal phenomena, connected to the plant development processes, plant reactions to diverse stresses (Cicalov, 2011), including pathogen attacks (Dascaliuc A., Voineac 2006). Utilization of *Reglalg* for seeds treatment showed that this NGR is involved in such physiological processes as seed germination,

subsequently seedlings development (Dascaliuc A., 2007, Kiricenco E. 2013, Caus M. 2013).

Also, experiences were initiated for investigation of the influence of *Reglalg* on growth and development of plant explants and cells *in vitro*. Cultivation of explants *in vitro* represents a biological model, which provides the opportunity to study the mechanisms of cell dedifferentiation and differentiation, morphogenesis and cell interactions under controlled conditions.

In this work the influence of NGR *Reglalg* application at different grade of dilutions on *Rhodiola rosea* L. callus accumulation was investigated. The stable callus culture of *R. rosea* L., of leafs origin, was used as model for studies. Callus of *R. rosea* L. is distinguished by the high rate of callus growth. The later is an important factor for the tissue culture establishment with a high level of uniformity, used in research experiences under control conditions (9).

MATERIAL AND METHODS

Callus, used in the experiments, derived from the leaves of *in vitro* grown *Rhodiola rosea* L. Callus was cultivated on agar supplemented with basal Murashige and Skoog (MS) (10) medium, supplemented with PGR including 6-benzylaminopurine (BA) and α-naphthylacetic acid (NAA). In order to maintain and cultivate the callus culture for routine necessities, every 40 days the fragments of callus were transferred on freshly prepared MS media. All the media used in the experiences were autoclaved at 121°C/18 psi for 22 min. Before autoclaving, the media was adjusted to pH 5,8. Callus growth was effectuated at 26°C and the light cycle was 16 h light (intensity of 2000 lux) and 8 h dark.

PGR Reglalg, tested in the experiences, was diluted with the basal MS media at the following ratios: 1/200, 1/400, 1/800 and 1/1000. Control variants included basal MS medium containing BA and NAA, but without *Reglalg*. Also, in order to clarify, whether the NGR *Reglalg* can substitute basal phytohormones (BA, NAA) used in MS medium, experiments were done with supplemented basal MS media with either alone (*Reglalg* , dilution 1/1000) or in combination with BA and (or) NAA (figure 2, 3) .

Callus cell growth rate. Callus was harvested at the following time intervals (days): 0, 12, 16, 21, 38, and 52.

Statistic analysis. Results represent the arithmetic means and their standard deviations (SD) of at least three different experiments.

RESULTS AND DISCUSSIONS

The pronounced beneficial effects of *Reglalg* were observed at dilution 1/1000 at all terms of observations. At lower dilutions of *Reglalg*, initially the inhibition of

cells proliferation dominated. With increasing the duration of cultivation in these variants the degree of inhibition decreased, the fact that demonstrate the "*adaptation*" of callus cells to the presence of active components of *Reglalg.* The dilution 1/1000 conducted to increased callus biomass accumulation, compared to the control (without *Reglalg* in cultivation medium), throughout all period of cultivation (figure 1). It has been observed that during cultivation the intensity of callus green color was higher in the variant on medium with *Reglalg* diluted with nutritive medium in ratio 1/800 and 1/1000. At the 52th day of cultivation the content of green pigments (chlorophyll a + chlorophyll b) in callus of the control variant was 2.02 mg/g of fresh biomass, while in variants cultivated on media supplemented with the *Reglalg* in dilutions 1/800 and 1/1000 the values of this parameter was respectively 2.94 and 3.57 mg/g of fresh weight.

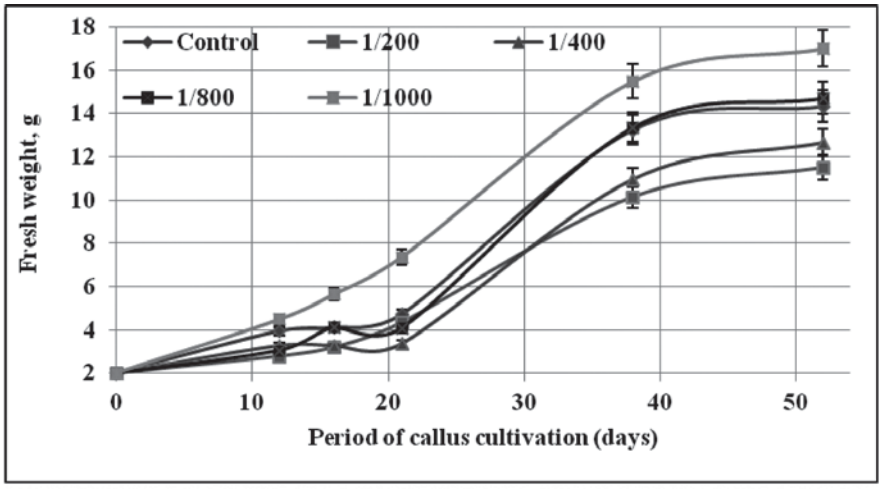

Figure 1. Effects of NGR *Reglalg* in different dilutions on growth rate of *R. rosea* callus.

The experiments with inclusion, or exclusion, of phytohormones and *Reglalg* in the MS cultivation medium (figure 2, 3) demonstrated that *Reglalg* cannot substitute the main growth regulators (BA, NAA), characteristics for this cultivation medium (2). At the same time, the supplementation of cultivation medium, containing BA and NAA, with diluted solution in ratio 1/1000 of *Reglalg* essentially stimulates the cells proliferation and callus biomass accumulation (figure 2, 3).

The callus of *R. rosea* cultured on MS medium supplemented with BA, NAA and *Reglalg* in different combinations (figure 3) showed differences in callus color in dependence of both presence of PGR and period of callus cultivation.

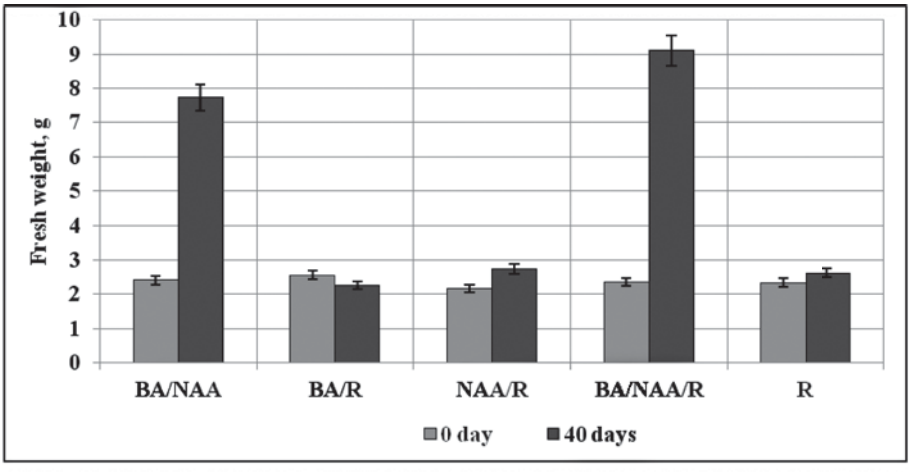

Figure 2. The influence of different combinations of basal phytohormones (BA, NAA) and tested NGR *Reglalg* on callus growth of *R. rosea* leaf origin after 40 days of cultivation.

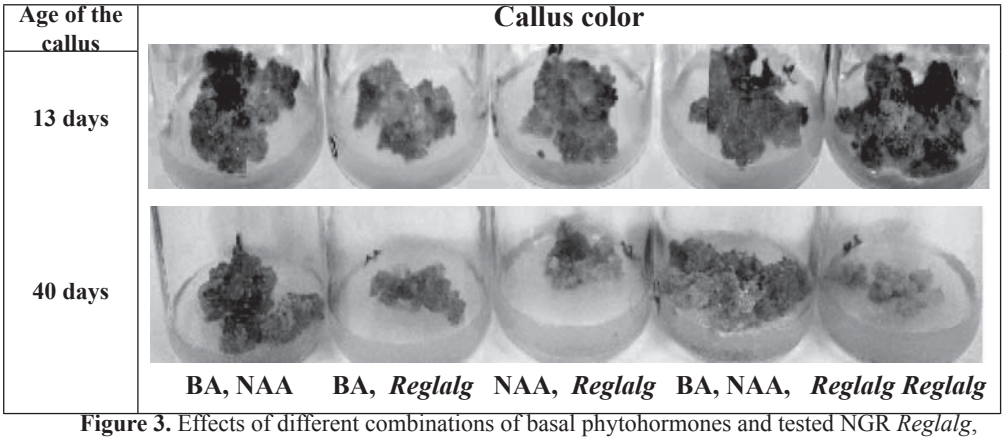

Figure 3. Effects of different combinations of basal phytohormones and tested NGR *Reglalg*, included in the MS medium, on the growth and color of *R. rosea* callus, evaluated after 13 and 40 days of cultivation.

The medium supplemented with *Reglalg* alone or in combination of *Reglalg* with BA or NAA induced yellowish green or yellowish brown callus after 13 and 40 days of cultivation (figure 3). The callus obtained in the medium fortified with BA, NAA or BA, NAA and *Reglalg* was light to dark green in color after both 13 and 40 days of cultivation (figure 3).

CONCLUSIONS

1. The callus biomass accumulation can be increased by introduction of *Reglalg* in high dilutions.

2. NGR *Reglalg* cannot substitute the main phytohormones (BA, NAA) characteristics for MS nutritive medium, but the supplementation of the complete medium with *Reglalg* stimulates the process of callus growth.

BIBLIOGRAPHY

1. Caus M. Creşterea plantulelor de castravete în funcție de concentrația regulatorului natural de creştere în diferite modificații // *Teze. Al III-lea Simpozion Național cu participare Internațională "Biotehnologii Avansate – Realizări şi Perspective", 24-25 octombrie 2013, Chişinău, RM, p. 88. (in Romanian).*

2. Călugăru-Spătaru T. Optimizarea mediului de cultivare a calusului de *Rhodiola rosea* L. şi marcarea proteică a etapei de dezvoltare a lui. In: *Conservarea diversității plantelor // Materialele simpozionului ştiințific internațional consacrat aniversării a 60-a de la fondarea Grădinii Botanice (Institut) a AŞM, Chişinău, 7-9 octombrie 2010, Chişinău, p. 50-55(in Romanian)*.

3. Cicalov V., Călugăru-Spătaru T., Dascaliuc A. Reacția de creşterе ca indice al eficienței reglatorilor naturali în protecția plantelor la temperaturi ridicate // *Conferința ştiințifică „Genetica si fiziologia rezistenței plantelor". In memoria academicianului Anatolie Jacotă. Chişinău, 2011, p. 26 (in Romanian).*

4. Dascaliuc A., Voineac V., Ralea T. Native substances in plant protection // Bul. Acad. de Ştiințe a Moldovei, Ştiințele vieții, 2006, N3, (300), p.46-51*(in Romanian).*.

5. Dascaliuc A., Ralea T. Reglalg, a new plant growth regulator with perspective of utilization in organic agriculture. 2nd International Symposium „Plant Growth Substances: Intracellular Hormonal Signaling and Applying Agriculture", Ukraine, Kyiv, 8-12 october, 2007, p.120.

6. Dascaliuc A., Voineac V., Ralea T. Rolul reglatorilor naturali de creştere în agricultura organică // *Conferința ştiințifico-practică internațională. Chişinău,, 2011, p. 297-303 (in Romanian).*

7. Gaspar T., Kevers C., Penel C., Greppin H., Reid D.M., Thorpe T.A. Plant hormones and plant growth regulators in plant tissue culture // *In Vitro Cell. Dev. Biol., 1996, vol.32, p. 272-289.*

8. Kiricenco E. V., Griniuk S. A. Rostreguliatornaia activnocti akstrakta vodoroslei // *J. „Biotechnologia Acta" , 2013, t.6, (5), s.143-149(in Russian)*

9. Al Khayri, Jameel M.; Al Bahrany, Abdulaziz M., 2001: In vitro micropropagation of Citrus aurantifolia (lime) // *Current Science, 2001, vol. 81, (9), p.1242-1246.*

10. Murashige T., Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue culture // Physiol. Plant. - 1962. - V. 1. - P. 473 - 497.

PEROXIDASE ACTIVITY IN CUCUMBER (*CUCUMIS SATIVUS* L.) SEEDLINGS OBTAINED FROM SEEDS TREATED WITH NATURAL GROWTH REGULATOR *REGLALG*

Caus M., Dascaliuc A.

Institute of Genetics, Plant Physiology and Protection, Academy of Sciences of Moldova, 20 Padurii str., MD-2002, Chisinau, Republic of Moldova

Abstract. From cucumber seeds treated with solutions of the three modifications of natural growth regulator (NGR) Reglalg (R1, R2, R) seedlings were obtained. The influence of Reglalg on peroxidase (PO) activity of apoplast fluids proteins, soluble proteins, and cell walls proteins from seedlings roots were investigated. It was established that treatments of cucumber seeds with different modifications of NGR Reglalg induce the beneficial effects on seedling growth. The activity of ionic and cationic cell walls bound PO was inversely related to Reglalg-mediated biomass accumulation by cucumber seedlings.

INTRODUCTION

Plant peroxidases (PO) are known to catalyze the oxidation of a wide variety of substrates using H_2O_2 or other peroxides. Due to their capacity to be expressed as multiple isoforms, PO are considered to mediate a diverse plant metabolic activities, including synthesis of the plant cell walls, growth, morphogenetic and organogenetic processes, as well as responses to abiotic and biotic stresses (2, 3, 6). There are numerous reports about the PO activity in relation to the state of endogenous plant hormones (1, 5, 7). The results of exogenous application of plant growth regulators (*PGR*), extracted from higher plants, including from algae, represent particular interest. Studies of plant responses to application of *PGR* are important, because the appropriate treatments are essential for the improvement of the parameters of plant growth and resistance to abiotic and biotic stress factors. The aim of this work was to study the effects of spraying of *Cucumis sativus* L. seeds with solutions of *Reglalg*, *PGR* extracted from algae *Spirogyra* sp., on seed germination, seedling growth, and PO activity in different compartments of root cells.

MATERIALS AND METHODS

Plant material

In all experiments was used the cucumber cultivar Concurrent. Before germination the seeds were disinfected with 0, 02% KMnO4, then immersed during 24 hours at 4^{o}C in water (control), or in solutions with different modifications of *Reglalg*, diluted with water in ratios 1/100, 1/200, 1/400. The modifications of the

preparation *Reglalg* were extracted from algae collected in the *s*pring (*R1*), summer (*R2*) and in autumn (*R3*). Subsequently, the seeds were germinated in the darkness at 25ºC. At the age of three days the influence of *Reglalg* on seedlings growth, patterns and activity of roots soluble and bound PO was evaluated.

Preparation of protein fractions

Isolation of enzymatic extracts from roots apoplast. The roots of cucumber seedlings were cut into segments of 2 mm length, then exposed to vacuum infiltration (60 kp) for 5 minutes with 0,05M sodium phosphate buffer, containing 1,5% (w / v) polyvinylpolypyrrolidone, 1 mM EDTA and 0, 5 mM phenylmethylsulphonyl fluoride, pH 6,8. Then, the apoplast fluids were recovered by centrifugation during 20 minutes at 500 g and 4 °C.

Preparation of soluble symplastic and cell wall - bounded protein fractions. Root samples were washed with distilled water and homogenized in mortar with pestle containing 0, 05 M sodium phosphate buffer, pH 6,8; 1,5% (w/v) polyvinylpolypyrrolidone; 1 mM EDTA, and 0,5 mM phenylmethylsulphonyl fluoride. The homogenates were centrifuged (1600 rpm, 20 min, 4 °C), giving the *soluble protein fractions* as supernatant. The pellets from centrifugations were washed several times with cold deionised water (4°C). Precipitates, free of PO activity, were treated with 2% Triton X-100. After 1 hour of incubation in non ionic detergent, the suspensions were centrifuged (16000g, 20 min, 4 °C) and *fractions of proteins bound to cell membranes by hydrophobic bonds* were obtained. Resulted precipitates, free of PO activity, were dispersed in solution of 1M NaCl, shaken gently for 1 h and then were centrifuged (16000g, 20 min, 4 °C) to give the *fractions of ionically wall-bounded proteins*. Afterwards, the pellets, washed several times with deionised water and free of PO activity, were incubated overnight with 0,05 M sodium phosphate buffer (pH 6,8), 0,6% cellulase and pectinase at 27°C. After 24 hours, the suspensions were centrifuged (16000g, 20 min, 4 °C) and separated supernatants were used as *fractions of covalently wall - bound proteins*.

Assay of peroxidase (PO) activity. The activity of peroxidase (PO, EC 1.11.1.7) in protein fractions was determined by determining the increase of the absorbance at 334 nm in result of benzidine oxidation in the presence of H_2O_2 (11) within 3 min at 25ºC. Extinction coefficient of benzidine is equal to 3, 8 mM^{-1} cm^{-1}.

Isozymes assays. Isoenzymatic patterns of soluble and bound peroxidises were obtained by native electrophoresis in 7% polyacrylamide gel electrophoresis (PAGE) according to (4). Protein bands displaying peroxidase activity were revealed utilizing 1,3 mM benzidine and 0,3% H_2O_2 as substrates.

Statistic analysis. Each experiment was performed three times in triplicate. The arithmetic means and their standard deviations (SD) were calculated.

RESULTS AND DISCUSSIONS

It was established that the modifications *R1*, *R2* and *R3* of *Reglalg,* utilised in three dilutions (1/100, 1/200, 1/400), exerted beneficial effects on seedlings growth and biomass accumulation. In this regard, the modification *R2,* diluted with water in ratios 1/200, was the most effective (figure 1).

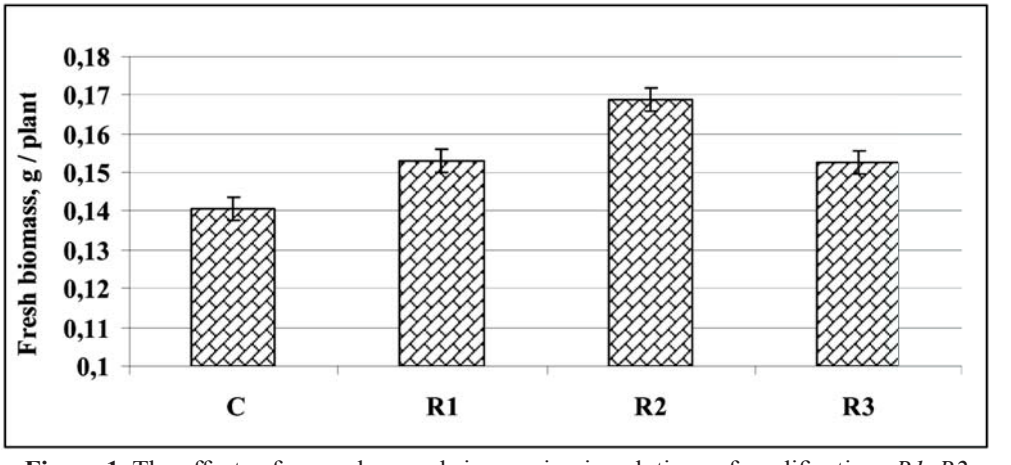

Figure 1. The effects of cucumber seeds immersion in solutions of modifications *R1, R2,* and *R3* of the preparation *Reglalg* diluted with water in ratio 1/200 on seedling biomass accumulation.

Seed treatments with solutions of *R1*, *R2* and *R3* in dilution 1 /200 did not influence significantly the activity of roots soluble PO (figure 2) and as well their isoform patterns (figure 4A). The PO activity in apoplast fluids also was not significantly affected by seed treatment with solutions of *Reglalg (*figure 2).

At the same time, the changes in activity of PO, embedded in the cell walls and linked to their biochemical residues by hydrophobic, ionic and cationic bonds were significant (figure 3). The changes of the level of activity of ionic and cationic PO, bounded on root cell walls, was marked in roots of seedlings obtained from seeds treated with all three modifications of *Reglalg*. The activity of PO linked by hydrophobic bonds to cells walls in the roots of experimental plants was comparable with that in the roots of control plants (figure 3). Apparent differences were observed in isoenzymes patterns of cells walls bound PO (figure 4).

Peroxidase isozymes in the fractions membrane proteins undergo noticeable quantitative and qualitative changes in dependence on modification of *Reglalg*, utilized for seed treatments (figure 4).

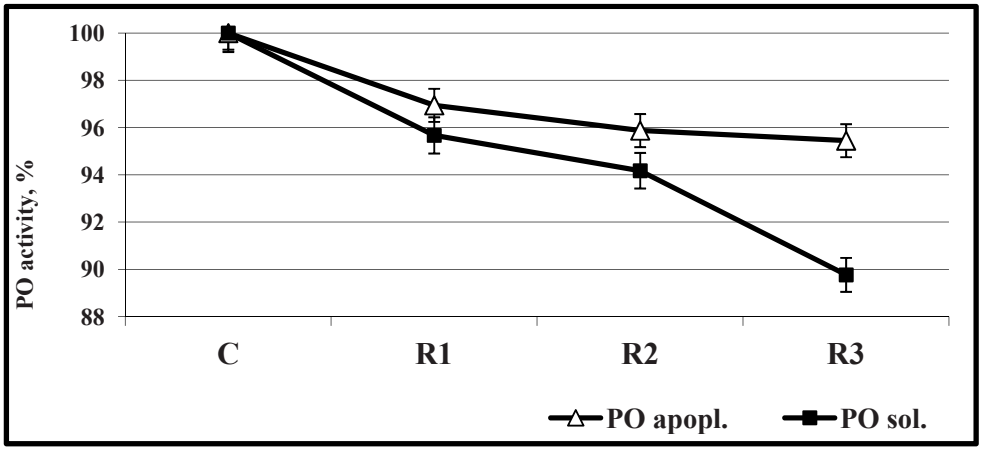

Figure 2. PO activity in fluids of apoplast and soluble proteins fractions, separated from the roots of three day old cucumber seedlings, obtained from the control seeds and treated with solutions of modifications *R1*, *R2*, and *R3* of the preparation *Reglalg,* diluted with water in ratio 1/200.

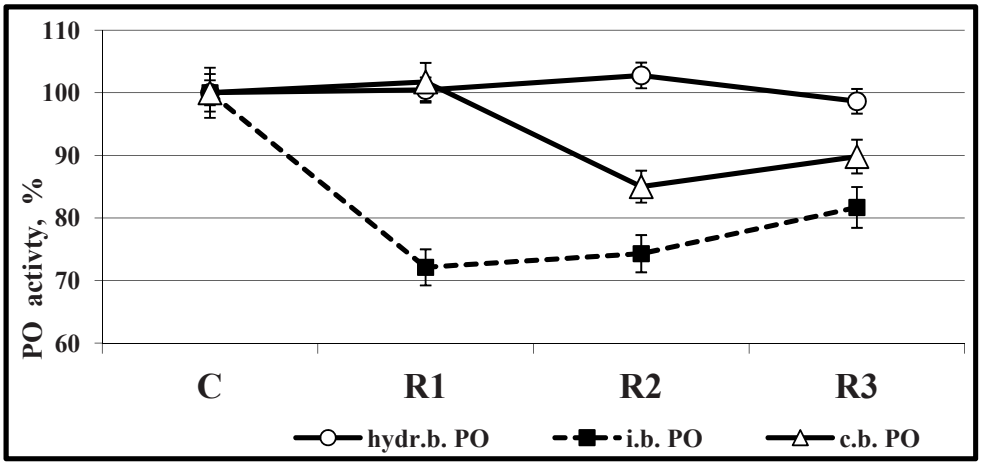

Figure 3. PO activity of proteins embedded in the root cell walls and bounded to root cell membranes, separated from the roots of three days old cucumber seedlings, obtained from the control seeds and treated with solutions of modifications *R1, R2*, and *R3* of the preparation *Reglalg,* diluted with water in ratio 1/200.

Isoenzyme patterns (figure 4, B, C, D) indicated the clear distinction of the influence of different *Reglalg* modifications on PO isoform patterns in proteins extracted from the cucumber root cell walls. They differ by electrophoretic mobility and intensity of the bands displaying the PO activity.

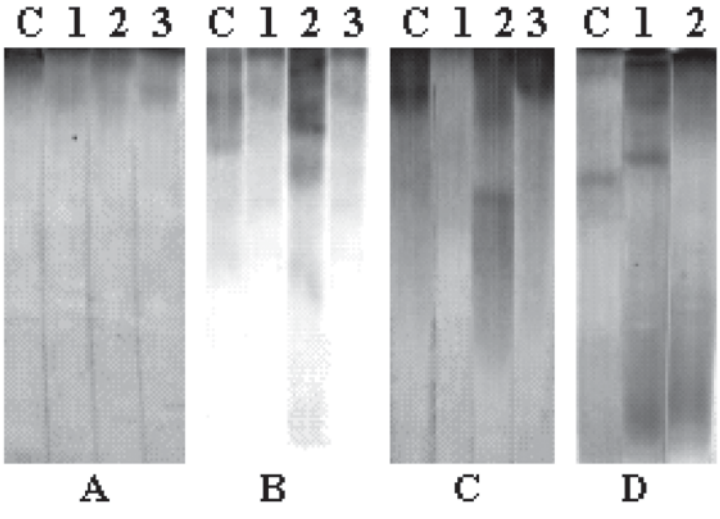

Figure 4. Zymogram of PO isozymes of roots soluble protein fractions (A), proteins isolated from the cell walls after treatments with Triton X 100 (B), NaCl (C), cellulase and pectinase (D). Seedlings were obtained from seeds treated with solutions of modifications *R1, R2*, and *R3* of the preparation *Reglalg*, diluted with water in ratio 1/200. Native electrophoresis was provided in 7% PAAG. Lanes: C – Control; 1 – *R1;* 2 – *R2* and 3 – *R3*.

So, our results showed, that treatment of cucumber seeds with solutions of *Reglalg* caused positive effect on seedling biomass accumulation (figure 1), but was not accompanied with significant increasing of PO activity of proteins from roots apoplast fluids and PO activity of soluble proteins (figure 2). However, as it is mentioned above, enhancing biomass accumulation by *Reglalg* solutions is accompanied by decreasing the level of the PO of proteins ionically and cationically bounded to cell membranes. Based on our results and those indicated in scientific literature (7, 8, 9, 10) we can suggest, that peroxidases, founded in multiple forms in cell walls and linked to membrane by hydrophobic, ionic, and cationic bonds, have an important role in the redox processes related to the cross-linking of matrix polymers in the cell walls of growing roots, that is important for a normal plant growth and development.

CONCLUSIONS

1. Treatments of cucumber seeds with different modifications of *PGR Reglalg* induce the beneficial effects on seedling growth and development.

2. The activity of ionic and cationic cells walls bounded PO is inversely related to *Reglalg*-mediated biomass accumulation of cucumber seedlings.

BIBLIOGRAPHY

1. Abeles F. B., Biles C. L., Dunn L. J. Hormonal regulation and distribution of peroxidise isoenzymes in the Cucurbitaceae // *Plant Physiology, 1989, vol. 91, p.1609-161.*

2. Almagro L., Gomez Ros L.V., Belchi-Navarro S., Bru R., Ros Barcelo A., Pedreno M.A. Class III peroxidases in plant defence reactions *// J. Exp. Botany, 2009, vol. 60, (2), p. 377–390.*

3. Cosio C., Dunand C. Specific functions of individual class III peroxidase genes // *J. Exp. Botany, 2009, vol. 60, (2), p. 391- 408.*

4. Davis B. J. Disc electrophoresis. Method and application to human serum proteins // *Ann. New York Acad. Sci., 1964, vol. 121, p. 404 – 427.*

5. Gharib F. A., Hegazi A. Z. Salicylic acid ameliorates germination, seedling growth, phytohormone and enzymes activity in Bean (*Phaseolus vulgaris* L.) under cold stress // *J. American Science, 2010, vol. 6, (10), p.675-683.*

6. Gaspar T., Penel C., Hagege D., Greppin H. Peroxidase in plant growth, differentiation, and development processes *// In: Lobarzewski J., Greppin H., Penel C., Gaspar T. (Eds.) Biochemical, Molecular, and Physiological Aspects of Plant Peroxidases. Geneva, University of Geneva, 1991, p.249 - 280.*

7. Gaspar T., Kevers C., Hausman J., Ripetti V. Peroxidase activity and endogenous free auxin during adventitious root formation // In *Physiology, Growth and Development of Plants in Culture. Eds. P.J. Lumsden, J..R. Nicholas and W.J. Davies. Kluwer Academic Publishers, Dordrecht, 1994, p. 289 – 298.*

8. Kukavica B.M., Veljovic-Jovanovic S., Menckhoff L., Luthje S. Cell wall-bound cationic and anionic class III isoperoxidases of pea root: biochemical characterization and function in root growth // *J. Exp. Botany*, 2012, vol. 63, (12), p. 4631 –4645.

9. Liyama K., Lam, T. B. - T., Stone B. A. Covalent Cross-Links in the Cell Wall // *Plant Physiology, 1994, vol. 104, p. 315-320.*

10. Syros T, Yupsanis T, Zafiriadis H, Economou A. Activity and isoforms of peroxidases, lignin and anatomy, during adventitious rooting in cuttings of *Ebenus cretica* L. // *J. Plant Physiology, 2004, vol. 161, p. 69-77.*

11. Seevers P. M., Daly J. M., Catedral F. F. The role of peroxidase isozymes in resistance to heat stem rust disease // *Plant* Physiology, 1971, vol. 48, p. 353-360.

PRODUCTIVITY AND BIOCHEMICAL COMPOSITION OF THE *SILYBUM MARIANUM* DEPENDING ON DIFFERENTIATION OF ELEMENTS OF THE TECHNOLOGY OF GROWING UNDER THE CONDITIONS OF IRRIGATION OF THE SOUTH OF UKRAINE

Fedorchuk V.G., Doctor of Philosophy
Kokovichin S.V., Doctor of Agricultural Sciences
Ryabukha I.M., Associate Professor
Filipova I.M., graduate student
Kherson State Agricultural University, Ukraine

Abstract. The results of researches of Silybum marianum grown on the irrigated lands of south of Ukraine are given in the article. The optimal values of depth of soil treatment, sowing terms, row-spacing and doses of mineral fertilizers influencing the productivity of the investigated crop are defined. Through the usage of dispersible and cross-correlation-regressive analysis equity participation of factors on the indices of the productivity is set and the optimal range of width of row-spacing and doses of nitric fertilizers is found.

Keywords: Silybum marianum, soil tillage, row-spacing, terms of sowing, mineral fertilizers, productivity

INTRODUCTION

The favorable soil-climatic terms of the South Steppe of Ukraine and ARE Crimea, which are very similar to the conditions in other world districts of cultivation of volatile-oil-bearing plants and medical plants, allow to cultivate the large set of these cultures, enable with success to replace the imported perfume products, spices, medicinal preparations with domestic ones, and also to a great extent to extend their assortment and bring a cost down. Medical properties of plants depend on the presence in them of the substances with various chemical structure and therapeutic action. The content of these substances enables the medical plants to perform various vital functions of man: satisfaction of nutritive necessities, mobilization of protective forces of human organism. It is led to by scientific researches, that not all possibilities of medical plants are presently used. On information which is resulted in the special literature, with 2000 types of plants of flora of Ukraine, near 500 species were studied thoroughly for the purpose of their use in scientific medicine, and 230 species are used only. This circumstance compels to extend scientific researches on the medicinal plants, to study more detailed than property already the known species, their reaction on different elements of technologies of growing.

MATERIALS AND METHODS

The task of researches was to study influence of basic agrotechnical factors (systems of soil tillage, space-rowing, terms of sowing and background of mineral feeding) on productivity of plants of *Silybum marianum* at its growing under the conditions of irrigation in the South of Ukraine.

The field and laboratory researches were conducted during 2010-2012 in the Institute of Rice NAAN of Ukraine. Experimental areas were laid using the method of split areas according to existent methods of experimentalism. Soil is represented by dark-chestnut solonetsous residual soil. Humus content in 0-20 cm layer of soil was 2.06%.

As for weather conditions, the years of researches differed both by temperature and by the precipitations. For example, 2010 and 2011 were characterized by favourable weather conditions, and in 2012 the sharp deficit of precipitations was marked on the background of the high air temperature.

Agrotechnics of experiments was generally accepted for growing *Silybum marianum* on irrigation lands except for the explored factors.

RESULTS AND DISCUSSIONS

The depth of soil tillage poorly affects the productivity of *Silybum marianum*. After the analysis of the level of productivity of the explored culture, the tendency of positive influence on productivity of plants of expansion of spaces between rows from 30 to 45, and, especially, to 60 cm was established. In addition, exposed conformity to the law of decline of productivity of *Silybum marianum* at the delay with sowing and moving of its terms from the third ten-day period March on the third ten-day period April, and the maximum performance of plants was at sowing at the end of March. The mineral fertilizers substantially multiplied the indexes of productivity of *Silybum marianum*, however, this increase was disproportionate, as it is comparative with the unfertilized variant at the use of $N_{45}P_{45}$ productivity grew on 34.1%, and it is comparative between areas with bringing $N_{45}P_{45}$ and $N_{90}P_{90}$ – an increase was 16.9% only.

Analysis of the obtained data showed that productivity of *Silybum* has changed under the influence of the explored factors; however this influence was not similar.

Comparison of plants' productivity indices concerning the factor A (soil tillage) shows unimportant influence of replacement of shallow soil tillage (depth 14-16 cm) with ploughing depth 20-20 cm. Under such change of soil tillage, element productivity of *Silybum* grew from 11.2 to 11.7 cwt/ha, or by 4.5%. It is necessary to notice that such increase is insufficient from the point of view of recoupment of additional outgo of fuel for conducting of ploughing, in comparison to shallow soil tillage.

There was the tendency of positive influence of expansion of spaces between

rows from 30 to 45 cm on plants productivity in the years of researches, and, especially, to 60 cm. For example, at row-spacing 30 cm, productivity of *Silybum* made 10.7-11.1 cwt/ha, at the increase to 45 cm this index was multiplied by 4.7-5.4% (or to 11.2-11.7 cwt/ha). Expansion of spaces between rows from 30 to 60 stimulated greater increase of productivity – by 1.1-1.3 cwt/ha (or by 10.3-11.7%). Consequently, taking into account the biological features of the crop, it is found that the best productivity of plants was formed at sowing with the row-spacing of 60 cm.

Concerning the terms of sowing regularity, a productivity decline of *Silybum marianum* was found at the delay of sowing and moving of its terms from the third ten-day period of March to the third ten-day period of April. Maximal productivity at the level of 13.5-14.2 cwt/ha is defined at sowing at the end of March, row-spacing 45-60 cm and conducting of ploughing with depth of 20-22 cm. Minimum values 8.5-8.9 cwt/ha were shown in the areas with shallow soil tillage with depth of 14-16 cm, row-spacing 30-45 cm and move of sowing terms to the end of April. It is necessary to notice that difference between the best and the worst variants was 51.7-67.1% that testifies to substantial influence of sowing terms on productivity of plants of *Silybum marianum*.

The mineral fertilizers (factor D) positively affected productivity of *Silybum marianum*, For example, in a variant without the fertilizers the productivity made up 8.8 cwt/ha, and on areas with bringing in $N_{45}P_{45}$ and $N_{90}P_{90}$ it rose up to 11.8 and 13.8 cwt/ha. It is necessary to underline that the increase of plants productivity was disproportionate. For instance, in comparison with the unfertilized variant, the use $N_{45}P_{45}$ provided growth of *Silybum* productivity by 34.1%; and comparing areas with bringing-in $N_{45}P_{45}$ and $N_{90}P_{90}$ – an increase was only 16.9% (or 2.0 times less), that testifies to the decline of recoupment of mineral fertilizers and needs clarification of their doses on the planned level of productivity.

Using variance analysis, it was proved that the rate of influencing of factors has substantial differences in relation to forming of plants productivity of *Silybum marianum* on the irrigated lands.

Soil tillage (factor A) and row-spacing (factor B) have an insignificant influence on productivity of the explored crop – only 3.3 and 5.3%, accordingly. Influence of sowing terms (factor C) substantially grew to 26.2% that exceeds first two factors 4.9-8.0 times. The greatest influence on plants productivity during the experiment had mineral fertilizers, as a rate of their influence was 39.2%.

When analyzing indices of combination of factors ABCD, the most influencing one constituted 5.2%, which proves the positive influence of optimization of all explored elements of growing technology on irrigated lands. The least couple co-operation 0.5% is marked for connection of factors A and D (soil tillage and background of mineral feeding), and the most one at the level of 2.6% – between factors C and D (terms of sowing and background of mineral feeding).

Using the correlation-regressive analysis there was defined the difference of influence on productivity of *Silybum marianum* of theoretically calculated quantitative characteristics of the factor B (row-spacing) and factor D (background of mineral feeding) (figure 1-2).

When width of spaces between rows is expanded from 30 to 60 cm there can be observed substantial increase of plants productivity, which represent the lines of polynomial trend. Under the range from 65 to 73 cm, the increase of productivity is substantially slowed, and after the point of 76 cm – the decline of the explored index is marked. Consequently, the results of statistical experimental data analysis proved, that 60 cm is the optimum width of spaces between rows for *Silybum*, and its growing with the greater width of spaces between rows causes the decline of plants' productivity.

Similar tendency was observed at comparison of theoretical line of trend of explored culture productivity level with the doses of nitric fertilizers.

Under the increase of nitric fertilizers dose from 30 to 90 kg of agent per 1 hectare growth of productivity was swift, that is conditioned by substantial stimulant action of this most important element on productivity of plants. In the range from 130 to 165 kg of a/ha substantial deceleration of growth of productivity indexes was noticed, and after exceeding of N_{168} – negative action of increase of nitrogen dose on the explored index shows up. Thus, taking into account the results of correlation-regressive analysis it is possible to draw a conclusion that the highest efficiency is shown by the use of nitric fertilizers with a dose from 30 to 90 kg of agent per 1 ha.

The depth of soil tillage poorly affects productivity of *Silybum marianum*. According to the analysis of productivity level of the explored crop's tendency, it was observed a positive influence on plants productivity of expansion of spaces between rows from 30 to 45, and, especially, to 60 cm. In addition, the regularity of productivity decline of *Silybum* was found at the delay of sowing and moving of its terms from the third ten-day period of March to the third ten-day period of April, and the maximum performance of plants was at sowing at the end of March. The mineral fertilizers substantially multiplied the productivity indices of *Silybum*, however this increase was disproportionate, as comparing the unfertilized variant and the use of $N_{45}P_{45}$ productivity grew by 34.1%, and comparing areas with bringing-in of $N_{45}P_{45}$ and $N_{90}P_{90}$ – the increase was just 16.9%.

Using the analysis of variance it was proved that soil tillage and row-spacing have an insignificant influence on productivity of the explored crop with the rate of influence – just 3.3 and 5.3%. The terms of sowing had an influence on productivity of plants at the level of 26.2%, and the greatest value from the point of view of productivity level forming had mineral fertilizers, as the rate of their influence was 39.2%. Using the correlation-regressive analysis we found out that the optimum range of row-spacing makes up 15-60 cm, and for the doses of nitric fertilizers – from 30 to 90 kg a/ha.

It is led to by the analysis of variance, that soil tillage and width of spaces between rows have an insignificant influence on productivity of the explored culture with little influence – only 3.3 and 5.3%. The terms of sowing had an influence on productivity of plants at the level of 26,2%, and the greatest value, from the point of view forming of level of productivity, had the mineral fertilizers, as a particle of their influence was 39.2%. By the correlation-regressive analysis is set, that the optimum range of width of spaces between rows makes 15-60 cm, and for the doses of nitric fertilizers – from 30 to 90 kg d.r./ha.

CONCLUSIONS

By the biochemical analyses considerable differentiation of organic acids is proved in the butter of seeds of the *Silybum marianum* linoleic acid (56.45%) spotted with advantage and, opposite, by minimum maintenance of pentadecanoic (0.03%) and linolenic (0.04%) acids. The presence of 2.3-degidrosilibinum, maintenance of which from the common amount of dominant flavolignan is 2.5-3.0%, is exposed in the garden-stuffs of plants of *Silybum marianum*. Due to its biochemical composition, the Yugoslav variety is promising for the industrial growing.

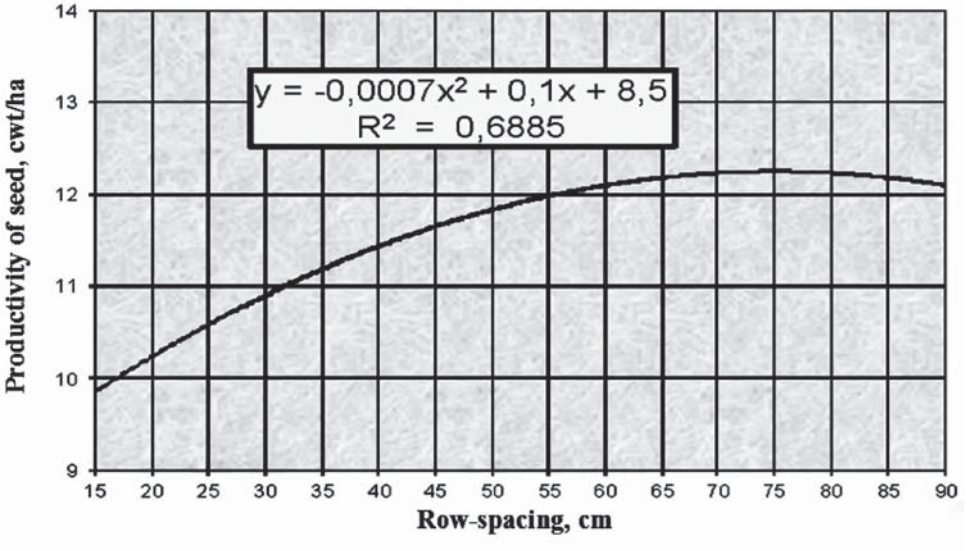

Figure 1. Correlation-regressive dependence between the indices of productivity of seed of *Silybum marianum* and width of row-spacing

BIBLIOGRAPHY

1. Arnone A. Constituents of *Silybum marianum*. Structure of isosilybum and stereochemistry of isosilybin / A. Arnone, L. Merlini, A. Zanarotti // J. Chem. Soc. (Chem. Commun.).- 1999.-Vol. 41, № 7.-P. 696-697.

2. Bosisio E. Effect of the flavanolignans of *Silybum marianum* L. on lipid peroxidation in rat liver microsomes and freshly isolated hepatocytes / E. Bosisio, C. Benelli, O. I'irola // Pharmacological Research.- 1992.- Vol. 1, № 25.- P. 147-154,

3. Bruneton J. Pharmacognosy, phytochemistry, medicinal plants / J. Bruneton.- Paris: Lavoisier, 1995.- P. 267.

4. European pharmacopoeia. – 3rd ed,- Strasbourg: Council of Europe, 1996.- P. 2416.

5. Lahiri-Chatterjee M. A flavonoid antioxidant, silymarin, affords exceptionally high protection against tumor promotion in the SENCAR mouse skin tumorigenesis model / M. Lahiri-Chatterjee, S.K. Katiyar, R.R. Mohan, R. Agarwal // Cancer Res.- 1999.- Vol. 59, № 3.- P. 622-632.

6. Morazzoni P. *Silybum marianum* (Carduus marianus) / P. Morazzoni, E. Bombardelli // Fitoterapia. – 1995.- № 66.-P. 3-42.

7. Wagner H. Plant drug analysis / H. Wagner, S. Bradt.- Berlin: Springer-Verlag, 1995.- P. 376.

APPLE FRUIT CALLUS: CELLULAR AND SUBCELLULAR ORGANIZATION

Marinescu M., Kolesnikova L.

Institute of Genetics, Plant Physiology and Protection of ASM

Abstract. The analysis and observations upon light microscopy pictures, especially scanning electron micrographs of apple uncoated callus, have provided additional information about cell proliferation, its assembly and callus growth. Investigations on the nuclear cytology of callus induction and development show that cultured cells commonly undergo progressive changes in the chromosomal constitution, which lead to the formation of a tissue containing a mixture of diploid, polyploid and, frequently, aneuploid cells. The cytological study revealed different types of abnormalities in the mitotic metaphase chromosomes.

INTRODUCTION

The callus tissue of plant origin has been examined widely for its cytological and morphological characteristics but very little is known about the differences between the cells of intact tissue and the corresponding cells grown on an artificial medium. The present study compares the anatomical and cytological structure of apple fruit explants and apple callus, especially about changes in the chromosomal constitution, which lead to the formation of tissue with diverse ploidity.

MATERIALS AND METHODS

Mature fresh fruits of apples *Malus domestica* Borkh were used in the experiments. Apple callus was obtained from the fruit by aseptic culture on modified MS medium containing the supplements; 2,4-D, 2 ppm; kinetin, 0·3 ppm; thiamine, 10 ppm; pyridoxine, 5 ppm; casein hydrolysate, 0·05%. The cultures were maintained at 26°C. Actively growing callus mass was collected between 45 and 50 days of culturing from the 3rd subculture (130 days after callus initiation). Fresh apple callus was analyzed in stereoscopic or phase-contrast microscope. For light microscopy two types of cytological preparations were used: the propion-lacmoid stain of Kaptari (1967) produced temporary squash preparations with swollen chromosomes, which were easily located and counted, and the Haidenhain hematoxylin stain (Pausheva, 1988) gave permanent mounts. We used for light microscopy the sections embedded in epoxy resins. Sections of 2-4 μm thickness were stained with 0.05% toluidine blue and viewed with a light microscope, applying immersion oil directive to the section itself. Moreover, clusters or sections of the cell line callus were examined in modular confocal microscope system Cl (Nikon Corporation) and confocal laser scanning microscope Leica DM IRB/E with relative rhodamine, phalloidin fluorochrome treat-

ments and pseudo-coloured cytoskeletal and nuclear cytoplasmic constituents, by V.M.Laurent etc., 2002.

For general data about the anatomy, cell division, growth and senescence of the apple fruits, including preparation of sections and structural analysis, we have used procedures from previous publications (Rotaru, 1972).

RESULTS AND DISCUSSIONS

Apple fruit callus and cell lines

According to our observations, the initiation of callusing in Malus explants occurred within 35-45 days. After 3-4 subcultivation carpoexplants formed three types of callus: green and white, comparatively homogenous, friable, and rose-colored, more compact and dense. It was established that the initiation of cell proliferation was localized in the subepidermal layer. Later, in secondary callus, only the cells of the peripheral layers induce the production of a new layer of active divisions surrounding the inner tissue. The growing of green and white calli, compared to the rose-colored callus, was more intensive. During 40 days fresh weight increased 3-5 times as much.

The cytological study of the calli originating from pericarp revealed the presence of parenchymatous cells of different shapes and sizes in the samples of all types. The cell structure in all calli was dependent on the growth phase and topographic localization. Comparative microscopical analysis of green callus cells showed that this tissue type consists of small thin walled isodiametric cells (80-100 μm) with large intercellular spaces. The green callus was characterized by spherical cells which contained a considerable quantity of starch, which was localized mainly in plastids around the nucleus. The white callus consists of prosenchymatous and worm-like cells and protein intraplastidial crystal-like inclusions (Matienco et al., 1997). Cells containing anthocyanins were rarely observed.

The rose-colored callus was characterized by a predominance of spherical and slightly oval vacuolated cells (60-120 μm), which contained anthocyanins. Intensively stained cell groups were placed on the callus' surface. The sizes of the cells did not correlate with anthocyanin accumulation.

A structural analysis of the white callus showed that this callus type consisted of large irregular spherical, oval and elongated (prosenchymatous) cells (77x350-600 μm) with large intercellular spaces.

In all types of callus, binucleate and polynucleate cells and relatively numerous nuclear pore complexes were observed. As noted by Zabel et al. (1996), the cells stopped their growth in response to the reduction of nuclear pore complexes.

Tissue polyploidization. Typical and atypical multiplication

It was thus established that the increase of callus volume is the result of cell divisions or multiplication after mitosis, with the same deviations and cell enlargement. The mitotic index in apple callus ranged from 1.8% to 2.1% for rose-colored callus and from 1.9% to 2.3% for green and white callus, respectively. Variability in chromosome number frequently occurs during *in vitro* dedifferentiation and growth of plant cell populations. Investigations on the nuclear cytology of callus induction and development show that cultured cells commonly undergo progressive changes in the chromosomal constitution, which lead to the formation of a tissue containing a mixture of diploid, polyploid and, frequently, aneuploid cells (Rumyantseva et al., 1998; Matienco, Marinescu, Colesnicova, 2000). Cytological study revealed different types of abnormalities in the mitotic metaphase chromosomes, whereas one or more chromosomes lying outside the equatorial plate, aneuploidy with different chromosome numbers: 2n=33, 35 instead of the normal chromosome number (2n=34). Observed mitotic abnormalities were also chromosome lagging, forestalling, anaphase and telophase bridges (Marinescu, Matienco, Kolesnikova, 2004). Chromosomes rearrangements and aberrations are linked to the generation of numerous gains and losses of genetic material characteristic to aneuploidy. Endomitotic and amitotic pictures are rather undepicted, whereas binucleate and seldom multinucleate cells were found very frequently. Binucleate cells were widely described *in vivo* for apple fruit and *in vitro* in the callus of leaf, stem, root and fruit origin (Kordan,1977; Colesnicova and Matienco, 1992; Marinescu, Matienco, 1994; Zaprometov et al., 1994; Matienco, 1999; Rumyantseva et al., 1998; Marinescu et al., 2002). Sometimes this phenomenon may be the effect of parhomology, because the ranging of microscope optic field planes induced the errors. Nevertheless, many researches and our micrographs indicated, on the widespread, binucleate cells in callus tissue, and which occurred independently from its organogene specificity. In our scanning electron-micrographs of callus lines of uncoated (without gold) specimens recorded in SEM LEO 435 VP with variable pressure advantage, which provided the translucent visualization of intact protoplast configuration through transparent cell envelope, the binucleate phenomenon was confirmed. Nagl (1978), Brodsky and Uryvaeva (1985), Colesnicova and Matienco (1992) had been drawing attention on the "attempt" of tissue to multiply the worked cell elements in case of parenchyma hypertrophy, certainly with ulterior cell enlargement of proliferative pool.

Another factor of cell enlargement is the incomplete cell plate formation. According to Garcia-Florenciano et al. (1992), rod-like cells in suspension of cultured grape berry are a widely documented phenomenon, when incomplete cell plate formation keeps the new sister cells into mother space. Thus, the volume and reshaping of cell increase by elongation of non-dedoubled initial parenchymatic

element. This phenomenon represents the same multiplication of cell organelles number in comparison with binucleate cells.

Interestingly, those binucleate cells were previously found *in vivo* in parenchyma cells of succulent apple pericarp (Colesnicova, Matienco, 1994) of different species and cultivars, i.e. in natural conditions. We have presumably connected this process with the evolution phenomenon of hypergenesis or hyperthely, gigas excessive growth (Matienco, 1969). This biological instrument might be conditioned by endogenous hormones synthesized by giant fruits and transmitted (shifted) through carpoexplants to callus proliferative potential *in vitro*.

CONCLUSIONS

Thus, tissue callus polyploidization represents one of the pushing forces in parenchyma proliferation *in vivo* and was all present *in vitro* with its multiplicity, variability of the expression. Consistent with this idea, the proliferation *in vitro,* under nutritional stress, the presence of exogenous hormones (auxins and cytokinins) and other factors exploit the same multiplication mechanism (model, evolution benefit, advent, constructs) that was established *in vivo,* during the evolution of hypertrophy and hyperthely.

BIBLIOGRAPHY

1. Brodsky V.Ya., Uryvaeva I.V. *Genome multiplication in growth and development*. Cambridge Univ.Press, 1985: 305 p.

2. Colesnicova L.S., Matienco B.T. *Somatic polyploidization of pericarp tissues of some apple cultivars*. Rev. of AS of RM, Biol.and chem.., 1992, 1: 7-12.

3. Colesnicova L.S., Matienco B.T. *Investigation of particularities of binucleation in pericarp cells of old apple cultivars*. Rev. of AS of RM, Biol.and chem.., 1994, 1: 30-32.

4. Garcia-Florenciano E., Calderon A.A., Munoz R., Barcelo R.A. *The decarboxylative pathway of indole-3-acetic acid catabolism in grapevine protoplasts*. J. Exp. Bot., 1992, 43: 715-721.

5. Kaptari S.G. *Rapid propion lacmoid method preparation and staining of temporary cytological slices for counting of plant chromosomes.* Cytology and Genetics, 1967, 4.

6. Kordan H.A. *Mitosis and cell proliferation in lemon fruit explants incubated on stimulated nutrient solutions*. New phytology, 1977, 79: 673-677.

7. Marinescu M., Matienco B. *Cytological analysis of tomato fruit callus culture*. Proc. VIII Intern. Congr. Plant Cell Culture. Firenze, 12-17 June, 1994: 266.

8. Marinescu M., Matienco B., Kolesnikova L. *Carpoculture Cucurbita moschata Duch. In vitro*. Lucr. Stint. Simp. III Soc. Fiziol. Bioch. Veget. RM, 28 mai, 2004: 159-160.

9. Matienco B. *Comparative anatomy and ultrastructure of Cucurbita fruits*. Kishinev: Cartea Moldoveneasca, 1969: 407 p.

10. Matienco B. *Towards the intact image of parenchyma cells by improved scanning electron microscopy methods*. Proc. 1st Congr.Croation Soc.Electron Microsc., Zagreb, 13-16 May, 1999: 20-21.

11. Matienco B., Maximova E., Brezeanu A., Marinescu M. *Visualization of intracellular traffic of phenolic compounds during its biosynthesis and translocation in grape berry callus.* Proc. 13th Nat. Electron Microsc. Congr. Ankara, 1-4 Sept., 1997: 167-173.

12. Matienco B., Marinescu M., Colesnicova L. *Structure and ultrastructure of carpocallus cell nuclei*. Proc. 7th Asia-Pacific Electron Microsc. Conf. Life Sci., Singapore, 26-30 June, 2000: 219-220.

13. Nagl W. *Endopolyploidy and polyteny in differentiation and evolution*. North Holland, Amsterdam, 1978:614-615.

14. Pausheva Z.P. *Handbook of plant cytology*. M., Agropromizdat, 1988: 272 p.

15. Rotaru G.I. *Comparative anatomy of pericarp in subfamily Pomoideae*. Kishinev: Stiinta, 1972: 138 p.

16. Rumyantseva N.I., Valyeva A.I. et al. *Peculiarities of lignification of cell walls of buckwheat calli with different morphogenic abil*ity. Tsitologiya (in Russian), 1998, 40: 835-843.

17. Zabel U., Doye V., Tekotte H. et al. *Nic96p is required for nuclear pore formation and functionally interacts with a novel nucleoporin Nup188p*. J. Cell Biol., 1996, 133: 1141-1152.

18. Zaprometov M.N., Subbotina G.A., Nikolaeva T.N. *Action of kinetin on the phenolic compounds formation and on the ultrastructural organization of tea plant tissue culture*. Plant Physiology (in Russian), 1994, 41: 354-358.

THE SEASONAL ACTIVITY OF PHOTOSYSTEM II AND ENZIMES OF DEGRADATION OF HYDROGEN PEROXIDE AND DURING DEHYDRATION OF *BUXUS SEMPERVÍRENS* L. LEAVES

Nina Zdioruk, Tudor Ralea, Natalia Jelev, Petru Cuza*, Gheorghi Florența*

Institute of Genetics, Physiology & Protection of Plants ASM
*Institute of Ecology and Geography ASM**

Abstract. The transition of plants from the active growing season in the summer to the autumn rest and their transition from winter rest to the spring growth were studied. These changes were accompanied with increasing of the activity of H_2O_2 decomposing enzymes. In all four seasons the activity of H_2O_2 decomposing enzymes is higher in one year old leaves in comparison with two year old leaves. The water content in the boxwood leaves is one of the most important factors determining the activity of FS-II. Reduced activity of FS-II is accompanied by a corresponding decrease in the activity of decomposition H_2O_2, which demonstrates the correspondence of the activity of photosystem II and of H_2O_2 depredating enzymes.

INTRODUCTION

Boxwood (*B. sempervirens* L.) is one of the ancient ornamental plants. The most commonly it is used in horticulture. The plant is characterized by slow growth and long duration of life (up to 500 years). Boxwood leaves are evergreens, varying in shape and color, but they have almost the same characteristics - they are bare, green and shiny on the top and light green or light yellow on the bottom, simple, elliptical. There is no doubt that over a long period of evolution boxwood acquired specific qualities that contribute to its survival in a variety of adverse environmental conditions. This may explain its plants high resistance to extreme summer and winter temperatures and unpretentiousness to soil. These characteristics demonstrate the unique qualities of boxwood that can be essential for using it as a test plant for assessing the problems of plants survival under the threat of global warming.

It is known that for adaptation of plants to changing external conditions is important the regulation of redox system enzymes activity. The stress reactions of plants include enhancing the processes of free-radicals and *reactive oxygen species* (*ROS*) quenching. In the process of evolution in plants was established the system of protection against *ROS*, such as superoxide and hydrogen peroxide, using antioxidant enzymes (4). These enzymes include superoxide dismutase, catalase, and peroxidases (1). For determining plant resistance to adverse environmental factors, often the activity of catalase and peroxidases is analyzed. Basically, catalase is considered as

one of the terminal oxidases, responsible for the decomposition of peroxides in plant cells (5). Changes in the composition and activity of redox enzymes may provide some indication about the reaction of the plant systems to stress conditions and to specific environment. These parameters may serve as a criterion for assessing the level of plant adaptation to the conditions of life (2).

In different seasons of year and during dehydration of boxwood leaves we have investigated the characteristics of redox processes. The change of leaves functional state in different seasons and during dehydration was estimated by the yield photosystem II.

MATERIALS AND METHODS

Total activity of peroxide degradation enzymes activity was determined in extracts of two-year old boxwood leaves, collected in the winter. Boxwood leaves dehydration was provided in air thermostat at 25°C and relative humidity of air 60%. The recovery of leaves humidity was realized in desiccators at 25°C and air relative humidity 100%. The water content in the samples was determined by weighting leaves during dehydration and rehydration (3). Activity photosystem II was determined by the fluorometer PAM 2100 (Germany). To obtain the extracts, 200 mg fresh weight leaves were homogenized in 2 ml of 0.2 M tris-buffer, pH 7.4, centrifuged for 15 minutes at 4000 g. The enzymatic reaction is initiated by adding 40 µl of supernatant and 60 µl of 0.05% H_2O_2 to 1.5 ml of tris-buffer. Analysis of the content of dissolved oxygen in extracts was provided with laboratory analyzer YSI 5300A (USA).

RESULTS AND DISCUSSIONS

The obtained data, Fig.1, indicate that reduction of the water content in the leaves from 80% to 5% is accompanied with decreasing of the photosystem II *Yield* from 0.34 to 0. In this period also occurs the diminution of the activity of H_2O_2 degradation enzymes from 0. 0079 to 0.0049 U of catalase per mg of leaves wet weight. When leaves were rehydrated from 5% up to 72%, the recovery of the activity of H_2O_2 degradation enzymes from 0.49 to 0.089 U / mg wet weight and the increasing of photosystem II Yield from 0 to 0. 76 were observed. It is interesting to note that the recovery of the activity of peroxide splitting enzymes precedes the restoration of the activity of photosystem II *Yield* in leaves. In the complex, the obtained data confirm the assumptions that the activity of H_2O_2 degradation enzymes can be an important indicator of leaves functional state during dehydration or rehydration.

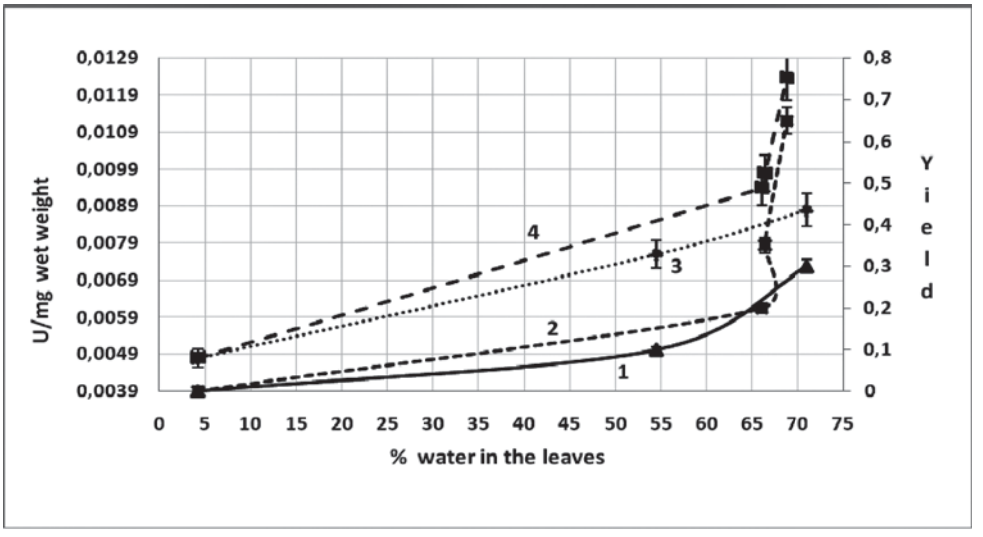

Figure 1. The Yield of photosystem II and activity of decomposition of H_2O_2 in extracts from boxwood leaves (expressed as catalase U per extract from 1 mg of leaves wet weight) during dehydration and subsequent rehydration. Legend: 1-2, photosystem II activity during dehydration and rehydration; 3, 4-activity of degradation of during dehydration and rehydration.

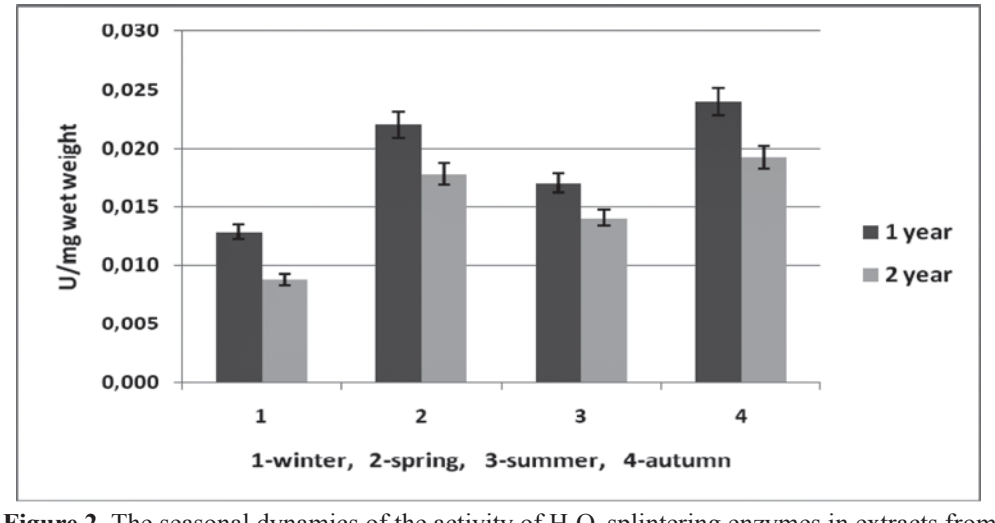

Figure 2. The seasonal dynamics of the activity of H_2O_2 splintering enzymes in extracts from 1 year and 2 year old leaves.

It was found that the total activity of peroxide destroying enzymes varies depending on the season of the year and the age of the leaves, Fig. 2. These data

indicate that the activity of enzymes which degrade the peroxide is increased in the seasons of transition from heat to cold (in the fall) and from cold to heat (in the spring). In all seasons the activity of these enzymes in the one year old leaves was higher than in two year old leaves. These data indicate that the adaptation of the leaves to the changed environmental conditions is associated with increasing the activity of the enzymes that are splintering of peroxide. In young leaves, these processes are more pronounced than in old leaves. This indicates that the participation of hydrogen peroxide degradation enzymes in leaves of plants adapted to seasonal changes in temperature and lightening.

CONCLUSIONS

1. The transition of plants from the active growing season in the summer to the autumn rest and their transition from winter rest to the spring growth are accompanied with increasing of the activity of H_2O_2 decomposing enzymes. In all four seasons the activity of H_2O_2 decomposing enzymes is higher in one year old leaves in comparison with two year old leaves.

2. The water content in the boxwood leaves is one of the most important factors determining the activity of FS-II. Reduced activity of FS-II is accompanied by a corresponding decrease in the activity of decomposition H_2O_2, which demonstrate the correspondence of the activity of photosystem II and of H_2O_2 decomposing enzymes.

BIBLIOGRAPHY

1. Bryuhanov A.L. , Netrusov A.I. Kataliza i SOD rasprostranenie, svoistva i fiziologicheskaia roli v kletkah strogih anaerobov *// Biochimia 2004 S. 68-69, № 9.*

2. Voskresenskaia O.L. Bolishoi praktikum po bioekologii. Ch.1. Uchebnoie posobiie *// Voskresenskaia O.L., Aleabisheva E. A., Polovnikova M.G. Mar.gos.un-t; Ioshkar-Ola, 2006 C.61.*

3. Gudskov N.L., Obrucheva N.V. Fotosintez i bioproduktivnosti: metodi opredeleniia *// Moskva 1989, s.104-107.*

4. Sarbayeva E.V., Voskresenskaia O.L. Izmenenie aktivnosti jelezosoderjachih oksidaz u decorativnih rastenii v usloviiah urbanizirovannoi sredi. *// 2009, s.16-18.*

5. Sarbayeva E.V., Grosheva N.P. Izmenenie aktivnosti antioksidantnih fermentov nekotorih dekorativnih rastenii v usloviiah urbanizirovannoi sredi. *// Mariiskii gos.un-t, Rossia, ecology@marsu.ru.*

PROGESTERONE 5B-REDUCTASE FROM HORSERADISH (*ARMORACIA RUSTICANA*): CLONING, SEQUENCE COMPARISON AND MOLECULAR MODELLING

[1]Oleg Budeanu, [2]Catarina Costa, [2]Andreas Loebers, [2]Peter Bauer, [2]Jennifer Munkert, [2]Frieder Müller-Uri[*], [2]Wolfgang Kreis

[1]University of the Academy of Science of Moldova, Academiei 3/2, MD-2009 Chisinau, Moldova Republic

[2]Lehrstuhl für Pharmazeutische Biologie, Department Biologie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Staudtstrasse 5, 91058 Erlangen, Germany

Abstract. Protein extracts prepared from *Armoracia rusticana* leaves exhibited progesterone 5β-reductase activity (P5βR). Using primers deduced from known P5βR genes a full-length cDNA encoding a progesterone 5β-reductase (ArP5βR, EC 1.3.99.6) was isolated from Armoracia rusticana leaves. A SphI/SalI ArP5βR PCR fragment was cloned into the pQE30 vector and transformed into *Escherichia coli*. The recombinant His-tagged fusion protein gene was expressed in *E. coli* M15. Progesterone was enantio selectively reduced to 5β-pregnane-3,20-dione. The ArP5βR of *A. rusticana* (JF460011) showed 92 and 91% sequence identity to steroid 5β-reductases isolated from *Arabidopsis thaliana* and *Erysimum crepidifolium*, respectively. A three-dimensional model of ArP5βR on the basis of the *Digitalis lanata* P5βR (PDB ID: 2V6G, DlP5βR) highlights the close structural relationship to Vein Patterning 1-encoded enone reductases described previously.

Key message. Further evidence is provided that VEP1-encoded enone reductases (progesterone 5β-reductases; P5βR) are distributed ubiquitously in the angiosperms and that VEP1 may be used as a molecular marker to infer taxonomic relationship in Brassicaceae.

Keywords. *Armoracia rusticana*, Brassicaceae Steroid reductase, Protein alignment, Metabolism, Molecular modelling

Abbreviations

GC-MS	Gas chromatography/mass spectroscopy
HPLC	High performance liquid chromatography
IPTG	Isopropyl-β-D-thiogalactopyranoside
NADPH	Nicotinamide adenine dinucleotide phosphate
P5βR	Progesterone 5β-reductase
RT-PCR	Reverse transcription polymerase chain reaction
SDR	Short-chain dehydrogenase/reductase
StR	Steroid reductase

Rezumat. Extractele proteice preparate din frunze de *Armoracia rusticana* au prezentat activitate a enzimei progesteron 5β-reductaza (P5βR). Folosind primeri specifici a genelor cunoscute P5βR au fost efectuată amplificarea pe ADNc, care codifică progesteron 5β-reductaza (ArP5βR, CE 1.3.99.6). Un fragment SphI / SalI ArP5βR PCR a fost clonat în vectorul pQE30 şi transformată în *Escherichia coli*. Gena proteinei His recombinante din fuziune a fost expresată în *E. coli* M15. Progesteronul a fost redus selectiv la 5β-pregnan-3,20-dionă. ArP5βR de *A. rusticana* (JF460011) a demonstrat 92 şi 91% identitate de secvență cu steroizi 5β-reductaze izolate din *Arabidopsis thaliana* şi *Erysimum crepidifolium,* respectiv. Un model

tridimensional al ArP5βR pe baza de *Digitalis lanata* P5βR (PPB ID: 2V6G, DIP5βR) evidențiază relația structurală apropiată cu reductazele descrise anterior. Sunt furnizate dovezi suplimentare care demonstrează că enon reductazele VEP1 (progesteron 5β-reductaze; P5βR) sunt răspândite larg în angiosperme şi VEP1 poate fi utilizat ca un marker molecular pentru a deduce relația taxonomică în Brassicaceae.

Cuvinte-cheie. *Armoracia rusticana* Brassicaceae Steroid reductaze Alinierea proteinelor Metabolizm Modelare moleculară

INTRODUCTION

The evolution of bioactive plant natural products is an issue of fundamental and practical interest. Many of these compounds still are important drugs used in therapy, such as paclitaxel, morphine, quinine, scopolamine and digoxin. The occurrence of several so-called secondary metabolites seems to be restricted to certain taxa whereas others are produced by many plant species. 5β-Cardenolides, *i.e.*, a group of cardioactive steroid glycosides, have been detected in many orders of the angiosperms (Kreis and Müller-Uri, 2010) but it is rather the rule than the exception that only one or few genera within a given plant family produce cardenolides. This has been termed an 'erratic' occurrence since the biosynthetic origin of cardenolides in taxonomically distant taxa has yet to be elucidated (Kreis and Müller-Uri, 2010). Investigating genes and enzymes involved in cardenolide biosynthesis in different plants may help to understand evolution of the cardenolide trait and shed light on the conservation and/ or multiple evolution of genes coding for cardenolide-biosynthetic enzymes. Steroid 5β-reduction is a mandatory step in the biosynthesis of 5β-cardenolides. Progesterone 5β-reductases (P5βR, Fig. 1) are capable of converting progesterone and other steroids into their 5βH-configured derivatives (Herl et al., 2006a, b; Schebitz et al., 2010). P5βRs, which are members of the short chain dehydrogenases/reductase (SDR) super family of proteins (Kavanagh et al., 2008), are supposed to be involved in the biosynthesis of 5β-cardenolides (Gärtner et al., 1990; Kreis et al., 1998; Gavidia et al., 2007). SDRs typically are composed of 230 – 350 amino acids with low sequence identity but a conserved protein scaffold. P5βRs are substrate-promiscuous enone reductases ubiquitously distributed in angiosperms. These enzymes are encoded by Vein Patterning 1 (VEP 1) genes (Herl et al., 2009). VEP 1 genes occur in a wide range of plant species independent of the ability of a respective plant to produce cardenolides or not. VEP 1 can therefore be used as a molecular taxonomic marker (Herl et al., 2008; Bauer et al., 2010; Munkert et al., 2011). VEP 1 genes have been isolated from members of several angiosperm families. Several *P5βR*s have already been cloned and functionally expressed in *E. coli* (Gavidia et al., 2007; Herl et al., 2006b, 2007, 2009; Bauer et al., 2010). In the *Brassicaceae* only few genera are known that contain cardenolides (*Draba, Erysimum (incl. Cheiranthus and Syrenia*, Makarevitch et al., 1994) while all of them contain glucosinolates. Cardenolides have a bitter taste and show a high toxicity in many organisms (Agrawal et al., 2012). Cardenolides can help plants defend themselves against insects, fungi and other pests. Glucosinolates

represent another potent group of plant natural products in the *Brassicaceae* that might be involved in chemical ecology and co-evolution. They contribute a pungent taste to condiments, such as mustard and horseradish. Cardenolides act in a highly specific manner, inhibiting Na+/K+-ATPase, whereas glucosinolates possess a less specific toxicity and may, for example, exhibit goitrogenic or antithyroid activity (Polat, 2010). More recently members of both classes of compounds have also been described as potential anticancer drugs (Talalay and Fahey, 2001; Newman et al., 2008). Glucosinolates are widely distributed in *Brassicaceae* while cardenolides are found in few genera only and one may ask whether cardenolides originated several times in the *Brassicaceae* or whether they were lost during evolution. This question may be addressed by studying genes and proteins involved in cardenolide biosynthesis in *Brassicaceae.*

Based on sequence homology it was estimated that about 20% of the genes involved in the formation of plant secondary metabolites also occur in *A. thaliana* (Sommerville and Sommerville, 1999). Since sequence homology alone is an insufficient criterion on which to predict the specific function of an enzyme the detailed functional characterization of every single gene product is mandatory (Facchini, 2006). In continuation of the effort to characterize and compare VEP1 genes and P5βR enzymes in the angiosperms we here isolated a VEP1 of *Armoracia rusticana* (*Ar*P5βR). Its nucleotide sequence and the derived amino acid sequence were aligned with known and putative VEP1-encoded P5βRs. We modelled the new enzyme on the crystal structure of *Dl*P5βR (PDB ID: 2V6G, *Dl*P5βR) and compared the active site of the enzyme with that of known P5βRs (Bauer et al., 2010; Thorn et al., 2008). In addition, we over-expressed the *Ar*P5βR gene in *E. coli* and we tested the catalytic function of the recombinant protein.

MATERIALS AND METHODS

Plant material, chemicals

A. rusticana (Gaertn., Mey., & Scherb.) plants were grown under standard seasonal conditions (no additional illumination) in the greenhouse of the Department of Biology, FAU Erlangen-Nürnberg, Germany (latitude 49.600, longitude 11.015). Steroids were purchased from Steraloids INC (Newport. USA). All other chemicals used were obtained from Sigma (Munich, Germany) or Merck (Darmstadt, Germany).

RNA extraction

Plant leaves were ground to a fine powder in liquid nitrogen using mortar and pestle. Total RNA extraction was carried out using E.Z.N.A. Plant DNA and RNA Mini Kits (Peqlab GmbH, Erlangen, Germany). All other molecular biology methods were performed according to Sambrook et al. (1998).

Cloning and expression of *Armoracia rusticana* P5βR

The synthesis of cDNA was performed employing SuperScript™ III First-Strand Synthesis Kit for RT PCR (Invitrogen, Darmstadt, Germany) using oligo $(dT)^{15}$-Pimers and *Armoracia rusticana* RNA isolated from mature leaves as the starting materials. Semi-quantitative PCR was carried out using ATGAGTTGGTGGGGGGCT (dir) and ATGTCTGGAAAAAGATTGGATGG (rev), which were derived from GenBank sequence of *Ec*P5βR (Acc.-Nr. GU354236), as well as two actin-specific primers. Each reaction (50 µL total volume) contained 2.5 units SAWADY Taq-DNA-Polymerase (Peqlab, Biotechnologie GmbH Erlangen, Germany), 1x reaction buffer S, 0.5 mM of each dNTP, 2 µM of primers and 0.2 µg of cDNA. A Personal Cycler 20 (Biometra GmbH, Göttingen, Germany) was used for amplification according to the supplier's recommendation. 30 cycles of 30 s denaturation at 95°C, followed by 30 s annealing at 63°C and 1 min extension at 72°C. Finally, a 10 min extension at 72°C was added to complete the amplification. PCR products were analysed by 1% agarose gel electrophoresis in TAE buffer system. Gels were stained with ethidium bromide and visualized by illumination at UV_{365}.

The full-length cDNA was amplified using the two *Ec*P5βR-specific primers shown above. Primers for over-expression were designed by adding the cloning sites *Sph1* and *Sal1* sequences as follows: 5'-ATAT***GCATGC***ATGAGTTGGTGGGGGGCT-3' (*AmSph*dir) and 5'-TATA***GTCGAC***ATGTCTGGAAAAAGATTGGATGG-3' (*AmSal*rev).

Recombinant *Ar*P5βR was isolated and purified in native form following instructions in the manufacturer's manual (QIAexpressionist, Hilden, Germany). The Ni-NTA matrix was washed with 20 mM imidazole buffer and the recombinant protein was eluted with 250 mM imidazole buffer. Progesterone 5β-reductase activity of the recombinant His-tagged fusion enzyme was determined using the enzyme assay described earlier and using TLC to detect product formation (Herl et al., 2006a, b; Bauer et al., 2010).

TLC and GC-MS analysis

For the GC-MS analysis the evaporated assays were dissolved in 100 µL dichloromethane and subsequently 3 µL of this solution were tested according to Bauer et al. (2010). The AUCs of the 5β-pregnane-3,20-dione and the progesterone peak were used to calculate the relative activities using the formula $A_{product} / (A_{product} + A_{substrate})$. For the TLC experiments the residue was dissolved in 50 µL methanol following the procedure of Herl et al. (2006a).

In silico analysis

After sequencing (MWG Eurofins AG, Ebersberg, Germany), the data were analyzed using appropriate software packages (European Bioinformatics Institute).

For searching and sequence analysis BLAST® search of the GenBank™ data base was carried out. The nucleic acid sequences as well as the translated, deduced sequence (Transeq® http://www.ebi.ac.uk/Tools/emboss/transeq/index.html) amino acid sequences were aligned using ClustalW (http://www.ebi.ac.uk/clustalw/index.html).

Modelling of the *Ar*P5βR structure

For modelling the *Ar*P5βR, the experimentally determined crystal structure of the homologous protein *Dl*P5βR in complex with $NADP^+$ from *D. lanata* (Thorn et al., 2008) was used as a template (PDB ID: 2V6F-G; Berman et al., 2002). SWISS-MODEL, a fully automated protein structure homology-modelling server, was used in the automated mode (Kiefer et al., 2009). The substrate molecule progesterone was placed into the active site. For this purpose 250 random progesterone positions and orientations were generated differing only slightly (mean r.m.s.d.: 0.7Å) from the coordinates of progesterone previously docked into the active site of *Dl*P5βR from *D. lanata* (Thorn et al., 2008).

Supplementary materials can be found online at http://www.pharmbio.nat.uni-erlangen.de

RESULTS AND DISCUSSION

Alignment and sequence comparison

Progesterone 5β-reductase (P5βR) has first been demonstrated in the cardenolide-producing plant *D. purpurea* and was therefore proposed to play a key role in the biosynthesis of cardenolides (Kreis et al., 1998; Gärtner et al., 1990; Kreis and Müller-Uri, 2010). P5βR catalyzes the conversion of progesterone to 5β-pregnane-3,20-dione (Fig. 1), representing the committed first stereo-specific step in 5β-cardenolide biosynthesis (Gärtner et al., 1990, 1994). The functional and structural characterization of various VEP 1-encoded progesterone 5β-reductases (Roca-Perez et al., 2004; Herl et al., 2007; Thorn et al., 2008; Perez-Bermudez et al., 2009; Kreis and Müller-Uri, 2010) led to the question whether orthologous *P5βR* genes from other plants also code for functional progesterone 5β-reductases. GenBank™ searches (BLAST) revealed a very high number of putative *P5βR* genes using *At*St5β-R (EF579963; At4g24220) as a query sequence (Herl et al., 2009; Bauer et al., 2010). No homologues were found in the *Armoracia rusticana* gene/protein sequences available. This caused us to demonstrate progesterone 5β-reductase in leaf extracts and to isolate a VEP 1 gene encoding a progesterone 5β-reductase.

Protein extracts prepared from leaves showed progesterone 5β-reductase activity (data not shown). After having demonstrated progesterone 5β-reductase activity *in vitro* we directly aimed at the isolation of a VEP 1 cDNA from *A. rusticana* leaves. RNA was isolated and served as the template for cDNA synthesis. Oligonucleotide primers deduced from the published GenBank™ sequence of *Erysimum crepidifolium*

(GU354236; Munkert et al., 2011) were used for RT-PCR amplification. Gel electrophoresis following RT-PCR amplification showed a single DNA band of about 1200 bp which was isolated and sequenced (GenBank™ JF460011). *In silico* analysis showed that the gene product, termed *Ar*P5βR (GenBank™ AEX31541.1), was highly homologous to previously isolated P5βR genes (Herl et al., 2009; Bauer et al., 2010). The new gene proved to be 89 and 91% identical with the *E. crepidifolium* and *A. thaliana* homologue, respectively. High sequence identity (91 and 92%, respectively) was also seen on the amino acid level. We aligned the deduced protein sequence (Fig. 2) of the new *Ar*P5βR with those of *Ec*P5βR, *At*St5βR and two putative P5βRs from *Brassica oleracea* (*Bo*P5βR, GenBank™ JQ608337) and *Lunaria annua* (GenBank™ *La*P5βR, JN638575) recently isolated in our group. This comparison confirmed that *Ar*P5βR belongs to the VEP1-like proteins of the SDR super family (Persson et al., 2003). *Ar*P5βR was also aligned with other VEP1-like proteins (data not shown) and demonstrated to possess all of the 6 typical sequence motifs (Filling et al., 2002; Kallberg et al., 2002; Thorn et al., 2008). The motifs I to III are present in all NADPH-dependent SDRs and are involved in the binding of the co-substrate NADPH to the double Rossmann fold, typical for SDRs (Oppermann et al., 2003). The remaining three motifs IV to VI (Thorn et al., 2008) and in particular the characteristic NFYYxxED motif (Perez-Bermudez et al., 2009) are not present in the standard SDRs but in all VEP1-like proteins. These motifs are also present in the VEP-1-like proteins of other *Brassicaceae*, such as various *Erysimum sp., Lunaria annua* and *Brassica oleracea*. All 6 motifs are highly conserved and the *Ar*P5βR differs in only 5 positions from the *At*StR (Fig. 2). This means that the cluster (WGxKxFxYxxMF) that was suggested to be part of the substrate-binding pocket (Bauer et al., 2010) is also present in *Ar*P5βR. The role of individual amino acids within the active site of P5βRs has recently been addressed by site-directed mutagenesis experiments (Bauer et al., 2012).

Progesterone 5β-reductase genes in *Brassicaceae*

P5βR genes have been used to infer phylogenetic relationship in the genus *Digitalis* (Herl et al., 2008). Since P5βR genes have been identified in many other angiosperms it was suggested to include P5βR gene analysis in molecular phylogeny (Herl et al., 2008; Munkert et al., 2011). In the *Brassicaceae* P5βR genes have been reported in the genera *Arabidopsis*, *Erysimum*, *Brassica*, *Lunaria* and *Armoracia* (according to GenBank™). Other species (e.g., *Draba*, *Hesperis*, *Sisymbrium*, *Capsella*) are under investigation and their sequences almost completed (data not shown). Bailey et al. (2006) provided a supermatrix phylogenetic analysis of the Brassicaceae family based on several molecular markers (ITS, adh1, ndhF, matK, rbcL, leafy). We here tried a preliminary cladistic analysis using the P5βR cDNA sequences (Fig. 3). A possible evolutionary history was created using the neighbor-joining method (Saitou and Nej, 1987). The tree was rooted using *Digitalis lanata* as outgroup. The cladogramme

generated correlated nicely with the assumed phylogenetic relationship of the species. The genus *Lunaria* was not included in the *Brassicaceae* analysis (Bailey et al., 2006) but showed a high coincidence to the genus *Armoracia* (high bootstrap value). Multiple forms of P5βR genes may occur Brassicaceae as has already been demonstrated for Arabidopsis with paralogs on different chromosomes. Our primer design probably only allowed the isolation of closely related homologues and one may assume that they have evolved from a common ancestral gene. The P5βR available may therefore be used to infer phylogenetic relationship within the *Brassicaceae* family (Fig. 4). P5βR has already been tried as a molecular marker to infer phylogenetic relationship in *Erysimum* (Munkert et al., 2011). So far P5βR genes have been isolated from a few *Brassicaceae* and hence bootstrap analysis is not yet fully conclusive.

Functional analysis and substrate selectivity

The catalytic function of newly described genes or proteins should not be determined solely by its degree of sequence identity to known proteins, although this is currently common practice (Pichersky and Gang, 2000; Friedman and Pichersky, 2005). Ringer et al. (2005) stressed that homology-based cloning is challenging but suggested that direct functional screening from enriched cDNA libraries is the most promising approach to identify new genes involved in secondary metabolite formation. In the *Brassicaceae* P5βR activity has been demonstrated for recombinant forms of P5βRs from *Arabidopsis* (Herl et al., 2009), *Erysimum* (Munkert et al., 2011) and here for the first time also for *Armoracia*. We introduced the *Ar*P5βR cDNA into *E. coli* for heterologous expression. *Ar*P*5βR* was cloned into the pQE30 vector for over-expression as a His-tagged-fusion protein. Over-expression of a recombinant form of *Ar*P5βR, termed r*Ar*P5βR, was initiated by IPTG induction following protein isolation and affinity purification on Ni-NTA. The calculated molecular mass of r*Ar*P5βR was 43.914 Da and the pI was 5.12.

*Ar*P5βR displayed progesterone 5β-reductase activity in the presence of NADPH but not NADH (Fig. 4). 5β-Pregnane-3,20-dione was the only product formed as verified by GC-MS. Progesterone 5β-reduction could not be detected in protein extracted from bacteria containing the empty pQE vector. Hence, *Ar*P5βR qualifies for an enantio selective progesterone 5β-reductase (Fig. 5). This fact corroborates our previous findings from other P5βRs (Herl et al., 2009; Bauer et al., 2010; Munkert et al., 2011). The specific activity of *Ar*P5βR, as determined semi-quantitatively by TLC and GC-MS was lower than that reported for native or recombinant progesterone 5β-reductases from *A. thaliana* (Herl et al., 2006a, b).

Protein modelling

Results from structural genomics projects demonstrated that even if experimental crystal structures are available, it is not possible to reliably predict substrate selectivity

and specificity. In standard SDRs, a catalytic tetrad composed of the four residues asparagine (Asn), serine (Ser), lysine (Lys) and tyrosine (Tyr) has been proposed to be essential for catalysis (Filling et al., 2002). Interestingly in *Ar*P5βR, a Ser residue was missing near the active site, but all other catalytic residues are present and conserved. A glutamine (Gln-181) substitutes for leucine (Leu) in motif V, at a position similar to the asparagine (Asn) in standard SDRs (Herl et al., 2009).

The high degree of protein sequence similarity and the presence of highly conserved motifs indicate that all P5βR proteins described so far also share similar molecular structures. Hence, we modelled the atomic structure for *Ar*P5βR using the 2.3Å crystal structure of P5βR from *D. lanata* (PDB ID: 2V6G) as the template (Herl et al., 2009) (Fig. 6). In addition, several other P5βRs were modelled on the available crystal structure. They all fitted well to the template showing QMEAN-scores as high as 0.70 to 0.79 (*e.g.*, *At*StR: 0.77/0.01 and 0.76/-0.17 for *Ec*P5βR) and Z-scores of +0.23 to -1.18. The domains containing the cosubstrate binding site and the substrate binding site can easily be recognized. NADPH is bound along the double Rossmann fold contributing the ionic and polar interactions necessary to bind the co-substrate's phosphate group.

Bauer et al. (2010) demonstrated that P5βRs have a similar core area clustered around the catalytic pocket including seven highly conserved amino acids. These amino acids (*At*StR: tryptophan-106, glycine-145, lysine-147, phenylalanine-153, tyrosine-178, methionine-214, phenylalanine-342) are also found in the new P5βR member isolated and characterized here (Table 1). Only four amino acids in the substrate binding-site differ from the structure modelled for *At*StR (Herl et al., 2009) (Fig. 6). Bauer et al. (2012) compared the activities of P5βR from Digitalis lanata, Mentha piperita, *Erysimum crepidifolium*, *Gomphocarpus fruticosus* and *Arabidopsis thaliana* and used their protein sequences to identify a set of residues within the catalytic site responsible for strong enone reductase-activity. Mutagenesis screening of these residues resulted in two positions (tyrosine-156, asparagine-205) responsible for 2-8 fold elevated catalytic efficiency. In ArP5βR an asparagine resides in position 156 explaining its low enone reductase activity since highly active enzymes possess a hydrophobic residue (e.g., valine in the A. thaliana AtStR) in this position. As in the weak *Erysimum* P5βRs (Munkert et al., 2011) a threonine resides in position 205 which was also identified as a hot spot responsible for weak enzyme activity. In the strong AtStR a methionine resides in position 205. The catalytic efficiency of DlP5βR could be increased considerably when substituting threonine-205 for methionine (Bauer et al., 2012). The substitution of leucine-205 for methionine increased the enone reductase activity of EcP5βR (data not shown). Hence, the prediction made by Bauer et al. (2012) concerning the distinction of weak and strong P5βR is valid for the new ArP5βR described here.

Acknowledgements

We thank Gabriele Fischer for excellent technical assistance. BMBF funding is acknowledged for financial support to O. Budeanu within the framework of collaboration with the University of the Academy of Sciences of Moldova (MDA09/029). Plant material was kindly provided by Schamel Meerrettich GmbH and Co. KG, Baiersdorf, Germany.

Nucleotide accession numbers

*Ar*P5βR (*Armoracia rusticana*), *Bo*P5βR (*Brassica oleracea*) and *La*P5βR (*Lunaria annua*) sequences were submitted to GenBank under the Acc.Nr. JF460011, JN638575 and JQ608337, respectively.

REFERENCES

1. Agrawal AA, Petschenka G, Bingham RA, Weber MG, Rasmann S (2012) Toxic cardenolides:chemical ecology and coevolution of specialized plant-herbivore intewractions. New Phytol 194:28-45.

2. Bailey CD, Koch MA, Mayer M, Mummenhoff K, O'Kane Jr SL, Warwick SL, Windham AD, Al-Shehbaz LA (2006) Towards a global phylogeny of the Brassicaceae. Mol Biol Evol 23:2142-2160.

3. Bauer P, Munkert J, Brydziun M, Burda E, Müller-Uri F, Gröger H, Muller Y, Kreis W (2010) Highly conserved progesterone 5β-reductase (P5βR) genes, from 5β-cardenolide-free and 5β-cardenolide-producing angiosperms. Phytochemistry 71:1495-1505.

4. Bauer P, Rudolph K, Müller-Uri F, Kreis W (2012) Vein patterning 1-encoded progesterone 5β-reductase: Activity-guided improvement of catalytic efficiency. Phytochemistry, doi:101016/J.phytochem.2012.01.022.

5. Facchini PJ (2006) Regulation of alkaloid biosynthesis in plants. Alkaloids Chem Biol 63:1-44.

6. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evol 39:783-791.

7. Filling C, Berndt KD, Benach J, Knapp S, Prozorovski T, Nordling E, Ladenstein R, Jörnvall H, Oppermann U (2002) Critical residues for structure and catalysis in short-chain dehydrogenases/reductases. J Biol Chem 277:25677-25684.

8. Friedman E, Pichersky E (2005) Metabolomics, genomics, proteomics, and the identification of enzymes and their substrates and products. Curr Opin Plant Biol 8:242-224.

9. Gavidia I, Tarrio R, Rodriguez-Trelles F, Perez-Bermudez P, Seitz HU (2007) Plant progesterone 5β-reductase is not homologous to the animal enzyme. Molecular evolutionary characterization of P5βR from *Digitalis purpurea*. Phytochemistry 68:853-864.

10. Gärtner DE, Wendroth S, Seitz HU (1990) A stereo-specific enzyme of the putative biosynthetic pathway of cardenolides. Characterization of a progesterone 5β-reductase from leaves of *Digitalis purpurea* L.. FEBS Lett 271:239-242.

11. Gärtner DE, Keilholz W, Seitz HU (1994) Purification, characterization and partial peptide microsequencing of progesterone 5β-reductase from shoot cultures of *Digitalis purpurea.* Eur J Biochem 225:1125-1132.

12. Herl V, Fischer G, Müller-Uri F, Kreis W (2006a) Molecular cloning and heterologous expression of progesterone 5β-reductase from *Digitalis lanata* Ehrh.. Phytochemistry 67:225-231.

13. Herl V, Fischer .G, Bötsch R, Müller-Uri F, Kreis W (2006b) Molecular cloning and expression of progesterone 5β-reductase (5β-POR) from *Isoplexis canariensis*. Planta Med 72:1163-1165.

14. Herl V, Frankenstein J, Meitinger N, Müller-Uri F, Kreis W (2007) Δ^5-3β-Hydroxysteroid dehydrogenase (3βHSD) from *Digitalis lanata*. Heterologous expression and characterisation of the recombinant enzyme. Planta Med 73:704-710.

15. Herl V, Albach DC, Müller-Uri F, Bräuchler C, Heubl G, Kreis W (2008) Using progesterone 5β-reductase, a gene encoding a key enzyme in the cardenolide biosynthesis, to infer the phylogeny of the genus *Digitali*s. Pl Syst Evol 271:65-78.

16. Herl V, Fischer G, Reva VA, Stibritz M, Muller YA, Müller-Uri F, Kreis W (2009) The VEP1 gene (At4g24220) encodes a short-chain dehydrogenase/reductase with 3-oxo-Δ4,5-steroid-5β-reductase activity in *Arabidopsis thaliana* L.. Biochimie 91:517-525.

17. Kallberg Y, Oppermann U, Jörnvall H, Persson B (2002) Short-chain dehydrogenases/reductases (SDRs). Co-enzyme based functional assignments in completed genomes. Eur J Biochem 269:4409-4417.

18. Kavanagh KL, Jörnvall H, Persson B, Oppermann U (2008) Medium- and short-chain dehydrogenase/reductase gene and protein familiesThe SDR superfamily: functional and structural diversity within a family of metabolic and regulatory enzymes. Cell Mol Life Sci. 65:3895–3906.

19. Kiefer F, Arnold K, Künzli M, Bordoli L, Schwede T (2009) The SWISS-MODEL Repository and associated resources. Nucleic Acids Res 37:D387-D392.

20. Kreis W, Hensel A, Stuhlemmer U (1998) Cardenolide biosynthesis in foxglove. Planta Med 64:491-499.

21. Kreis W, Müller-Uri F (2010) Biochemistry of Sterols, Cardiac Glycosides, Brassinosteroids, Phytoecdysteroids and Steroid Saponins. Biochemistry of Plant Secondary Metabolism. Ed., Wink, M., Oxford: Wiley-Blackwell, p. 304-363.

22. Makarevitch IF, Zhernoklev KV, Slyusarskaya TV, Yarmolenko GN (1994) Cardenolide-containing plant of the family Cruciferae. Chem Nat Comp 30:275-289.

23. Munkert J, Bauer P, Müller-Uri F, Kreis W (2011) Progesterone 5β-reductase of *Erysimum crepidifolium*: cDNA cloning, expression in *Escherichia coli*, and reduction of enones with the recombinant protein. Phytochemistry 72:1710-1717.

24. Newman RA, Yang P, Pawlus AD, Block KI (2008) Cardiac glycosides as novel cancer therapeutic agents. Mol Interv 8:36-49.

25. Oppermann U, Filling C, Hult M, Shafqat N, Wu X, Lindh M, Shafqat J, Nordling E, Kallberg Y, Persson B, Jörnvall H (2003) Short-chain dehydrogenases/reductases (SDR): the 2002 update. Chem Biol Interact 143-144:247-253.

26. Perez-Bermudez P, Moya Garcia AA, Tunon I, Gavidia I (2009) *Digitalis purpurea* P5βR2, encoding steroid 5β-reductase, is a novel defense-related gene involved in cardenolide biosynthesis. New Phytol 185:687-700.

27. Persson B, Kallberg Y, Oppermann U, Jörnvall H (2003) Coenzyme-based functional assignments of short-chain dehydrogenases/reductases (SDRs). Chem Biol Interact 143-144:271-278.

28. Pichersky E, Gang DR (2000) Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. Trends Plant Sci 5:439-445.

29. Polat U (2010) The effects on metabolism of glycosinolates and theirs hydrolysis products. J Biol Environ Sci 4:39-42.

30. Ringer KL, Davis EM, Croteau R (2005) Monoterpene metabolism. Cloning, expression, and characterization of (-)-isopiperitenol/(-)-carveol dehydrogenase of peppermint and spearmint. Plant Physiol 137:863-872.

31. Roca-Perez L, Boluda R, Gavidia I, Perez-Bermudez P (2004) Seasonal cardenolide production and *Dop5βr* gene expression in natural populations of *Digitalis obscura.* Phytochemistry 65:1869-1878.

32. Saitou N, Nei M, (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406-425.

33. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning. A laboratory manual, 2nd edn. New York: Cold Spring Harbor Laboratory Press.

34. Schebitz P, Nothdurft L, Hensel A, Müller-Uri F, Kreis W (2010) Norcholanic acids as substrates for recombinant 3β-hydroxysteroid dehydrogenase and progesterone 5β-reductase, enzymes of the 5β-cardenolide biosynthesis. Tetrahedron Lett 51:367-370.

35. Somerville C, Somerville S (1999) Plant functional genomics. Science 285:380-383.

36. Talalay P, Fahey JW (2001). Phytochemicals from Cruciferous Plants Protect against Cancer by Modulating Carcinogen Metabolism. AICR's 11th Annual Research Conference on Diet, Nutrition and Cancer. J Nutr 131:3027S-3033S.

37. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetic analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596-1599.

38. Thorn A, Egerer-Sieber C, Jäger CM, Herl V, Müller-Uri F, Kreis W, Muller YA (2008) The crystal structure of progesterone 5β-reductase from *Digitalis lanata* defines a novel class of short-chain dehydrogenases/reductases. J Biol Chem 283:17260-17269.

39. Zuckerkandl E, Pauling L (1965) Evolutionary divergence and convergence in proteins. Evolving Genes and Proteins. Eds Bryson V, Vogel HJ, Academic Press, New York, pp.97-166.

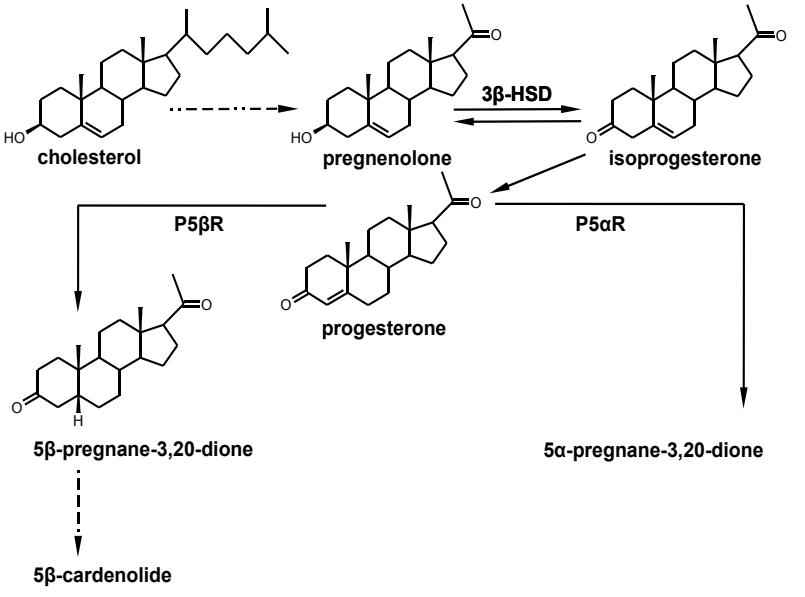

Fig. 1. Early steps in the cardenolide biosynthesis and the reaction catalysed by P5βR.

```
                                  motif I
Armoracia MSWWGAGAIGAAKKKLDEDEPTQTYESVALII**GVTGIVG**NSLAEILPLSDTPGGPWKVY**G**
Erysimum  ----------------D---S-SY---------------------------------- 60
Arabidopsis ----W------------D---S-SF---------------------------------- 60
Brassica  ----W------------DY--S-SY-----------I---------------------- 60
Lunaria   ----G----------FEE-----SY---------------------------------- 60

                                                                  # #
          motif II          motif III
Armoracia **VARR**PRPSWNADHPIDYIQCDVS**DADD**ARSKLSPLTDVTHVFYVTWTSRESEHDNCEANG 120
Ecrep     -------S---------------N-E--------------------K----SE------ 120
Arabidopsis -------T---------------S-A-D------------------N----SE------ 120
Brassica  --PS---T--------------N-EE ----------------C-D----SE------ 120
Lunaria   -------S-----------------------------------N-P----S----- 120

                                  #
                                motif IV                  motif V
Armoracia SMLRNVLQAIVPHAPDLRHVCLQ**TGTKHYIGP**FDNNGRSRHDAPFTEDMPRLQIQ**NFYYT** 179
Ecrep     ----------V-H-—N---I---------L---S-L-GP---P----------------- 179
Arabidopsis ----------I-Y-—N---V---------L---T-V-GP---P----------------- 179
Brassica  -T-----R-V--S-KN---V--------YY---T-V-GP---P-----------NN---- 179
Lunaria   -------R----N--N------------L-----L-K-Q-HD----------------- 179

                                  # #
                               motif VI
Armoracia **LED**VLFEEIKKKES-VT**WSIHRP**NTIFGFSPYSLMNIVGTLCVYAAICKHEGLPLLFPGS 238
Ecrep     Q-I----------IS---------T--------------------------S------- 239
Arabidopsis Q-I--------I-T----------M--------------------------S------- 238
Brassica  --------V-------T-------T---------T-V-----------Q--S------- 238
Lunaria   --DI---------G—S-----------------------------------S—V----- 238

Armoracia KNAWEGFTAASDADLIAEQQIWAAVDPYAKNEAFNCNNADIFKWKHLWKFLAEQFGIEEY 298
Ecrep     K-----TT-----------------------------------------I---------- 299
Arabidopsis K-----MT-----------------------------------------I---------- 298
Brassica  E-----AA------V----------------------G-V--------V---R---K-F- 298
Lunaria   K------------V-----------------------------M—V----------- 298

Armoracia GFEEGKNLGLVEMMKGKERVWEEMVKENQLQGTKLEEVGVWWFADVIHGVEGLIDSMNKS 358
Ecrep     -------------------------------EK-------------L----M------- 359
Arabidopsis -------------------------------EK-------------L----M------- 358
Brassica  -------V---K-----------I--K---KDR---D--------DVL----M------- 358
Lunaria   ----------Q------------------ERR------------L-G--M-------- 358

Armoracia KEYGFLGFRNSNNSFISWIDKYEAFKIVP 388
Ecrep     --H--------------------K------ 389
Arabidopsis --Y--------------------K------ 388
Brassica  --H--------K----------K------ 388
Lunaria   --H------------S—V----K------ 389
```

Fig. 2. Alignment of the deduced P5βR amino acid sequences from *Armoracia rusticana* (JF460011, *Ar*P5βR), *Erysimum crepidifolium* (GU354236, *Ec*P5βR), *Arabidopsis thaliana* (EF579963, *At*StR), *Brassica oleracea* (*Bo*P5βR, JQ608337) and *Lunaria annua* (*La*P5βR, JN638575). # - variable amino acid position in the motif III to V are indicated.

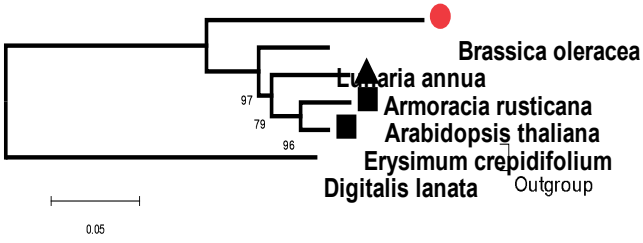

Fig. 3. Phylogenetic analysis of *Brassicaceae* modified after Bailey et al. (2006). The supermatrix analysis of data sets using molecular markers (adh1, atpB, chalcone synthase, ITS, matK, ndhF, pistilla intron, rbcL, leafy and tml-F).

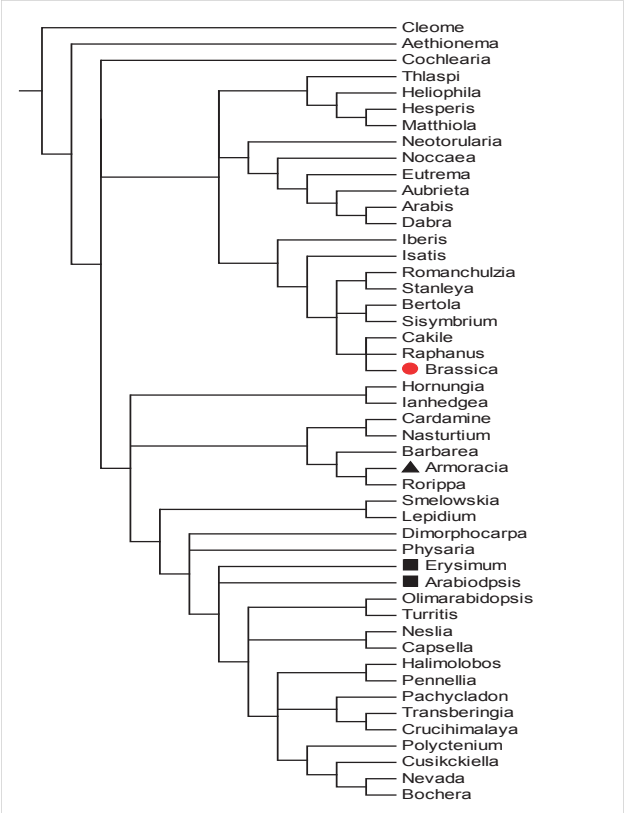

Fig. 4. Evolutionary relationship of six *Brassicaceae* taxa. The history was inferred using the neighbor-joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 0.58997 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test was shown next to the branches (Felsenstein, 1985). It is drawn to scale with branch lengths in the same units as those of the evolutionary distances (using the Poisson correction method, Zuckerkandl and Pauling, 1965) used to infer the phylogenetic tree. Phylogenetic analyses were conducted in MEGA4 (Tamura et al., 2007). Functional P5βRs are marked with asterisks.

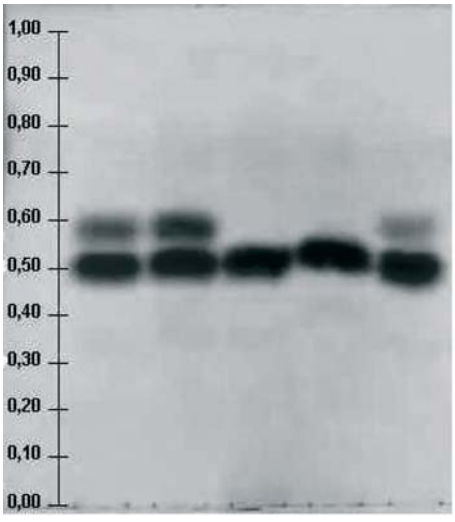

Fig. 5. TLC analysis of the enzymatic P5βR activity assays (12 hours). Lane 1 – active assay of *Ec*P5βR; lane 2 – active assay of *At*StR; lane 3 - control after treatment at 100°C for 10 min.; lane 4 – progesterone reference, $R_f = 0.50$; lane 5 – active assay of *Ar*P5βR, final product 5β-pregnane-3,20-dione in yellow, $R_f = 0.58$.

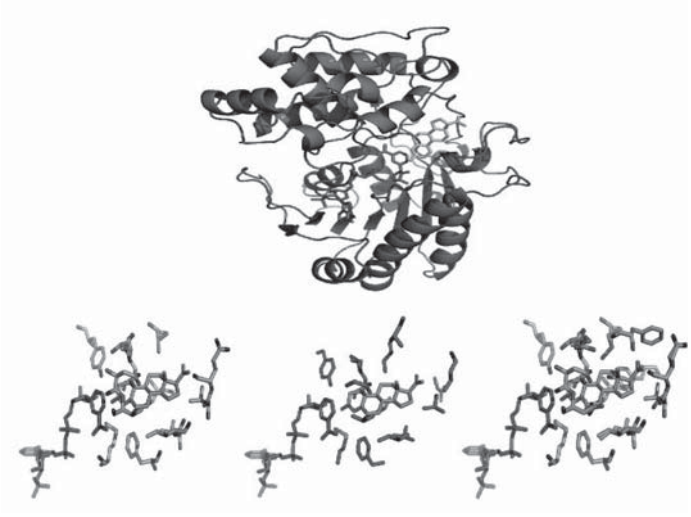

Fig. 6. Upper part - Atomic model of the *Ar*P5βR (green) on the basis of PDB 2V6G (Qmean score 0.76; z-score -0.15). Both, progesterone and co-substrate NADPH (in yellow) are supplemented to the catalytic site of the enzymes. Lower part - Active site modelling of ***At***StR (left, red), ***Ec***P5βR (middle, green) and ***Ar***P5βR (right, blue); co-substrate and substrate (yellow). Relevant amino acids in the close vicinity (4Å) of the substrate are shown.

Table 1. Amino acids suggested to be part of the binding pocket of P5βR reductases (P5βRs). All of the protein sequences deduced here are shown together with those of the P5βRs of *A. thaliana* (Herl et al., 2009), *E. crepidifolium* (Munkert et al., 2011), *E. rhaeticum* (Bauer et al., 2010), *A. rusticana* (JF460011), *Brassica oleracea* (JQ608337) and *Lunaria annua* (JN638575). Structurally conserved amino acids are highlighted.

	Amino acid residue (numbers as for P5βR form *A. thaliana*)																
Species	106	145	146	147	150	153	156	179	204	205	215	343	346	347	350	351	353
A. thaliana	W	G	T	K	L	F	V	Y	N	M	M	F	V	I	V	E	M
E. crepidifolium	W	G	T	K	L	F	L	Y	N	T	M	F	V	I	V	E	M
E. rhaeticum	W	G	T	K	V	F	L	Y	N	T	M	F	V	I	V	E	M
A. rusticana	W	G	T	K	I	F	N	Y	N	T	M	F	V	I	G	E	L
B. oleracea	*C*	G	T	K	Y	F	I	Y	N	T	T	F	D	V	V	E	M
L. annua	W	G	T	K	L	F	L	Y	N	T	M	F	V	I	G	E	M

IMPACT OF SUCROSE CONCENTRATION ON *IN VITRO* CULTURE LONG-TERM STORAGE OF RARE SPECIES

Sedcenco Maria

Botanical Garden (Institute), Academy of Sciences of Moldova

Summary: This article describes the peculiarities of long-term in vitro preservation of rare spontaneous plants from Moldova. In our experiment, we have used a low-cost substance, i.e. sucrose, as an agent that inhibits growth of plantlets in tubes and increases their viability after long-term storage. The plantlets were maintained in an environment without any phytohormones applied while various concentrations of sucrose were used. After 12 months of cultivation, the amount of regenerated plants which survived was assessed, and their visual analysis was performed. To maintain *Lilium martagon* L. as a species *in vitro* for a long term, MS-100% environment supplemented with sucrose concentration of 100 g/l was applied. To maintain Fritillaria montana and Bellevalia sarmatica as a species, according to the viability estimates, the most optimal environment is MS- 100% with sucrose in an amount of 90 g/l. Hence, higher sucrose concentrations in nutrient environments can be used successfully for in vitro long-term maintenance of rare species.

INTRODUCTION

Conservation measures, along with the basic *in situ* conservation strategy for vulnerable species, are significantly oriented towards microcloning, and, particularly, their *in vitro* programming for obtaining a greater number of plants and developing a technology aimed at long-term *in vitro* preservation of plantlets [1]. This plant material propagating technique has many advantages and can significantly advance conservation of plants. Currently, advanced modern methods of plant biotechnology are applied on a larger scale for preserving plant genetic resources. It is important to mention that these modern methods do not exclude traditional *in situ* or *ex situ* conservation used as complementary means of preservation. A wide range of modern methods such as *in vitro* biotechnology, molecular methods of genome analysis, cryopreservation protocols, or immunological diagnostic methods are currently used to characterize collections of plants, propagate and cultivate them, detect diseases, preserve, distribute, or exchange plant resources. Therefore, the basic task of our research is creating favorable conditions for applying morphogenetic potential to *in vitro* conditions, experimental simulation of the regeneration process, and creating conditions for maintaining the viability of explants in combination with the maximum increase in the duration among subcultures.

MATERIAL AND METHOD

The objects of the research under discussion are rare spontaneous flora species from the Republic of Moldova which have a known ornamental value. *Lilium martagon*

– the forest lily – is one of the most beautiful and delicate plants in the *Liliaceae* family. It is a rare plant declared to be a monument of nature and protected by law. It is a wild perennial plant originating from Europe and blooming in May-June. Its flowers have different colors: red, pink or violet, and a single forest lily can have 10-20 flowers overall. *Fritillaria montana* – the multicolored tulip – is a flower that delights the eye and each spring. In Moldova and Romania, it is declared to be a monument of nature. *Bellevalia sarmatica (Georgi) Woronov* is a herbaceous perennial plant. It is included in the list of protected plant species in the territory of the Republic of Moldova, and the species is listed in the Red Book [3]. Its brown and gray flowers are deflected horizontally in the fruit area and persist for a long time.

The biotechnological research methodology was based on generally accepted methods of working with classic tissue culture isolates and plant organs [4].

For the induction of cultures of the species studied bulb fragments of genetically stable types of explants were used [5].

The *in vitro* cultivated material consisted of flower-bearing bulb explants taken from every species. For this sake, a portion of the scale was detached. The size of the explant varied from 0,5 cm to 1,5 cm, depending on the size of the initial bulb. Explants taken from the donor material (bulbs or bulb pieces) were washed in running tap water. For preliminary sterilization, solution of KMnO4 (0.05%) + Tween-20 was applied for 10 minutes. For sterilization, diacid (0.01%) solution was applied for 6-7 minutes, followed by three washes with sterile distilled water.

After disinfecting the plant material depending on the size of the explant, they were inoculated in the culture environment in tubes and large vials, so that the bulbs could have enough space for growing.

In all the variants of the experiment, the MS (Murashige and Skoog, 1962) basic medium was used [6], and certain hormonal components and additions were applied for each species. Initially, the experiment was based upon a large number of culture environments. Explants were kept in the dark. An individual cultivation regime was developed for each taxonomic category.

RESULTS AND DISCUSSION

After the culture environments for induction and multiplication of new plants were established [8,9], we stopped at the stage of development environments and conditions allowing us to maintain explants growing at a slow pace by increasing the interval between subcultivations. To reduce the risk of eventual somaclonal variability, we decided to exclude all phytohormones from the nutrient media. After studying literature in the field of *in vitro* plant conservation, we found promising data on using sucrose [4,7]. It is known that increasing the concentration of sucrose as carbon source can speed up the growth of plantlets, but a certain amount of sucrose in

the environment can be used as a preservative which inhibits growth of plantlets due to osmosis.

Some authors have observed that the cultivation in an environment enriched with sucrose at the last stages of reproduction leads to a significant increase in viability of micro plantlets at the stage of adapting *ex vitro* conditions and accelerates their subsequent development.

Based on the foregoing, the need to establish the optimal concentration of sugar which would protect the viability of the explants after prolonged subculture and ensure long-term storage condition became evident. After neoplantlets were obtained, they were transferred to a phytohormone-free environment with different sucrose concentrations – 20, 30, 60, 90, 100 g/l. Plants were kept in a culture chamber (20 °C) in the dark.

During the year under discussion, the observations made showed that the three populations developed in a relatively uniform manner. 30 days after the transfer to the environment with sucrose concentration of 20, 30 g/l, the inoculated plants formed many roots, which was undesirable and too early for that period of time. In the environment with the average sucrose concentration of 60 g/l, bulbs increased 1,5-2-fold as compared with other environments. However, as time passed, as the nutrient medium was drying out, in 3-4 months, the bulbs stopped developing (arrested development). In the environment with the peak concentrations of sucrose (90, 100 g/l), plantlets apparently stopped developing, and their size hardly changed. 60 days later, they started having roots, but their number and length was much lower and they looked rather fragile. After 12 months, the amount of regenerated plants which survived was assessed, and their visual analysis was performed. At the average concentration of sucrose of 20-60 g/l, a greater number of plantlets were necrotized because of vitrification. In other medium, a small percentage of plantlets could not be preserved because of tissue necrosis. The experimental data is presented in Table № 1.

Table № 1

The viability of the plants on media with different concentrations of sucrose,%

Sucrose concentration in the medium MS-100%, g/l		**20**	**30**	**60**	**90**	**100**
Viability of species,%	***Lilium martagon***	**52**	**54**	**70**	**89**	**94**
	Fritillaria montana	**55**	**56**	**73**	**92**	**89**
	Bellevalia sarmatica	**50**	**50**	**68**	**94**	**93**

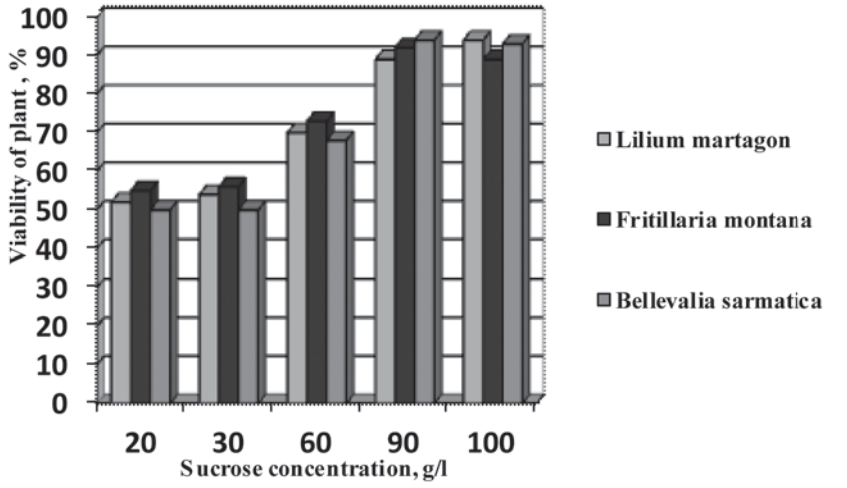

Figure 1. Effects of sucrose concentration on percentages of viability of the plants.

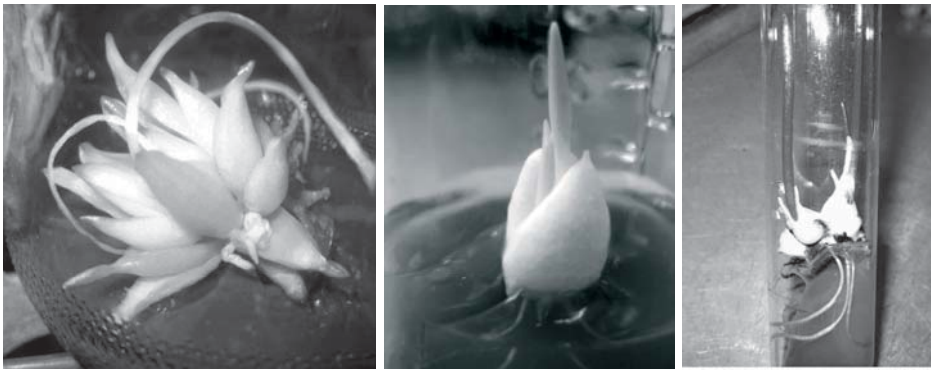

Figure 2. Species *Lilium martagon, Fritillaria montana, Bellevalia sarmatica* after 12 months of long-term storage *in vitro* culture.

CONCLUSIONS

Due to the research results, we have achieved *in vitro* conservation purposes of reducing growth, which allows extending the duration of the two subcultures. The works aimed at *in vitro* maintenance of plants have been practiced in accordance with the principles of the active gene bank, as the entire material has been passing through a cyclical process of multiplication.

Therefore, to maintain *Lilium martagon L.* as a species *in vitro* for a long term, after the propagation phase, bulbs can be transferred to MS-100% environment supplemented with sucrose concentration of 100 g/l. To maintain *Fritillaria montana*

and *Bellevalia sarmatica* as a species, according to the viability estimates, the most optimal environment is MS-100% with sucrose in an amount of 90 g/l. If we intend to obtain larger and more viable bulbs in the short term, MS-100% environment with sucrose concentration of 60 g/l can be used.

Physical and chemical parameters, which cause slow *in vitro* growth of plantlets for long-term storage of *in vitro* culture, which can be used to create a gene bank of rare plants in the conditions of the Biotechnology Laboratory of the Botanical Garden of the Republic of Moldova have been revealed.

We have developed protocols for mass clonal propagation of these species, and determined the optimal conditions for *in vitro* introduction, cultivation, and adaptation.

BIBLIOGRAPHY

1. Zăpârțan, M, Conservarea florei spontane prin înmulțire in vitro. Ed. Alc Media Group, Cluj –Napoca, 2001.

2. Pence V.C. The application of biotechnology for the conservation of endangered plants // Plant Conservation Biotechnology / Ed. E.E. Benson. Chapter 15. London: Taylor and Francis, 1999. P. 227–241.

3. Negru A., G. Şabanov şi alt.//Plantele rare din flora spoantana a Rebublicii Moldova.// CE USM, Chişinau, 2002.

4. Бутенко Р.Г. Биология клеток высших растений in vitro и биотехнологии на их основе. М.: ФБК-ПРЕСС,1999.160 с.

5. Takuo Furyuya, Koji Nomura, 2004 Rapid Production of Lilium auratum Bulbs from Zygotic Embryos. pp 39-42. Asia Pacific Journal of Molecular Biology and Biotechnology. Vol. 12.

6. Murashige T., Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures // J. Physiol. Plant. 1962. V. 15. P. 473–497.

7. Plant Propagation by Tissue Culture. Handbook and Directory of Commercial Laboratories. Eds. Edwin F. Georg, Paul D.Sherrington. 1984. P. 695.

8. Чоркинэ Н., Седченко М. Особенности микроклонирования *in vitro* редкого растения *Lilium martagon L.*// Вісник Київського Національного Університету імені Тараса Шевченка.//25-29, 2009 стр.157-158.

9. Sedcenco M., Ciorchina Nina, Doina Clapa, Al. Fira.//Conservation of rare species of Bellevalia sarmatica (Georgi) Woronov by vitroculture methode.// Materialele Simpozionului ştiințific Internațional, Conservarea diversității plantelor in situ şi ex situ. Iaşi, 2011.

II. CONSERVATION OF BIOLOGICAL DIVERSITY

IN VITRO ASSESSMENT FOR CONSERVATION OF FUNGAL DIVERSITY

Balaeş T., Tănase C.

„Alexandru Ioan Cuza" University of Iasi, „Anastasie Fatu" Botanical Garden

Abstract. 51 species of lignicolous macromycetes were isolated from natural substrates, screened and then were preserved through in vitro culturing and cryopreservation. The isolation was performed using dikaryotic mycelium from the fruit-bodies and the pure cultures were deposited in the Culture Collection of Research Laboratory for fungi with applications in ecological reconstruction of heavy metals polluted soils – RECOSOL. In this paper, the in vitro characterization of 8 isolates is presented. The mycelium's growth rhythm and macroscopic features of the colony, along with microscopic features of mycelium were recorded. The cryopreservation of fungal mycelium was performed using glycerol 10% as cryo-protective agent, after the cultivation of mycelium onto liquid media and homogenization. Cryopreservation proved to be effective.

INTRODUCTION

The fungi form a heterogeneous taxonomic group of organisms with diverse ecological and morphological features. The natural fungal diversity is frequently assessed by identification of fruit-bodies, which are, very often, evanescent. This aspect creates difficulties for fungal species inventory and for developing effective conservation strategies. Isolation in pure culture and *in vitro* characterization of mycelium provide advantages for both conservation and study of fungi, especially when a selection of isolates with biotechnological potential (Petre & Tanase, 2013) is desired, considering the possibility of using fungal mycelium in various processes.

The preservation methods of fungal isolates are particularly important because in long-term some cultures might degenerate, losing some biochemical properties, such as the synthesis of enzymes. In this respect, the use of conservation methods that allow long-term storage of all the cultures while maintaining fungal properties is necessary. Such a method is represented by the fungal mycelium cryopreservation (Mantovani et al., 2012).

In the present study, more than 50 species of lignicolous basidiomycetes were screened and conserved by cryopreservation. Some of these isolates were previously characterized *in vitro* (Balaes & Tanase, 2012a,b), while 8 other isolates from Order Agaricales (Class Agaricomycetes, Phyllum Basidiomycota, Kingdom Fungi), were characterized and described in the present study. The mycelium grown on artificial

media was analysed in order to observe the macroscopic and microscopic features of the mycelium, the growth rhythm and the aspect of colony.

MATERIALS AND METHODS

The isolation was realised using dikaryotic mycelium from the fruit-bodies as source of inoculum, and afterwards the mycelium was grown onto malt extract-agar media (malt extract 20 g L^{-1}) and incubated at 25 °C, in the dark, for 6 weeks. The fruit-bodies were collected from wood, lyophilized (UniEquip freeze-drying equipment, UNICRYO MIC 4 L model) and deposited in the Faculty of Biology Herbarium, Alexandru Ioan Cuza University of Iasi, Romania, receiving a voucher registration code [I] (Table 1). The identification of species was performed based on analysis of fruit-bodies, involving macroscopic and microscopic methods according to the literature [Borgarino & Hurtado, 2001,2004; Breitenbach & Kränzlin, 1995; Hansen & Knudsen, 1992; Roux, 2006], and the used nomenclature is according to The Species Fungorum database. All the fungal isolates were deposited in the *Culture Collection* of *Research Laboratory for fungi with applications in ecological reconstruction of heavy metals polluted soils* – RECOSOL.

During incubation time, all the macroscopic and microscopic features related to the mycelium and colony aspects were recorded according to the method established by Stalpers (1978), weekly, for 6 weeks.

The *in vitro* conservation of fungi was done by subcultivation on artificial media and refrigeration at 4 °C or by cryopreservation, using glycerol 10% as cryo-protective agent. In this respect, a liquid medium (yeast extract – 7 g L^{-1}, malt extract - 7 g L^{-1}, glucose – 10 g L^{-1}) was used for cultivating the mycelium, then the culture was homogenized at 7000 rpm for 1 minute (SilentCrusher M, Heidolph). The homogenized suspension was distributed in 2 mL Eppendorf tubes, with the addition of glycerol to a final concentration of 10%, the tubes were kept at room temperature for 8 hours for the glycerol diffusion through cell membranes and then placed in the freezer. The viability of the mycelium was repeatedly checked (monthly).

RESULTS AND DISCUSSIONS

A great morphological variability of the mycelium grown on artificial media is caused by the ecological adaptations of fungi and is influenced by their different taxonomical position. Particular structures may be present in the culture, but their presence is not prerequisite for all the species. The analysis of the cultural characteristics of the mycelium offers additional information regarding the biology of the fungal nutrition and development and it might help identification of species without having fruit-bodies. The analysed fungal isolates differed by the presence of the different types of propagules, growth rhythm, by the general aspect of the mycelium, and the presence/absence of particular elements (Table 2).

Table 1.

Selected isolates deposited in the Faculty of Biology Herbarium

No.	FAMILY	SPECIES ISOLATED IN PURE CULTURE	HERBARIUM VOUCHER	COLLECTION VOUCHER*
1	Agaricaceae	*Lycopedon pyriforme* Schaeff., 1774	I 137384	RECOSOL 27
2	Strophariaceae	*Gymnopilus junonius* (Fr.) P.D. Orton, 1960	I 137370	RECOSOL 18
3		*Hemipholiota populnea* (Pes.) Bon, 1986	I 137393	RECOSOL 19
4		*Hypholoma fasciculare* (Huds.) P. Kumm., 1871	I 137371	RECOSOL 21
5		*Kuehneromyces mutabilis* (Schff.) Sing. & A.H. Sm.	I 137390	RECOSOL 26
6		*Pholiota alnicola* var. *salicicola* (Fr.) Holec, 2001	I 137391	RECOSOL 35
7		*Pholiota aurivella* (Batsch.) P. Kumm., 1871	I 137392	RECOSOL 37
8	Physalacriaceae	*Armillaria mellea* (Vahl.) P. Kumm., 1871	I 137387	RECOSOL 1

** Culture Collection of Research Laboratory for fungi with applications in ecological reconstruction of heavy metals polluted soils – RECOSOL*

The cultural characteristics of different macromycetes species have been studied by several authors (NOBLES, 1948; STALPERS, 1978, 1993), but the knowledge is far to be comprehensive. There are not known typical anamorphs among the tested species, but conidiogenesis have been reported for several species in Strophariaceae family (WALTHER & WEIß, 2005). Two of the tested species did not formed hyphae with clamp connection: *Gymnopilus junonius* and *Lycoperdon pyriforme*. The main macroscopic features of the tested isolates are presented in the following.

Armillaria mellea. The colony presents an aerial mycelium, homogeneous, smooth, sometimes felt, translucent-white. The mycelium subsequently becomes more compact and takes a slightly powdery aspects and colony edge is straight.

Gymnopilus junonius. The mycelium is homogeneous, felt-smooth, creamy white, fluffy on the walls, with areas powdery in appearance. It forms hyphal agglomerations cufflinks-like, more often together, forming dense areas.

Hemipholiota populnea. The mycelium is concentrically zoned, with areas less dense alternating with dense aerial mycelium rings, fluffy, white. The first ring is dense, soft, felt, compact and very dense to the wall, cream-colored. The second ring is similar to, but less dense. The third ring is lighter, heterogeneous, with hyphal clusters that are small in the middle and bigger, felt-compact to walls. Near the Petri

dishes walls, the mycelium gets a creamy-yellow aspect, forming areas felt and compact.

***Hypholoma fasciculare*.** The mycelium is fluffy-felt, radial disposed, forming compact networks, white or cream, sometimes yellowish. Near the walls formed a felt and thin mycelial ring is present. There were formed elongated hyphal clusters, felt, sometimes circular, and small cords or bundles also. Sometimes the mycelium presents a powdery appearance. Near the edge of colony, the mycelium is more homogeneous, felt.

***Kuehneromyces mutabilis*.** The mycelium is smooth, less dense, easily felt, forming an area with longer and thicker hyphae and some very small clusters of hyphae. The mycelium is dense and translucent in the distal and near the point of inoculation and to the side is forming a small area powdery-felt, cream. The submerged mycelium presents radial arranged bundles of hyphae.

Lycoperdon pyriforme. The mycelium forms a network with radial arrangement, translucent to white, with straight hyphae, submerged or smooth. Near the point of inoculation mycelium has many aerial hyphae, is rarefied, powdery. The colony edge is wavy, but smooth.

Pholiota alnicola* var. *salicicola. The mycelium is easily concentrically zoned, creamy to yellowish-grey, slightly smooth-pubescent in the proximal area, hirsute-pubescent in the middle, entangled in the distal area. Aerial mycelium is fluffy-felt, less dense, with short hyphae that are yellowish-cream in the proximal area and white in the distal area. The colony edge is straight.

Table 2

Cultural characteristics of the tested isolates

Species	Growth rhythm*, smell and revers	Hyphal characteristics	Reproductive structures	Particular elements
Armillaria mellea	> 6; strong mould-like smell; brown revers	generative hyphae, 1,5–4,5 µm; skeletal	chlamydospores of 10–15 (20) x 12–20 µm, spherical	swellings of 10 x 48 µm
Gymnopilus junonius	4; unchanged	generative hyphae, 1,5–4 µm; lateral dendroid branches	arthroconidia of 1,5–2,5 x 4–6 µm; chlamydospores of 8–11 x 10–15 µm, ellipsoidal	octahedral large crystals
Hemipholiota populnea	> 6; unchanged	generative hyphae, 1,5–3 µm; skeletal, de 4 µm	pigmented chlamydospores	swellings
Hypholoma fasciculare	4; rotten wood smell; unchanged	generative hyphae, 2–4 µm; skeletal, 1,5–3 µm	spherical or subspherical chlamydospores, 10–16 µm	swellings of 12–20 µm.
Kuehneromyces mutabilis	3; unchanged	generative hyphae, 3–4 µm; skeletal, 7–8 µm	colourless exsudates	

Lycoperdon pyriforme	> 6; unchanged	generative hyphae, 1,5–4,5(5) µm;		swellings of 10 µm; octahedral crystals, 12 x 12 µm; long crystals, 12–16 µm
Pholiota alnicola var. *salicicola*	4; unchanged	generative hyphae, 1,5–4 µm; binding hyphae; lateral finger-like branches		tetrahedral or octahedral crystals, of 5 x 8 - 13 x 13 µm
Pholiota aurivella	4; soil-like smell; brown revers	generative hyphae, 1,5–4,5 µm; skeletal		

* The needed time for covering the entire plate (in weeks)

Pholiota aurivella. Near the inoculation point the mycelium is smooth, in the middle is denser, forming a thick network. The aerial mycelium is yellow, forms thick bundles, divergently branched and over it a fluffy network is formed, more or less homogeneous, less dense. The mycelium is very easily concentric zoned, with fluffy denser areas to the walls. The colony edge is regular, straight.

Periodically (monthly), the viability of cultures was checked, revealing a 100% recovery on media culture for all the preservation methods: repeated subculturing, refrigeration or cryopreservation.

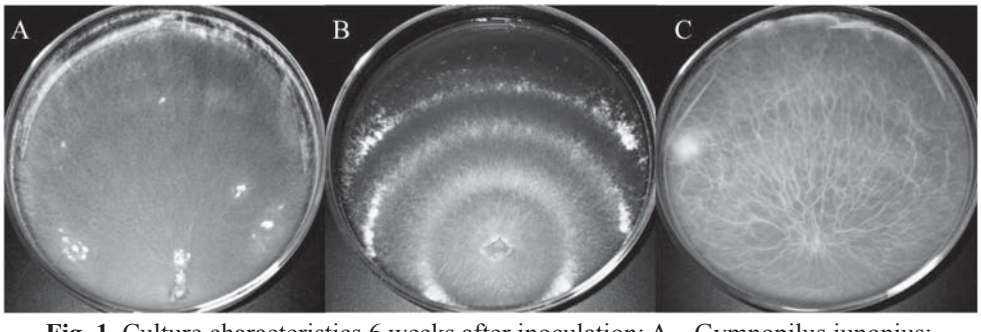

Fig. 1. Culture characteristics 6 weeks after inoculation: A – Gymnopilus junonius; B – Hemipholiota populnea and C – Pholiota aurivella

The first method requires a large consumption of culture media and an ongoing effort, and at the same time, there is a risk of mutating genome with isolates losing certain properties. Maintenance through refrigeration allows storing the cultures over a period of up to 8 months, but at the end it requires subculturing. Cryopreservation is the most effective and safe method of preservation, ensuring the maintenance of long-term biochemical properties with low maintenance costs.

CONCLUSIONS

The growth rhythm, the presence of particular structures (conidia, crystals, and swellings), the macroscopic features and types of hyphae were different from a species to another, but there were also similarities between species, as they belong to the same order, and most of them even to the same family.

Four of the tested isolates produced chlamydospores, while arthroconidia were seen only in the cultures of *Gymnopilus junonius*. *Armillaria mellea*, *Hemipholiota populnea* and *Lycoperdon pyriforme* grew very slow on artificial media.

There have been established effective methods for the *in vitro* conservation of the fungi. Among the tested methods, the cryopreservation proved to be appropriate in long term.

BIBLIOGRAPHY

1. Balaeş T., Tănase C., *Culture description of some spontaneous lignicolous macromycetes species***.** Journal of Plant Development, 2012, **19**: 83-98.

2. Balaeş T., Tănase C., *Description of in vitro cultures for some spontaneous lignicolous basidiomycetes species*. An. Ştiinț. Al. I. Cuza Iaşi Sect. II a. Biol. Veget., 2012, **58**(2): 19-29.

3. Borgarino D., Hurtado Cr., *Le guide des champignons en 900 photos et fiches*. Édisud, Aix-en-Provence, 2004, 1-450.

4. Borgarino, D., Hurtado, C., *Champignons de Provence, Haute-Provence et Midi Méditerranéen*. Édisud, Aix-en-Provence, 2001, 88-89.

5. Breitenbach J., Kränzlin F., *Champignons de Suisse*, *4. Champignons à lames, 2º partie*. Mykologia, Lucerne, 1995, 1-371.

6. Hansen L., Knudsen, H., *Nordic Macromycetes*, ***II****: Polyporales, Boletales, Agaricales, Russulales*. Nordsvamp, Copenhagen, 1992, 97, 123.

7. Mantovani T.R.D'A., Tanaka H.S., Umeo S.H., Zaghi L.L., do Valle J.S., Paccola-Meirelles L.D. Linde G.A, Colauto N.B., *Cryopreservation at -22 and -27 °C of Pleurotus ostreatus on grains*. Indian J Microbiol, 2012, **52**(3):484–488.

8. Nobles, M. K., Identification of cultures of wood-rotting fungi. Canadian Journal of Research, *1948,* **26**: 347-336.

9. Petre C.V., Tănase C., *Culture characteristics of 20 lignicolous basidiomycetes species that synthesize volatile organic compounds*. Analele Ştiințifice ale Universității "Al.I.Cuza" Iaşi, s.II a. Biologie Vegetală, 2013, **59(**2): 37-51.

10. Roux P., *Mille et un champinions*. Edition Roux, Sainte Sigolène, 2006, 431-434.

11. Stalpers J.A., *Identification of wood-inhabiting Aphyllophorales in pure culture*. Studies in Mycology, 1978, **16**: 1-248.

12. Stalpers, J.A., *Pleoanamorphy in Holobasidiomycetes. 205-206 pp.* In: Sugiyama, J., (Ed.). *Pleomorphyc fungi: the diversity and its taxonomic implications*. Elsevier, New York, 1987, 325.

13. Walther G., Garnica S., Weiß M., *The systematic relevance of conidiogenesis modes in the gilled Agaricales*. Mycol. Res., 2005, **109**(5): 525–544.

14. *** Species Fungorum database (www.speciesfungorum.org, accessed from 20th July 2013 to 25th January 2014)

COMPARATIVE ASPECTS REGARDING THE DIVERSITY OF WOOD-DESTROYING MACROMYCETES IN TWO OPEN AIR VILLAGE MUSEUMS FROM ROMANIA

Cojocariu Ana, Tănase Cătălin

„Alexandru Ioan Cuza" University of Iaşi - Romania, „Anastasie Fătu" Botanical Garden

Abstract: Our investigations highlighted the diversity of macromycetes involved in biodegradation of the monuments from the Bucovina Village Museum - Suceava and Village Museum - Sighetul Marmatiei (Romania), and allowed the identification of causes that increase the occurrence and the development of fungi. We proceed to an estimation of extension and degree of the attack on the main elements of the constructions. A comparative study was made regarding the diversity of fungi into the studied open air museums, and was highlighted the frequency of some species of macromycetes as important agents of wood biodeterioration.

INTRODUCTION

Open-air village museums represent objectives with a special status because they bring together architectural monuments represented by buildings that are no longer functional, having just the role of museum exhibits into the direct incidence of the environmental factors.

The Bukovina Village Museum is placed on a field in the near vicinity of the Musat Throne Citadel in Suceava, and exhibits the folk art architectonic heritage of the Northern Moldavia. The main roles of the museum is preserving, restoring and exhibiting the ethnographic cultural heritage as a cultural objective of first importance, representative for the folk culture of Bucovina. The idea of building the potential open air museum of traditional folk architecture was initiated in 1958, and researches to identify the representative monuments were started. Eight monuments have been purchased for the project to organize the open air museum; the first three objectives of Câmpulung Moldovenesc, Humor and Rădăuți ethnographic areas were transferred and restored in 1976. In its final shape, the Bukovina Village Museum will bring together through its 80 architectural objectives the cultural and spiritual atmosphere of Bukovina, tending to restore the complex ambiance through houses and annexes, technical installations and workshops crafts, buildings and annexes with spiritual, social and cultural purposes. In present, the open air exposition holds a number of 16 buildings in the main ethnographic areas, six of them with the traditional interiors and inventory (Photo 1).

Maramures Village Museum is an objective located in Sighetul Marmatiei town, Maramures County, which includes mainly a collection of houses specific

to Maramures area. The museum was inaugurated in 1981, after a long period for collecting the exhibits. The museum exhibit more than 30 houses, some completely furnished with original pieces. Houses and farms preserved are grouped by major sub-areas of the historical Maramures: Cosău - Mara, Viseu - Borsa and Ruscova basin. Besides the Romanian houses were reconstructed one Ukrainian house, one Hungarian house and two Hebrew village houses. The main attraction is the wooden church, the oldest building in the museum (XVI century), built with materials from an older church (some beams are dendrochronological dated between 1572 and 1614) from the Onceşti village (Photo 2).

Photo 1: Bucovina Village Museum – Suceava (SV) - Marginea workshop

Photo 2: Village Museum - Sighetul Marmatiei (MM) - The traditional wooden church from Oncesti village

The biotic factors can be considered essential for the process of wood biodeterioration due to the fact that the wood is an important source of organic matter and mineral elements for different organisms that alternate according to the deterioration of wood matter in time (6).

MATERIALS AND METHODS

The collection of sporocarps was made following periodical investigations of wood to all 16 exhibits from Bucovina Village Museum and 21 exhibits from Village Museum - Sighetul Marmatiei. For each sample collected from the construction wood have been made observations recorded into worksheets: the affected building element, position of sporiferous bodies above the ground, wood type, degradation degree (hard wood, nearly hard wood, degraded wood, heavily degraded), moisture of wood (dry, nearly dry, moist, humid). The identification of the species was made based on the analysis of the morphological macroscopic and microscopic characteristics (2, 3, 5, 7, 9, 10, 13, 14).

To emphasize the extent of the damage caused by macromycetes at the wooden structures selected for study, the affected parts of the construction, the specifics of the wood and the propagation area have been analyzed.

Observations about specific conditions that can determine the attack of macromycetes to the different wooden elements were made taking into account the level at which the item is located throughout the entire building: upper level, middle level, and bottom level (Fig. 1).

RESULTS AND DISCUSSION

The extension of wood resistance in time used in construction of historical monuments is a major concern for preserving cultural and historical heritage. Characteristic of this area of conservation is that the wood materials of the constructions are in an advanced state of degradation often containing destructive agents (fungi and insects) still in active status. Conservation issues that arise are complex, involving control measures, prevention and consolidation (possibly partial repairs and replacements) impractical due to the high volume wooden materials which are used to each objective.

A further difficulty is the fact that wood, at historical objectives in open air museums (houses, churches, industrial facilities, etc.) being incorporated into the whole construction, is not normally accessible to the treatment without complete disassembly or partially at least. Such issues are put both into our existing open air museums but also at the different constructions of historical monuments (houses and farms in different regions of the country), considered as cultural monuments, whose preservation must also be assured.

On construction's wood at the exhibits from Bucovina Village Museum – Suceava and Village Museum - Sighetul Marmatiei we have identified 28 species of macromycetes (Table 1). Regarding the distribution of the species in the two phylums (15), 89.3% of the identified species belong to the Basidiomycota and just 10.7% to the Ascomycota. The best represented is the Polyporales order, with 4 families, which holds 42% of the total identified species that belong to the Basidiomycota, followed by the Hymenochaetales with 18%.

In Bucovina Village Museum – Suceava, since 2007, were reported previous researches regarding the presence of macromycetes on construction woods, being cited a number of 26 species of macromycetes (1, 8).

Tab. 1

Species of macromycetes identified on construction wood and number of samples by construction level

Species	Bucovina Village Museum – Suceava (SV)			Village Museum – Sighetul Marmatiei (MM)		
	Number of samples					
	Upper level	Middle level	Bottom level	Upper level	Middle level	Bottom level
Xylaria longipes Nitschke	0	0	2	0	0	1
Xylaria polymorpha (Pers.) Grev.	0	0	0	0	0	1

Daldinia concentrica (Bolton) Ces. & De Not.	0	2	0	0	1	2
Dacrymyces deliquescens (Bull.) Duby	4	3	2	8	2	2
Calocera cornea (Batsch) Fr.	0	2	2	0	1	1
Schizophyllum commune Fr.	6	4	3	7	3	5
Haplotrichum aureum (Link : Fr.) Hol.-Jech.	0	0	2	0	0	0
Hymenochaete rubiginosa (Dicks.) Lév.	0	2	1	0	1	1
Phellinus ferruginosus (Schrad.) Bourd & Galz.	2	0	0	1	1	0
Phellinus contiguus (Pers.) Pat.	2	2	0	2	2	1
Hyphodontia arguta (Fr.) J. Erikss.	0	0	2	0	1	1
Hyphodontia breviseta (P. Karst.) J. Erikss. – Photo 6	0	0	1	0	1	1
Gloeophyllum sepiarium (Wulfen) P. Karst. – Photo 3	4	3	3	2	4	3
Gloeophyllum odoratum (Wulfen) Imazeki – Photo 4	0	0	0	0	1	2
Gloeophyllum abietinum (Bull.) P. Karst. – Photo 5	2	1	3	3	1	1
Gloeophyllum trabeum (Pers.) Murrill	2	0	3	2	0	2
Hapalopilus nidulans (Fr.) P. Karst.	0	1	1	1	1	0
Hyphoderma puberum (Fr.) Wallr.	0	2	1	0	2	0
Trametes versicolor (L.) Lloyd	1	2	3	1	1	3
Trametes pubescens (Schumach.) Pilát	0	0	2	0	1	2
Trametes hirsuta (Wulfen) Pilát	0	2	0	1	1	2
Datronia mollis (Sommerf.) Donk	0	0	1	0	0	0
Polyporus arcularius (Batsch) Fr.	0	0	2	0	0	0
Lenzites betulina (L.) Fr.	1	2	2	2	0	0
Dichostereum granulosum (Pers.) Boidin & Lanq.	0	0	2	0	0	0
Peniophora quercina (Pers.) Cooke	0	4	0	0	3	2
Stereum hirsutum (Willd.) Gray	1	3	3	0	4	5
Coniophora puteana (Schumach.) P. Karst.	0	0	0	0	0	1

The species *Gloeophyllum sepiarium* (Wulfen) P. Karst., *Trametes versicolor* (L. : Fr.) Pilát, *Stereum hirsutum* (Willd. : Fr.) S.F. Gray, *Schizophyllum commune* Fr. and *Dacrymyces deliquescens* (Bull.) Duby was commonly recorded. This result is explained by incidental environmental factors and growth conditions similar to those from the natural forest ecosystems.

In both open air museums, the identified causes which determine macromycetes occurrence are: Bottom level - represents the main area affected by macromycetes due to accumulation of moisture from soil and due to constructions defects or inadequate techniques for building foundation. Middle level – at this level is extended the local

conditions present at the bottom level and installation of fungal species takes a longer period. Upper level - strong infiltration observed here at the roof level, malfunctioning construction, deterioration of the protection area, lack of efficient ventilation.

Humidity of the air necessary to development of fungi which cause brown rot in buildings must be more than 95% during several months, and the wood moisture content 25%, unless otherwise moisture. According to these findings, the primary cause of fungal decay of this type at constructions must be a malfunction leakage to water sources for a long period (11).

Most vulnerable construction elements are presented in Table 2. The elements were grouped into three vertical levels (Fig. 1): upper level, middle level and bottom level.

Tab. 2:

The most affected wooden construction elements

Level	Elements
bottom level	area into connections with masonry, base support beams, planks of the floor, parquet, tiles for the ridge, bottom thresholds
middle level	upper thresholds, binding planks, wainscots, counter batten under the plaster, skirting boards, beams of outside walls
upper level	ceiling terminations, upper beam, roofs and bindings

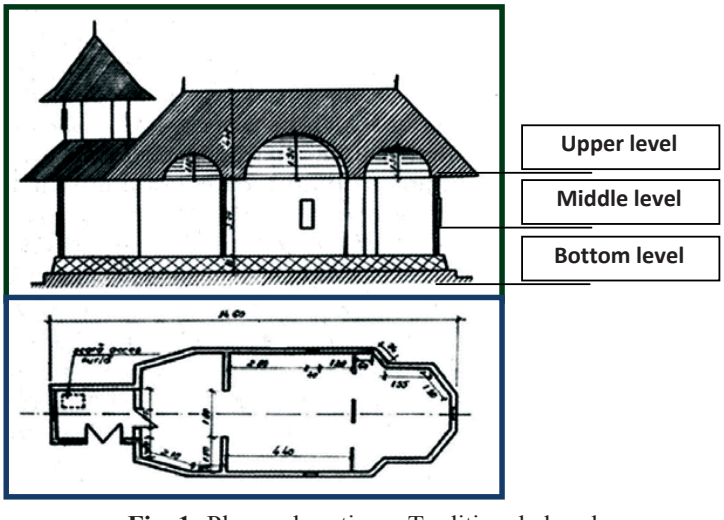

Fig. 1: Plan and section – Traditional church

The first species installed at the bottom level is *Stereum hirsutum* in the case of exhibits of Sighetul Marmatiei Vilage Museum, and in Bucovina Village Museum is specific the species *Trametes versicolor*. The both species represent specific fungi to

forest environment, in the both village museums all the construction being exposed in natural habitat. This species succeeds by *Hymenochaete rubiginosa*, and in some cases, but rather late, is installing *Gloeophyllum trabeum* when the wood is already heavily degraded. In the case of footbridges and exterior stair, often appear the species *Gloeophyllum sepiarium*, on deciduous wood used for these building elements.

At middle level, the initial deterioration of construction wood caused by certain species of macromycetes, can lead to a loss of material and the establishment of optimal condition for occurrence of other species of macromycetes, including the accumulation of organic material and installation of other groups of organisms. The lateral nave walls, which are made of thick oak or fir beams placed horizontally and joined in various techniques, thus providing a strong supporting base for the roof. However, grossly finished wooden beams provide gaps between them, which initially got walled up with moss and grass from the outside, but in time some of the beams would fall down (12).

For the upper level, in many cases we can find the species *Phellinus contiguus*, followed even in the second year by species of *Hyphodontia* genera. At exterior, there were found together species of *Hyphodontia* and *Dacrymyces* genera, which persist in accordance with substrate and atmospheric moisture. Subsequently, the species *Schizophyllum commune* and *Phellinus contiguus* are installing outside very fast, followed by other groups of organisms such as mosses and lichens.

At Sighetul Marmatiei was reposted a single case of attack of species *Coniophora puteana*, a specific and very destructive agent for biodeterioration of wood used in construction, but in this case the affected elements are represented by the exterior upstairs on a traditional house. The quality of exhibit for this buildings require a good monitoring for all types of biological agents of biodegradations, and the periodical investigation and treatments do not permit a extensive occurrence of fungi. Also, only in this open air museum some species as: *Xylaria polymorpha* and *Gloeophyllum odoratum* were found. In Bucovina Village Museum, there was a single reporting for species: *Haplotrichum aureum*, *Datronia mollis, Polyporus arcularius* and *Dichostereum granulosum.* These species did not appear as specific for construction wood as it is found in literature (4), but is important for the general description of wood biodeterioration in natural environment. The great diversity of wood essences used in masonry has as a consequence a great variety of biodegradation agents such as: bacteria, algae, fungi, lichens, moss, superior plants, insects and birds. Fungi are the most dangerous biodegradation agents in what structure wood is concerned, not only because of their damaging effect but also their rapid extension and frequent attack (4).

Although the specialists from Bucovina Village Museum, who worked at the Vama church in 2005, were confronted with a very difficult situation, namely an important damage caused by *Serpula lacrymans*, during our researches also did not

report the presence of this destructive species and any signs of a possible occurrence of *Serpula lacrymans*. This fungus attacked some construction elements from the inferior part (the foundation and floor). In this case the specialists resorted to the removal and the burning of the infected elements as well as of those in their vicinity. The other construction elements (old and new wood) were treated by immersion into a water solution (10%) of copper sulphate (55%) and potassium bichromate (45%) before the reconstruction. The treatment was repeated by spraying the areas with the same solution in May and September (1).

CONCLUSIONS

The wood was the main raw material used in achieving patrimony goods from house building and churches, decorative items or household products.

Biological factors involved in wood biodegradation at exhibited monuments occupy a special ecological niche that is not yet sufficiently studied. In the wood degradation research it is very important to know the interspecific relationships between fungi and other pests, contributing to a correct diagnosis of the state of conservation and finding optimal solutions for curative and preventive treatments.

On the construction wood in the both open air museums were identified 28 species of macromycetes. Species as *Gloeophyllum sepiarium*, *Stereum hirsutum*, *Schizophyllum commune* were reported very commonly, a situation explained by incidental environmental factors and growth conditions similar to those from the natural forest ecosystems.

Into the both open air museums, the identified causes which determine macromycetes occurrence are delimited by construction level with specific local condition - accumulation of moisture from soil, constructions defects, inadequate techniques for building foundation, infiltration at the roof level, malfunctioning construction, deterioration of the protection area, lack of efficient ventilation.

Photo 3: *Gloeophyllum sepiarium* (Wulfen) P. Karst.

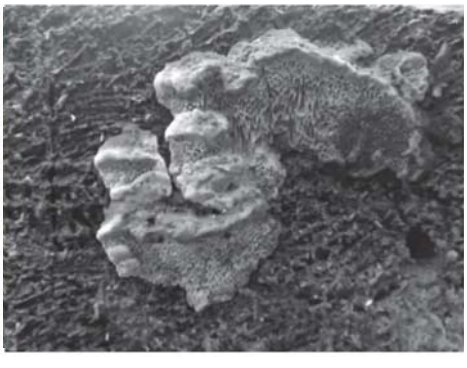

Photo 4: *Gloeophyllum odoratum* (Wulfen) Imazeki

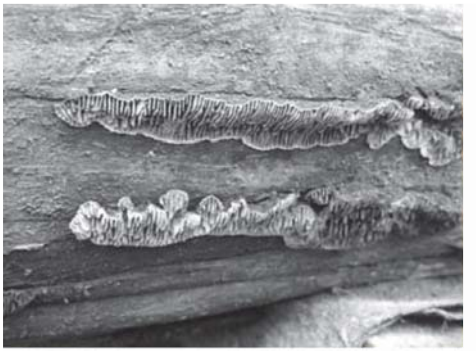

Photo 5: *Gloeophyllum abietinum* (Bull.) P. Karst.

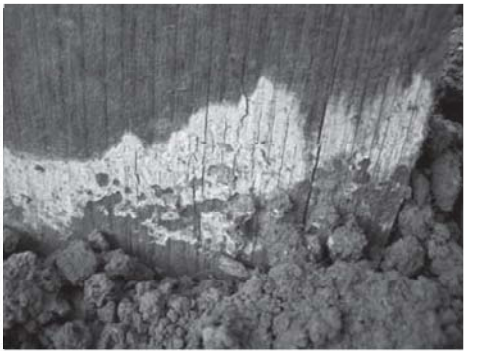

Photo 6: *Hyphodontia breviseta* (P. Karst.) J. Erikss.

BIBLIOGRAPHY

1. Axinte L. 2008. Aspects concerning the preservation state of Vama Church in the Bucovina Village Museum, Suceava. *European Journal of Science and Theology*. **4**(3): 71-78.

2. Bernicchia A. 2005. *Polyporaceae s.l.* Fungi Europaei 10. Edizioni Candusso, Alassio

3. Breitenbach J., Kränzlin F. 1986. *Fungi of Switzerland. A contribution to the knowledge of the fungal flora of Switzerland*. Vol. 2. *Heterobasidiomycetes, Aphyllophorales, Gasteromycetes,* Mycological Society of Lucerne, 414 pp.

4. Bucşa L., Bucşa C. 2005. *Agenți de biodeteriorare a lemnului la monumente istorice din România. Prevenire şi combatere*, Ed. Alma Mater, Sibiu: 127 pp.

5. Constantinescu O. 1974. *Metode şi tehnici în micologie*, Ed. Ceres, Bucureşti, 215 pp.

6. Dix N. J., Webster J. 1995. *Fungal Ecology*, University Press, Cambridge, Great Britain, 549 pp.

7. Eriksson J. 1958. *Studies in the Heterobasidiomycetes and Homobasidiomycetes – Aphyllophorales of Muddus National Park in North Sweden*, Uppsala, Symbolae Botanicae Uppsalienses, **XVI**: 1, 172 pp.

8. Jercan A., Tănase C., Mititiuc M., Chinan V. 2005. Wood-destroying macromycetes in the Bukovina Village Museum – Suceava, Procedings National Symposia of Romanian Mycological Society, *Sănătatea Plantelor*: 47-50.

9. Jülich W. 1989. *Guida alla determinazione dei funghi,*Vol. 2, *Aphyllophorales, Heterobasidiomycetes, Gasteromycetes*, Saturnia, Italy, 597 pp.

10. Jülich W., Stalpers J.A. 1980. *The resupinate non-poroide Aphyllophorales of the temperate northern hemisphere*, North-Holland Publishing Company, Amsterdam, Oxford, New York, 335 pp.

11. Paajanen L., Viitanen H. 1988. *Microbiological degradation of wood piles in building foundations*, Technical R.C. of Finland: 32 pp.

12. Raşcu P. E. 2005. Wood churches of Romania and Norway comparative considerations. *European Journal of Science and Theology*. **1**(3): 35-50

13. Ryvarden L. 1991. *Genera of Polypores. Nomenclature and taxonomy*, Synopsis Fungorum 5. Fungiflora – Oslo – Norway, 363 p.

14. Sălăgeanu G., Sălăgeanu A. 1985. *Determinator pentru recunoaşterea ciupercilor comestibile, necomestibile şi otrăvitoare din România*, Ed. Ceres, Bucureşti, 330 pp.

15. Şesan T. E., Tănase C. 2006. *Mycobiota. Sisteme de clasificare*, Ed. Univ. Al. I. Cuza, Iaşi: 251 pp.

NATURAL CROP PROTECTION BASED ON PLANT RESOURCES OF THE *JUNIPERUS SABINA* L. IN THE REPUBLIC OF MOLDOVA

Elisovetskaya Dina[1], Nastas Tudor[1], Bucatel Vasile[2], Galupa Dumitru[3]

[1]Institute of Genetics, Physiology and Plant Protection, Academy of Sciences, dina.elis.s@gmail.com

[2]The Botanical Garden (Institute) of the Academy of Sciences of Moldova

[3]Forest Research and Management Institute (ICAS) Chisinau

Summary: The studies have shown that the currently estimated resource of *J. sabina* materials in Moldova, obtained from the annual pruning only in the Botanical Garden and two nurseries (Jargara and and Teleneshti), is enough for treatment of 8.4 - 12.7 ha of potatoes (depending on the frequency of treatments - once or twice). It is possible to increase the calculated amount of raw material at least by 1.5 times at the expense of other areas (reserve Lozova; v. Kishkareni, district Sinjerey.) The cost of received by us *J. sabina* extract for the treatment of 1 hectare of potatoes from *L. decemlineata* is on average from 1200 lei. At the same time the average cost of insecticide ranges from 150 to 300 lei per 1 ha; per season for chemical treatment of 1 ha of potato producers spend 700-800 lei and more.

It was proved that the biological effectiveness of the extract, prepared from J. sabina, for three weeks has maintained at the level of chemical standard (Midash 200 SL, 0.3 l/ha). Meanwhile, the number of larvae did not exceed the thresholds and damage of the bushes was less than 5%.

INTRODUCTION

Over the last years there has been an essential increase of the topicality of using biorational resources in ecology-focused plants protection systems from pests and diseases. This is primarily due to environmental pollution, as well as increased resistance of pests to the used chemical agents. One of effective directions in plant protection is preparation and use of plant extracts containing biologically active substances with insecticidal and fungicidal properties. To the number of plants with insecticidal and antifeedant activity towards the phytophagans belong the representatives of the *Juniperus* L. species. We found that promising in this regard is the Cossack juniper - *Juniperus sabina* L. (Cupressaceae) [4, 5].

The objective of the study was aimed at determining the resource capabilities of *J. sabina* in Moldova for obtaining extracts that are biologically active against *L. decemlineata*.

MATERIALS AND METHODS

Analysis of *J. sabina* plantations in Moldova was carried out with help of Professionals of The Botanical Garden (Institute) of the Academy of Sciences of Moldova and Forest Research and Management Institute (ICAS) Chisinau [1].

Collection, drying, crushing, storage and preparation of raw materials and obtaining plant extract were performed according to standard procedures [6, 8].

J. sabina extract testing (15 g/l, 7.5 l/ha) to determine its biological effectiveness, in 2013, was conducted at the experimental field IGFZR ASM on potatoes ('Amerikanka' potato variety with a medium ripening period) in accordance with conventional methods [2, 7]. As a chemical standard on potatoes, we used 'Midash 200 SL' Sharda Worldwide Exports Pvt. Ltd India (imidacloprid, 200 g/l) insecticide, with a dose of 0.3 l/ha. 2.5% alcohol solution has been used as the reference. Treatment operations have been carried out using 'KWAZAR COR' knapsack sprayer with a capacity of 12 l. The consumption of working (spray) solution was 300 l/ha. During vegetation period agrotechnical measures of protection from weeds were carried out on this potato field.

Each variant (the treatment with *J. sabina*, chemical standard and reference) includes six replications - 20 plants for each. Pest population density has been recorded before treating plants and on the 1st, 3rd, 7th, 14th, 21st day after being treated. Biological efficiency has been determined according to standard methodology. Antifeedant activity (damage degree of potato plants) has been determined on the 1st, 3rd, 7th, 14th and 21st day according to standard methodology [2].

Mathematical processing of obtained data has been carried out according to the method of unifactor variance analysis [3].

RESULTS AND DISCUSSIONS

As a result of previously executed studies we found that extracts of *J. sabina* exhibit insecticidal and antifeedant action against Colorado potato beetle [4, 5]. Plants of *Juniperus* genus are the most frequently occurring on the territory of Moldova representatives of conifers. *J. sabina* genus is represented in Moldova in many varieties: Arcadia, Blue Danube, Broadmoor, Buffalo, Glauca, Cupressifolia, Mas, Rockery Gem, Tamariscifolia, Variegata, Nana, etc. Plants are grown in nurseries and are used for decorative landscaping in parks, backyards and along highway roadsides. *J. sabina* - evergreen shrub, moderately fast growing, hardy, drought-resistant, surviving urban climate, tolerates severe pruning. Needles are mostly bristly, prickly. Photophilous plant, survives little shading. *J. sabina* is undemanding to soil, it grows at all dry or fresh, drained, poor soils, from acidic to alkaline. Sizes of plants depending on the variety can vary widely - from 0.3 - 0.8 m to 2.0 - 4.0 m in height and from 1.2 - 2.0 m to 2.5-5.0 (up to 8 m) m in width. The annual growth rate is at least 10 cm in height, 20 cm in width. Some varieties might reach to 1.5 - 3.0 m of annual growth in width [9].

We assessed the largest areas of *J. sabina* in the Republic of Moldova. And using the results of this assessment, we have calculated possible volumes for obtaining raw materials for the production of the extract (Table 1).

Table nr.1

Assessment of *Juniperus sabina* areas in the Republic of Moldova, where it is possible to obtain raw materials for production of the extract

Availability of *Juniperus sabina* areas in the Republic of Moldova	**Size or the number of bushes**	**Quantity of green mass obtained during annual pruning of a bush / or m^2, kg**	**Quantity of dry mass obtained during annual pruning of a bush / or m^2, kg**	**Quantity of dry mass obtained at annual pruning from the entire area, kg**	**Quantity of *J. sabina* spray solution**
Yargora Reserve	1700 bushes	**0.5**	**0.1**	170	1700 l
Teleneshty Reserve	600 bushes	**0.5**	**0.1**	**60**	600 l
The Botanical Garden, Chisinau	0,5 ha 3 alleys of 150 m long	**Width for pruning 1 m** c 1 m^2 = 5 kg	**5 kg* 150 m = 750 kg**	**150**	22.5 kg d/o or 1500 l of spray solution
In total					3800 l

It was found that in total from all the evaluated areas it is possible to obtain about 380 kg of *J. sabina* dry plants or 3800 liters of spray solution (extract). Calculated amount of extract (3,800 liters) in a single treatment (spray solution consumption of 300 l/ha) is enough to protect 12.66 hectares of potatoes, in double treatment (working solution consumption of 450 l/ha) - for 8.4 ha.

Thus, it was found that from one hectare of *J. sabina* plantation it is possible to obtain an average of about 300 kg of dry mass, or 3000 liters of spray solution which suffices to protect 6.7 hectares of potatoes (two treatments and the overall consumption of 450 l/ha).

Additionally, we revealed the presence of juniper areas in other parts of Moldova - Reserve «LOZOVA» (0.5 ha or more) and at the north of Moldova village Kishkaren, district Sinjerey (along the route, the total length of 0.5 km), from which it is possible to obtain at least 1500 liters of *J. sabina* spray solution per year.

To determine the cost of the *J. sabina* plant extract we have calculated costs in an experimental way, which included: loss of ethanol for wetting raw extract and at the evaporation of the extract, as well as ethanol consumption for obtaining stock solution. As a result, it was found that the cost of *J. sabina* extract for the treatment of 1 hectare of potatoes from *L. decemlineata* in average is 1,200 lei (excluding the cost of electricity and operating personnel salaries). In industrial preparation of the extract

its net cost can be significantly reduced. However, the cost of chemical insecticides for treating 1 hectare of potatoes from *L. decemlineata* is several times lower, averaging from 150 to 300 lei. At the same time, taking into account the cost of the seed and the costs associated with agro-technical measures, the cost of a kilogram of potatoes in the country in 2013 ranged from 0.5-1 euro/kg (9-18 lei/kg) and higher. Therefore, we believe that, despite the high cost of the *J. sabina* plant extract, when using it to produce environmentally friendly products, the price of potatoes will grow within consumer demand.

Thus, we found that the currently estimated resources of *J. sabina* raw materials in Moldova, resulting from annual pruning, are enough for treatment of a minimum of 8.4 to - 12.7 hectares of potatoes (depending on the frequency of treatments - once or twice), and the calculated amount may be increased by at least one and a half times at the expense of other areas. At the same time, taking into account the cost of seed and other expenses, the cost of protecting potatoes with vegetable extracts will not significantly affect the cost of 1 kg of potatoes. Given that it is planned to create in Moldova a national park, with an area of 20 thousand hectares that will cover Orhei, Straseni and Criuleni regions and where the organic farming will be encouraged, the use of plant extracts can have a real prospect for the country.

As a result of field tests, it was found that the extract obtained from *J. sabina* (15 g/l) within three weeks after treatment effectively reduces the number of *L. decemlineata* larvae to pre-threshold level. Four weeks after treatment it was noted that its efficiency significantly decreases, but remains, however, at the level of the chemical reference. Simultaneously, there have been reduced antifeedant properties of the extract as well. The number of eggs laid by females increases, as well as the percentage of hatched larvae and survivors. Therefore, within a short time of 5 to 7 days the number of larvae of the Colorado potato beetle on plants significantly increases, which can lead to significant defoliation of plants. Therefore, it is appropriate to conduct the second treatment after 3-4 weeks after the first one, in case of increasing of the pest population above threshold level (Table 2).

A mathematical analysis of the data showed that under field conditions on potato the insecticidal activity of standardized *J. sabina* plant extract was comparable with standard chemical reference at three weeks after treatment. In the future, (28 days after treatment) the efficiency, both of the extract and the standard is significantly reduced, which is the basis for the second treatment when pest numbers exceed threshold levels.

Antifeedant activity of *J. sabina* extract and chemical standard was 1 point (up to 5% of leaf apparatus browsing), while in the control at that there was browsed more than 75% leaf apparatus (5 points).

Table nr.2

Biological efficiency of the extract of *Juniperus sabina* against *Leptinotarsa decemlineata* under field conditions in potatoes culture (Treatment date – 03.06 2013; Examination on – 06.06; 10.06; 17.06 and 24.06.2013)

Variant	Concentration s.a.,%	Biological efficiency on a corresponding day after being treated,%				
		3	7	14	21	28
Reference	-	-	-	-	-	-
Standard Midash 200 SL, (imidacloprid) 200 g/l	0,3 l/ha	100	99,5	100	99,3	89,3
Extract of *J. sabina* (extract 15 g/l)	4,5 kg/ha	**89,6**	**90,0**	**94,5**	**100**	**85,2**
						$HSD_{0.05}$ = 6.9

Thus, it is determined that the biological activity of the *J. sabina* extract against *L. decemlineata* larvae remains high for at least two to three weeks. So to protect potatoes from Colorado potato beetle is necessary to carry out one or two treatments against each pest generation depending on the *L. decemlineata* pest population density and climatic conditions of the year.

CONCLUSIONS

Due to the biological characteristics of *J. sabina* plant, it can serve as a source of raw materials for the plant extract. The currently estimated *J. sabina* materials resources in Moldova, obtained from annual pruning, are enough to treat a maximum of 20 hectares of potatoes. Despite the high cost of the plant extract, its biological effectiveness remains high (at the level of the chemical standard) for three weeks. Meanwhile, the number of larvae did not exceed the thresholds and damage of the bushes was less than 5%.

BIBLIOGRAPHY

1. Alexandrova V.D. *Dinamika rastitelinogo pokrova*. V kn. Polevaia geobotanika M., L.: Izd. AN SSSR, 1964, vol. 3, p. 300-450. [in Russian].

2. Doljenko V.I. et all. *Metodiceskie ukazania po registrationnym ispytaniam insecticidov, acaricidov, moliuscotidv i rodentodov v seliskom hozeaistve*. RASHN VNIIZR IZZR. S-Peterburg. 2004. 363 p. [in Russian].

3. Dospehov B.A. *Metodika polevogo opyta*. Moskva: Kolos, 1979. 338 p.

4. Elisovetskaya D.S, Nastas T.N. *Rastitelinye extract v kacestve effectivnyh sredstv dlea snijenia cislennosti coloradskogo juka*. Mat. mejd.naucino-pract. Conf. «Sostoyanie I perspectivy razvitia zascity rastenii». Kiev, 2013. p. 41.

5. Elisovetskaya D.S., Nastas T.N., Odobescu V.A. *Ispolizovanie rastitelinyh extractov dlea zascity cartofelea ot coloradskogo juka*. Mat. mejd.naucino-pract. Conf. «Fitosanitarnaya bezopasnosti i controli s-h productii». Inf.bull. JOBS EPRS, Boiany, 2013, nr. 44, p. 112-117.

6. Gosudarstvennaia pharmacopeia SSSR. 11-e izd. Moskva: Meditsina, 1989. 386 p. [in Russian].

7. Indrumări metodice pentru testarea produselor chimice şi biologice de protecție a plantelor de dăunători, boli şi buruieni în RM. Centrul de Stat pentru atest. produs. chimice şi biol. de prot. şi stimulare a creşterii plantelor. red. Lazari I. Chişinău: Tipografia Centrală, 2002. 286 p.

8. Muraviova D.A. et.al. *Pharmacognozia*. Moskva: Meditsina, 2007. 654 p. [in Russian].

9. Pisarev D.I. *Pharmacognosticescoe izucenie mojjevelinica dlinnohvoinogo I mojjevelinica cazatcogo*. Diss. Na soisc. Uc.step. cand. Pharm. Nauk, Peatigorsk, 2005. 146 p.

The study was performed within the project 11.817.04.15A financed by ASM.

THE SILVER FIR (*ABIES ALBA*) FOREST COMMUNITIES FROM EASTERN CARPATHIANS (ROMANIA)

Mardari Constantin, Tănase Cătălin, Bîrsan Ciprian, Balaeş Tiberius

„Anastasie Fătu" Botanical Garden, „Alexandru Ioan Cuza" University from Iaşi

INTRODUCTION

Abies alba, the second important conifer species in Romania, represents the (co)dominant tree in about 5% of Romanian forests (2). In the Eastern Carpathians of Romania it frequently forms mixed stands in co-dominance with *Picea abies* and *Fagus sylvatica*. More rarely, it edifies pure stands. Silver fir communities are classified in two vegetation classes: *Querco-Fagetea* and *Vaccinio-Piceetea* (7, 9, 23, 26). The article's purpose is to present a numerical classification of *Abies alba* communities, to detect the diagnostic species for the distinguished vegetation units and the main ecological factors influencing their floristic composition.

MATERIALS AND METHODS

In order to classify and characterize the silver fir communities, a set of 215 relevés (based on their assignment to *Abieti-Piceion* and a part of *Symphyto-Fagion*) was selected from literature (1, 4, 5, 6, 8, 10, 11, 12, 16, 17, 19, 20, 21, 22, 24, 25, 27, 28, 29, 30, 31, 32, 34, 35). From the initial dataset, only relevés in which the covering percentage of *Abies alba* was at least 15% were retained. Rare species (occurring in less than 5 relevés) were removed. Thus, the analyzed dataset included only 143 relevés (143 species). Data analysis was realized using the Flexible beta algorithm and Bray-Curtis distance in the VEGANA software package (3). Optimal number of clusters was determined using the *average mean Silhouette index*. Determination of the diagnostic species was realized using the *binary indicator value* coefficient (14). Square-rooted values of the *indicator values* were the subject of a permutation test (999 iterations) in order to observe which are the species significantly associated with the clusters (13). The threshold value for a species to be considered as diagnostic was set at 0.500 ($P \leq 0.05$). Detrended correspondence analysis (using non weighted average values of the Ellenberg indices for light, temperature, soil moisture, soil pH and nutrients (15) and altitude) has been performed in order to characterize vegetation from an ecological perspective. DCA was realized in CANOCO 4.5 (33). Box-plots with altitude and Ellenberg's indicator values were realized in PAST (18).

RESULTS AND DISCUSSION

In order to identify the optimum number of clusters, from the ultrametric matrix generated by the Bray-Curtis distance and Flexible beta algorithm were extracted 9 partitions (with 2-10) clusters, analyzed with the silhouette index. The silhouette statistic, showed local maxima for the partition with 2 clusters (0.152423) and partition with 6 clusters (0.126066) and, consequently, the 2 clusters partition was further analyzed at association level. Diagnostic species for each clusters allowed their relation to vegetal associations described in literature:

VACCINIO-PICEETEA Br.-Bl. in Br.-Bl. et al. 1939
ATHYRIO-PICEETALIA Hadač 1962
Abieti-Piceion (Br.-Bl. in Br.-Bl. et al. 1939) Soó 1964
Hieracio transsilvanico-Abietetum (Borhidi 1971) Coldea 1991
QUERCO-FAGETEA Br.-Bl. et Vlieger in Vlieger 1937
FAGETALIA SYLVATICAE Pawlowski in Pawlowski et al. 1928
Symphyto cordati-Fagion Vida 1963
Pulmonario rubrae-Fagetum (Soó 1964) Täuber 1987

Group 1: As. Pulmonario rubrae - Fagetum sylvaticae (Soó 1964) Täuber 1987

Diagnostic species (stat./P-value): *Fagus sylvatica* (0.848, P=0.001), *Galium odoratum* (0.744, P=0.001), *Rubus hirtus* (0.734, P=0.001), *Viola reichenbachiana* (0.680, P=0.001), *Salvia glutinosa* (0.663, P=0.023), *Impatiens noli-tangere* (0.632, P=0.001), *Carex sylvatica* (0.630, P=0.001), *Sanicula europaea* (0.616, P=0.001), *Stachys sylvatica* (0.590, P=0.001), *Pulmonaria rubra* (0.566, P=0.015), *Circaea lutetiana* (0.566/0.038), *Brachypodium sylvaticum* (0.562, P=0.001), *Carex remota* (0.527, P=0.001), *Epilobium montanum* (0.525, P=0.032), *Poa nemoralis* (0.521, P=0.024), *Glechoma hederacea* (0.514, P=0.007).

Abies alba and *Fagus sylvatica* edify vegetal communities (as co-dominant species) in lower montains areas, in an altitudinal range of 470-1120 m (mean altitude 703 m), on areas very variable in terms of slopes and aspects. They were described almost from all zones of the Eastern Carpathians. The trees layer includes alongside dominant species (*Fagus sylvatica* and *Abies alba*) also *Picea abies* (sometimes with significant covering percentages), *Acer pseudoplatanus*, *Acer platanoides*, *Populus tremula* etc. In the shrubs layer there are more frequent *Rubus hirtus, Corylus avellana* etc. The herbs layer presents variable coverages and includes numerous species (*Galium odoratum, Ajuga reptans, Gentiana asclepiadea*, *Paris quadrifolia* etc.). High constancy present also the characteristic species for *Symphyto cordati-Fagion* (*Symphytum cordatum, Pulmonaria rubra*, *Dentaria glandulosa*), *Fagetalia sylvaticae* (*Euphorbia amygdaloides*, *Lamium galeobdolon, Asarum europaeum* etc.) and *Querco-Fagetea* (*Moehringia trinervia*, *Geum urbanum*, *Viola reichenbachiana* etc.). In the floristic composition, most of the species preffer shaded places (mean 4.01) and are adapted to the climatic conditions of the low mountains sites (mean

5.08). They also prefer average humid (mean 5.39), moderately acid to neutral (mean 6.06) and rich in available nitrogen (mean 5.87) soils (Fig. 1).

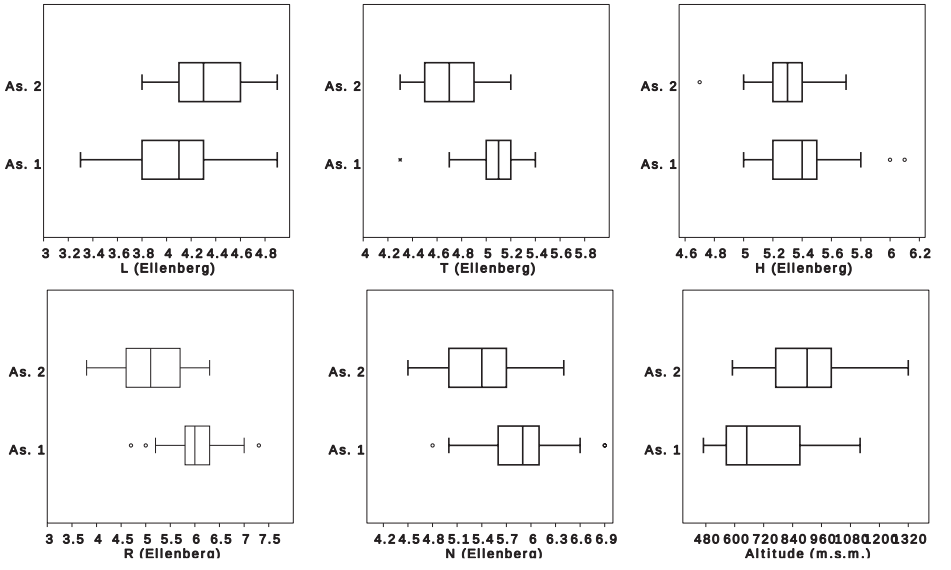

Fig. 1. Box (showing medians) with whisker plots (indicating the minimum and maximum values) of the average Ellenberg indicator values for relevés from the 2 clusters resulted in hierarchical clustering.

Group 2: As. Hieracio transsilvanico - Abietetum albae (Borhidi 1971) Coldea 1991

Diagnostic species (stat./P-value): *Picea abies* (0.911, P=0.001), *Hieracium transsilvanicum* (0.761, P=0.001), *Vaccinium myrtillus* (0.680, P=0.001), *Luzula luzuloides* (0.677, P=0.009), *Rubus idaeus* (0.582, P=0.002), *Maianthemum bifolium* (0.574, P=0.002), *Luzula sylvatica* (0.554, P=0.001), *Calamagrostis arundinacea* (0.535, P=0.002), *Veronica officinalis* (0.521, P=0.007), *Polygonatum verticillatum* (0.504, P=0.008).

Forests include communities edified mainly by *Abies alba*, developed from middle to upper mountain level, in an altitudinal range between 590 and 1320 m (mean 900 m) on terrains with medium slopes and various aspects. The trees layer is characterized by a high covering percentage (75-85%), is edified by *Abies alba* and sometimes *Picea abies* (as co-dominant species) nearby sporadically appear *Fraxinus excelsior*, *Sorbus aucuparia, Acer pseudoplatanus* or *Betula pendula*. The shrubs layer is generally less reprezented, including few species (e.g. *Spiraea chamaedryfolia*, *Vaccinium myrtillus*, *Rosa pendulina*, *Sambucus racemosa*, *Rubus idaues* etc.). The herbs layer is the most diverse, in some cases, some species can present higher cover percentages (*Hieracium transsilvanicum*, *Luzula luzuloides*, *Calamagrostis arundinacea*, *Dryopteris filix-mas*, *Salvia glutinosa* etc.). From the phytosociological

perspective, high constancies presents diagnostic species to *Abieti-Piceion* (*Epipactis helleborine*, *Lonicera xylosteum*, *Sanicula europaea*), *Athyrio-Piceetalia* (*Athyrium filix-femina*, *Mercurialis perennis*, *Daphne mezereum*) and *Vaccinio-Piceetea* (*Oxalis acetosella*, *Homogyne alpina*, *Moneses uniflora*, *Orthilia secunda*, *Picea abies* etc.). These communities include preponderantly shadow species (mean 4.31), adapted to the climatic conditions of the mountains areas (mean 4.68). Most of them prefer average dampness soils (mean 5.29), fairly acid (mean 5.10) and more nitrogen deficient (Fig. 1) comparing to *Pulmonario rubrae-Fagetum* (mean 5.33).

From another perspective, the spruce fir forests in Romanian Eastern Carpathian analyzed as a whole are developed in the low mountain range (mean 785 m) and include species preferring shaded places (mean 4.17), adapted to the climatic conditions of the low mountains areas (mean 4.92). Most of them prefer average dampness soils (mean 5.35), fairly acid (mean 5.66) and relatively nitrogen deficient (mean 5.33).

Detrended correspondence analysis (Fig. 2) showed that the first axis is the most important one (eigenvalue 0.278), explaining 8.2% of the cumulative percentage variance of species data and 39% of the cumulative percentage variance of species-environment relation. The second axis is less important (eigenvalue 0.150) and explains only 4.4% of the cumulative percentage variance of species data and 5.7% of the cumulative percentage variance of species-environment relation. Together, the first two axes explain 12.6% of the cumulative percentage variance of species data and 44.7% of the cumulative percentage variance of species-environment relation.

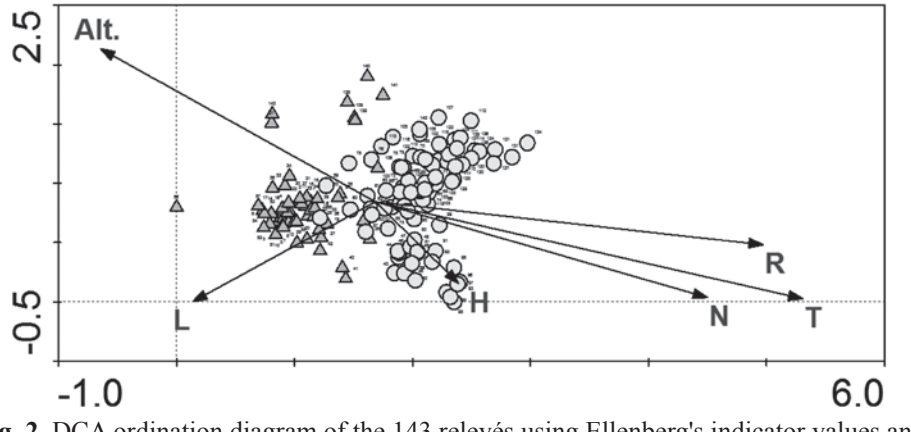

Fig. 2. DCA ordination diagram of the 143 relevés using Ellenberg's indicator values and altitude as passive variables (with clusters generated by hierarchical clustering colored as follows: *Pulmonario rubrae-Fagetum sylvaticae* - circles, *Hieracio transsilvanico-Abietetum albae* - triangles); first two axes presented. Eigenvalues: 1st axis: 0.278, 2nd axis: 0.150, total inertia: 3.410. Correlation of DCA axes with variables: L (1st axis: -0.3669, 2nd axis: -0.2573), T (1st axis: 0.8249, 2nd axis: -0.1599), H (1st axis: 0.1543, 2nd axis: -0.1753), R (1st axis: 0.7525, 2nd axis: -0.0416), N (1st axis: 0.6390, 2nd axis: -0.1711), altitude (1st axis: -0.5223, 2nd axis: 0.3101).

The first DCA axis is strongly positively correlated with EIVs for temperature, soil reaction and nutrients and negatively correlated with altitude (Fig. 1), suggesting that they could represent the main factors influencing the floristic composition of these forests. These ecological factors generate a differentiation among the communities from more increased altitudes, including species adapted to lower temperatures and low nutrients availability (*Hieracio transsilvanico-Abietetum*) from the left side of the ordinogram compared to the communities from lower altitudes including species with the ecological optimum in a higher range of temperature, developed on soils richer in nutrients (*Pulmonario rubrae-Fagetum*) situated in the right side of the ordinogram. The second DCA axis is mainly (negatively) correlated with EIVs for light indicating that floristic variation is affected also by light conditions but in a lower degree as temperature, soil reaction, nutrients and altitude.

CONCLUSIONS

Numerical analysis of the silver fir communities from Eastern Carpathians (Romania) has as result their classification in 2 major vegetation types (*Hieracio transsilvanico-Abietetum* and *Pulmonario rubrae-Fagetum*). Altitude, soil pH and nutrients are the main factors influencing the floristic composition of these plant communities.

BIBLIOGRAPHY

1. *Barabaş N. Contribuții la studiul vegetației din bazinul Tazlăului*. Stud. Com. Muz. Şti. Nat. Bacău. 1974, **7**: 93-178.

2. *Barbu I. and Barbu C. Silver Fir (Abies alba Mill.) in Romania*. Suceava, *Editura Technică Silvică. 2005, 220 p.*

3. *Bouxin G. Ginkgo, a multivariate analysis package*. J. Veg. Sci. 2005, **16**: 355-359.

4. *Chifu T. Cercetări micocenologice în asociația Abietum dacicum din Depresiunea Neamțului*. Stud. Com. Muz. Şti. Nat. Dorohoi. 1972: 57-66.

5. *Chifu T.* & *Ştefan N. Cercetări fitocenologice în pădurile din valea Nemțişorului (jud. Neamț)*. Stud. Com. Muz. Jud. Suceava, ser. Şti. Nat. 1973, **3**: 213-253.

6. *Chifu T.* & *Şurubaru B. Associations forestières caractéristiques à Obcina Mare (Suceava)*. Analele Şti. Univ. "Al. I. Cuza" Iaşi, s. II, a. Biol. veg. 1999, **45**: 133-139.

7. *Chifu T.*, *Mânzu C.* & *Zamfirescu O. Flora & Vegetația Moldovei (România)*. Iaşi. Edit. Universității Alexandru Ioan Cuza. 2006, vol. **2**. 698 pp.

8. *Coldea G. Munții Rodnei. Studiu geobotanic*. Bucureşti. Edit. Academiei Române. 1990, 183 pp.

9. *Coldea G. Prodrome des associations vegetales des Carpates du sud-est (Carpates Roumaines)*. Documents phytosociologiques. 1991, **13**: 317-539.

10. *Coroi A.-M. Flora şi vegetația din bazinul râului Milcov*. Iaşi. Edit. Tehnopress. 2001, 411 pp.

11. *Coroi M. Flora şi vegetația din bazinul râului Şuşița*. Iaşi. Edit. Tehnopress. 2001, 398 pp.

12. *Costică M.*, *Ştefan N.* & *Sârbu I. Contribution to the study of the wood vegetation from the Suhard Mountain*. Analele Şti. Univ. "Al. I. Cuza" Iaşi, s. II, a. Biol. veg. 2010, **56**: 65-81.

13. *De Cáceres M.* & *Legendre P. Associations between species and groups of sites: indices and statistical inference*. Ecology, 2009, **90**(12): 3566-3574.

14. *Dufrêne M*. & *Legendre P*. *Species assemblages and indicator species: the need for a flexible assymmetrical approach*. Ecological Monographs, 1997, **67**: 345-366.

15. *Ellenberg H*., *Weber H. E*., *Düll R*., *Wirth V*., *Werner W*. & *Paulißen D*. *Indicator values of plants in Central Europe*. Ed. 2. Scripta Geobotanica, 1992, **18**: 1-258.

16. *Filipaş L*., *Bărbos M*. & *Coldea G*. *A new syntaxon of fir forests (Abies alba) from South-Eastern Carpathians (Romania)*. Contrib. Bot., 2013, **48**: 69-73.

17. *Gurău M*. *Vegetația pădurilor şi tufărişurilor dintre râul Oituz şi muntele Ouşoru (Județul Bacău)*. Stud. Com. Muz. Şti. Nat. Bacău, 2004, **19**: 80-114.

18. *Hammer Ř*., *Harper D.A.T*. & *Ryan P*. *D*. *PAST: Paleontological Statistics Software Package for Education and Data Analysis*. Palaeontologia Electronica, 2001, **4**(1): 9pp.

19. *Horeanu C*. *Flora şi vegetația rezervației forestiere Cenaru (Jud. Vrancea)*. Ocrot. Nat., 1980, **24**(1): 21-28.

20. *Mardari C*. & *Chifu T*. *Asociații vegetale încadrate în clasa Vaccinio-Piceetea Br.-Bl. in Br.-Bl. et al. 1939 identificate în bazinul Negrei Broştenilor*. Stud. Com. Muz. Şti. Nat. Bacău, 2009, **22**: 36-46.

21. *Manzu C*., *Şurubaru B*. & *Chifu T*. *The Hieracio transsylvanici-Abietetum (Borhidi 1971) Coldea 1991 association from the "Obcinele Bucovinei" territory*. Rom. J. Biol. Plant biol., 2006-2007, **51-52**: 77-87.

22. *Mititelu D*., *Dăscălescu D*., *Ştefan N*., *Vițalariu G*., *Barabaş N*., *Davidescu G*., *Lupascu G*. & *Toderita M*. *Cercetări sinecologice în rezervația "Codrul Secular Slătioara"-Jud. Suceava*. Stud. Com. Muz. Şti. Nat. Bacău, 1979-1980, **13**: 135-182.

23. *Mucina L*. *Conspectus of classes of European vegetation*. Folia Geobotanica Phytotaxonomica, 1997, **32**: 117-172.

24. *Oprea A*. & *Sîrbu C*. *Munții Stânişoarei*. *Studiu fitosociologic*. Iaşi. Edit. Universității Alexandru Ioan Cuza, 2009, 220 pp.

25. *Oroian S*. *Vegetația defileului Mureşului între Toplița şi Deda*. Târgu-Mureş. Casa de Editură Mureş, 1998, 426 pp.

26. *Sanda V*., *Ollerer K*., *Burescu P*. *Fitocenozele din România*. *Sintaxonomie, structură, dinamică şi evoluție*. Bucureşti. Edit. Ars Docendi, 2008, 570 pp.

27. *Sămărghițan M*. *Flora şi vegetația Văii Gurghiului*. Târgu-Mureş. Edit. University Press. 2005, 510 pp.

28. *Sârbu I*., *Ştefan N*., *Coroi M*., *Tănase C*. & *Ciurăscu Ş*. *Rezervația naturală Cheile Nărujei-Lacul Negru (Jud. Vrancea)*. Bul. Grăd. Bot. Iaşi, 1997, **6**(1): 235-250.

29. *Ştefan N*. *Studiul fitocenologic asupra principalelor asociații vegetale lemnoase din bazinul superior al Râmnicului Sărat*. Stud. Com. Complex Muz. Jud. Vrancea, 1980, **4**: 463-469.

30. *Ştefan N*., *Mititelu D*., *Vițalariu G*., *Dăscălescu D*. & *Davidescu D*. *Contribuții la studiul geobotanic al pădurilor din bazinul Gemenea (Jud. Suceava)*. Anuarul Muz. Jud. Suceava, 1985, 83-98.

31. *Ştefan N*. & *Diaconu M*. *Contributions to the study of spruce forests in the Vrancea Mountains*. Analele Şti. Univ. "Al. I. Cuza" Iaşi, s. II, a. Biol. veg., 1994, **40**: 81-90.

32. *Ştefan N*., *Sârbu I*., *Coroi M*., *Oprea A*., *Tănase C*. & *Ciurăscu Ş*. *Rezervația naturală Cheile Tişiței (Județul Vrancea)*. Bul. Grăd. Bot. Iaşi, 1997, **6**: 251-274.

33. *Ter Braak C. J. F*. & *Šmilauer P*. *CANOCO reference manual and CanoDraw for Windows user's guide. Software for Canonical Community Ordination (version 4.5)*. – Microcomputer Power, Ithaca. 2002.

34. *Tomescu C. V*. & *Chifu T*. *Asociația Hieracio transsilvanici-Abietetum (Borhidi 1971) Coldea 1991 din bazinul râului Suceava*. Analele Univ. "Ştefan Cel mare" Suceava, ser. nouă, 2003, **2**: 51-60.

35. *Zamfirescu O*. & *Chifu T*. *Contributions to the study of class Vaccinio-Piceetea Br.-Bl. 1939 from the Oriental Carpathians*. Contrib. Bot., 2003, **38**: 37-45.

FLORA DIVERSITY OF THE PROTECTED NATURAL AREA "BLUEGRASS RIVER MEADOW"

Miron Aliona, Postolache Gheorghe, Titica Ghenadie
Botanical Garden (Institute) Academy of Sciences of Moldova

Summary. The paper presents the results of the study of flora referring to bluegrass meadow located on the riverside of Ciulucul Mic, village Bursuceni, district Sângerei.

INTRODUCTION

In the Republic of Moldova, there are some natural areas, which up to now remain fragmentary investigated. The protected natural area *bluegrass river meadow* fits in this context, where from the moment of establishment up to now, floristic and phytocenotic investigations haven't been performed. Our investigations, done in the above-mentioned protected area, had the target of flora and vegetation assessment. The results referring to flora investigations are presented in this article.

MATERIALS AND METHODS

The floristic investigations were performed in 2010. To identify the species of vascular plants, the floristic inventories and plant collections in diverse vegetation periods were made. The collected botanical material was herbarized and identified, employing speciality scientific works [1, 2, 3, 5 and 6]. Ecological indices according to the [4] source were taken.

RESULTS AND DISCUSSIONS

The *bluegrass meadow* represents a protected area of national interest which corresponds to the category: area with multifunctional management (representative sector with meadow vegetation). The above-named area is found on the administrative territory of Bursuceni village, district Sângerei, under public local authority management. The protected area has a surface of 12 ha and represents a sector distinguished by meadow, paludous and halophilous vegetation, situated in the meadow of the affluent of Ciulucul Mic river. The protected area has been established in 1998 according the Law on Fund of Natural Areas Protected by State.

Taxonomical analysis. The investigations attest the spread of 129 species of vascular plants, which belong to 97 genera and 32 families, were emphasized: ***Alliaceae:*** *Allium rotundum* L.; ***Apiaceae:*** *Daucus carota* L., *Falcaria vulgaris* Bernh.,

Peucedanum carvifolium Vill., *Peucedanum latifolium* (Bieb.) DC.; ***Asparagaceae:*** *Asparagus officinalis* L.; ***Asteraceae****: Achillea setacea* Waldst. et Kit., *Arctium lappa* L., *Artemisia absinthium* L., *Artemisia austriaca* Jacq., *Centaurea solstitialis* L., *Cichorium intybus* L., *Cirsium arvense* (L.) Scop., *Cirsium pannonicum* (L. fil.) Link, *Crinitaria villosa* (L.) Grossh., *Inula britannica* L., *Inula salicina* L., *Lactuca virosa* L., *Matricaria recutita* L., *Onopordum acanthium* L., *Senecio vernalis* Waldst. et Kit., *Sonchus palustris* L., *Tanacetum vulgare* L., *Taraxacum bessarabicum* (Hornem.) Hand.-Mazz., *Taraxacum officinale* Wigg., *Tragopogon dubius* Scop., *Xanthium strumarium* L.; ***Boraginaceae:*** *Cerinthe minor* L., *Echium vulgare* L., *Symphytum officinale* L.; ***Brassicaceae:*** *Capsella bursa-pastoris* (L.) Medik., *Cardaria draba* (L.) Desv., *Lepidium campestre* (L.) R. Br., *Lepidium latifolium* L., *Lepidium ruderale* L., *Rorippa austriaca* (Crantz) Bess., *Rorippa sylvestris* (L.) Bess., *Thlaspi arvense* L.; ***Caryophyllaceae:*** *Arenaria serpyllifolia* L., *Cerastium dubium* (Bast.) Guepin, *Saponaria officinalis* L., *Spergularia maritima* (All.) Chiov., *Stellaria graminea* L.; ***Chenopodiaceae:*** *Salicornia europaea* L.; ***Convolvulaceae:*** *Convolvulus arvensis* L.; ***Cyperaceae:*** *Bolboschoenus maritimus* (L.) Palla, *Carex otrubae* Podp., *Carex praecox* Schreb., *Carex vulpina* L.*, Scirpus tabernaemontanii* C. C. Gmel.; ***Euphorbiaceae****: Euphorbia stepposa* Zoz; ***Fabaceae:*** *Lathyrus pratensis* L., *Lathyrus tuberosus* L., *Lotus corniculatus* L., *Medicago lupulina* L., *Medicago sativa* L., *Melilotus officinalis* L., *Trifolium fragiferum* L., *Trifolium montanum* L., *Trifolium pratense* L., *Trifolium repens* L., *Vicia angustifolia* Reichard, *Vicia pannonica* Crantz, *Vicia sativa* L., *Vicia sepium* L., *Vicia tetrasperma* (L.) Schreb., *Vicia villosa* Roth; ***Iridaceae:*** *Iris halophila* Pall.; ***Juncaceae:*** *Juncus gerardii* Loisel.; ***Lamiaceae:*** *Acinos arvensis* (Lam.) Dandy, *Ajuga genevensis* L., *Phlomis tuberosa* L., *Salvia nemorosa* L., *Salvia verticillata* L., *Scutellaria hastifolia* L., *Teucrium chamaedrys* L.; ***Linaceae:*** *Linum austriacum* L.; ***Lythraceae:*** *Lythrum salicaria* L.; ***Malvaceae:*** *Hibiscus trionum* L., *Malva sylvestris* L.; ***Onagraceae:*** *Epilobium parviflorum* Schreb.; ***Papaveraceae:*** *Papaver dubium* L.; ***Plantaginaceae:*** *Plantago altissima* L., *Plantago lanceolata* L., *Plantago major* L., *Plantago media* L., *Plantago schwarzenbergiana* Schur; ***Plumbaginaceae:*** *Limonium gmelinii* (Walld.) E. Kuntze; ***Poaceae:*** *Agropyron pectinatum* (Bieb.) Beauv., *Agrostis tenuis* Sibth., *Alopecurus arundinaceus* Poir., *Alopecurus pratensis* L., *Bromus arvensis* L., *Calamagrostis epigeios* (L.) Roth, *Catabrosa aquatica* (L.) Beauv., *Dactylis glomerata* L., *Elytrigia intermedia* (Host) Nevski, *Elytrigia repens* (L.) Nevski, *Festuca pratensis* Huds., *Phragmites australis* (Cav.) Trin. ex Steud., *Poa angustifolia* L., *Poa pratensis* L., *Puccinellia distans* (Jacq.) Parl., *Puccinellia gigantea* (Grossh.) Grossh.; ***Polygonaceae:*** *Polygonum aviculare* L., *Rumex conglomeratus* Murr., *Rumex stenophyllus* Ledeb.; ***Ranunculaceae:*** *Adonis aestivalis* L., *Consolida paniculata* (Host) Schur, *Consolida regalis* S. F. Gray, *Ranunculus cassubicus* L., *Ranunculus illyricus* L., *Ranunculus repens* L.,

Ranunculus sceleratus L.; ***Rosaceae:*** *Agrimonia eupatoria* L., *Filipendula ulmaria* (L.) Maxim., *Filipendula vulgaris* Moench, *Fragaria vesca* L., *Potentilla impolita* Wahlenb., *Potentilla recta* L., *Potentilla reptans* L., *Rubus caesius* L.; ***Rubiaceae:*** *Galium verum* L.; ***Scrophulariaceae:*** *Veronica arvensis* L.,*Veronica austriaca* L.; ***Solanaceae:*** *Hyoscyamus niger L., Solanum nigrum* L.; ***Urticaceae:*** *Urtica dioica* L.; ***Verbenaceae:*** *Verbena officinalis* L.

The best represented genera are: *Vicia* (6 species), *Plantago, Ranunculus* and *Trifolium* (per 4 species each). The most representative families are: *Asteraceae* (23 species), *Fabaceae*, *Poaceae* (per species 16 each), *Brassicaceae* (8 species), *Lamiaceae* (7 species), *Cyperaceae* (5 species). The species of these 6 families, in number of 73, represent 58% from the vascular flora of this protected area.

The **analysis of bioforms** reveals the prevalence of **hemicryptophytes**, which constitute 56.3% of the floristic composition, thus attesting the protected area being situated into a region with temperate climate, where herbaceous formations that make up the grasslands are in abundance. The hemicryptophytes are followed by **therophytes** (27.0%) that confirm the persistence of a more or less arid climate and also of anthropogenic and zoogenous influences on flora and vegetation in this protected area. The **geophytes** totalize 10.3%. The **chamaephytes** are represented only by 1.6%, but the **hydrophytes** and **phanerophytes** realize a small percentage – by 0.8% each. The altitudinal index **Ka** possessing the value of 47.9% confirms a climate and anthropogenic moderate influences.

The **analysis of floristic elements** denotes the prevalence of **Eurasian** elements with 58.7%. **European** elements are represented by 9.5% among species. **Cosmopolitan** species comprise 7.9%, those **Pontic** – 7.1%, **circumpolar** – 6.3% and **mediterranean** – 3.2%.

Environmental analysis. The **analysis** of **humidity indices** established the predominance of **xero-mesophytes** ($U_{2-2,5}$) – 34.9%, **mesophytes** ($U_{3-3,5}$) – 17.5% and **meso-hygrophytes** ($U_{4-4,5}$) – 14.3%, these confirming the **xero-mesophyl** predominant character of vegetation from the protected area. **Hydrophyte species** ($U_{5-5,5}$) integrate 4.0%, those **xerophyte** ($U_{1-1,5}$) and **euryhydrophyte** (U_0) remark equal value – by 3.2% each.

Under the impact of species exigencies to **temperature (T)**, the flora of protected area is dominated by the **micro-meso-thermal** ($T_{3-3,5}$) – 41.3% species, followed by those **moderate-thermophyte** ($T_{4-4,5}$) – 16.7%, which reflect the temperate continental climate where the protected area is situated. The **amphitolerant** species (T_0) represent 12.7% of the floristic composition. The **micro-termal** ($T_{2-2,5}$) species and those **thermophyte** ($T_{5-5,5}$) have an insignificant value, 6.3% and 0.8%, respectively. From the point of view of the **soil reaction** (R), the species fall into five environmental categories. The species **low acid-neutrophyte** ($R_{4-4,5}$) – 35.7% are predominant, being

followed by those **amphitolerant** (R_0) – 27.0%. Fewer percentages performed the **acid-neutrophyte** species ($R_{3-3,5}$) and those **neutro- basophyte** ($R_{5-5,5}$) – by 7.1% each.

Concerning the plants exigencies to the soil trophicity (richness of nutrients), the species fall into four environmental categories. Their analysis remarks the prevalence of **eutrophic** species (38.9%), followed by those **oligotrophic** (12.7%). **Eurytrophic** species manifest low values – 3.2% and have high environmental amplitude towards trophicity of soil.

Duration of life. The flora of the protected area is dominated by the perennial plant species – 69.8%. The annual species totalize 20.6%, and the biennial species – 7.9%.

The economic importance. Under the aspect of the economical importance, the flora is divided in the following categories: food, fodder, melliferous, medicinal, industrial, toxic and decorative. The most representative economic categories are: **medicinal** – 46.0%, **fodder** – 39.7%, **melliferous** and **industrial** – by 34.1% each. The species with food value achieve 23.0%. The **decorative** species totalize 12.7%, but those **toxic** – 7.9%.

Rare plant species. In the protected area of *bluegrass meadow* was identified an *Asparagus officinalis* population – endangered species protected by the Law on Fund of Natural Areas Protected by State (1998).

CONCLUSIONS

In the protected area of *bluegrass meadow,* 129 species of vascular plants belong to 97 genera and 32 botanical families. Proceeding from the analysis of bioforms, the predominance of hemicryptophytes – 56.3% is established, identifying the predominance of herbaceous formations, thus forming the meadows. The share of the therophytes – 27.0% attests the presence anthropogenic and zoogenous influences. From the point of view of floristic elements, the investigated area belongs to the Eurasian areal, this fact being confirmed by the prevalence of Eurasian floristic elements – 58.7%. The results of the analysis referring to environmental indices have established that the flora of protected area is dominated by the following types of species: xero-mesophyte – 34.9%, mesophyte – 17.5% and meso-hygrophytes – 14.3%; micro-mesothermes – 41.3%; low acid-neutrophyte – 35.7% and amphitolerant – 27.0%.

Endangered species, *Asparagus officinalis*, was identified in the framework of the area of *bluegrass meadow*, and is protected by the Law on Fund of Natural Areas Protected by State (1998).

BIBLIOGRAPHY

1. Beldie Al. *Flora României. Determinator ilustrat al plantelor vasculare*. Bucureşti: Ed. Acad. Române, 1977-1979. Vol. I-II. 512 p.

2. Ciocârlan V. *Flora ilustrată a României*. Bucureşti, 1988-1990. Vol. I–II. 512 p.

3. Negru A. *Determinator de plante din flora Republicii Moldova*. Chişinău, 2007. 391 p.

4. Sanda V. şi alții. *Flora cormofitelor spontane şi cultivate din România*. Ed. Ion Borcea: Bacău, 2003. 316 p.

5. Гейдеман Т. *Определитель высших растений Молдавской ССР*. Кишинев,1986. 638 с.

6. Скворцов А. *Гербарий, пособие по методике и технике*. М.: Наука, 1977. 200 с.

7. Шабанова Г. и др. *Новый вид рода Plantago L. (P. schwarzenbergiana Schur) во флоре Молдовы.* Mediul Ambiant, Nr. 5(41), Chişinău, 2008. p. 38-39.

8. Legea privind fondul ariilor naturale protejate de stat nr 1538 din 25.02.1998.

CLASSIFICATION OF FOREST STANDS OF THE NATIONAL FORESTRY FUND DEPENDING ON INFLUENCE OF HUMAN ELEMENT

A. Palancean, V. Gogu

Rezumat. Articolul rezumă o analiză a arboretelor din fondul forestier național în dependență de influența factorului uman. Luând în vedere intensitatea schimbărilor în compoziție, structură, prezența speciei/speciilor principală în arboret şi modul lor de regenerare (generativ ori vegetativ), arealul speciei/ speciilor din compoziția şi corespunderea lor stațiunii au fost evidențiate 14 categorii de arboreturi încadrate în două tipuri şi şapte subtipuri care fac parte din „arborete gospodărite".

Cuvinte cheie: arboret, tip, categorie, gospodărit, natural, artificial.

INTRODUCTION

A considerable part of Moldavian forests are structured under the ecological and genetic report. We have too many unileveled and even aged monocultures, without typical undergrowth, derived vegetatively (about 57% in oak species forests), with reduced consistency, under 0,6; station improper, affected by different constructions, destroyed by snow and frost, invaded by secondary exotic species etc. The artificial forests, for the establishment of which, have participated both indigenous and exotic species (about 25 species of trees and bushes), forming a large diversity of stands, not always corresponding to the station, occupy over a third of the national forest fund.

From the beginning, it is necessary to take an urgent inventory of forest stands that require ecological reconstruction works. For this purpose a methodological research is needed to establish the criteria for classification of forest stands depending on their degree of removal from their optimal condition, as well as depending on the urgency of intervention (Giurgiu, 1995, Palancean, Gogu, 2013).

In „Technical rules concerning the ecological reconstruction of forest stands", published by the „Moldsilva" Agency, are stipulated stands that require ecological reconstruction works, falling under special categories, grouped depending on the technical methods of interventions:

1. Low productive stands – include stands from 4-5 degrees of production. These stands are derived from shoots, managed as young forest for several generations (genus Quercus), or are artificially made up from station improper species, mainly on rocky regions, weak soils, saline soils, carbonated soils, floodplain land etc.

2. Derived stands – include, both natural and artificial stands made up from secondary, accompanying or mixed species. In this category were also framed the young essential natural stands, (genus Quercus) in which the natural regeneration has

been compromised and forestry crops with the participation of introduced species as main species, including, compromised nut species, brought to a state of bushes or rare forest.

3. Degraded stands – include all the stands with the consistency under 0,3 regardless the (natural or artificial) forest character.

4. Unusable or waste stands - sum all the stands with the consistency between 0,4 – 0,6. These are all young natural or artificial stands with a weak achievement, with a reduced proportion of main species; or pre-exploitable or exploitable stands managed as young forest for several generations.

5. Artificial stands station improper – provide all types of stands consisting of species with improper growing conditions, in particular, acacia, from inside the forest bodies essentially natural, acacia forestry crops created in oak stations etc.

It is pointed out that „the purpose of this rule sums up the establishment of methods and technologies concerning restoration, replacement and improvement of low productive, derived, degraded, waste and with inadequate station conditions forest stands, in particular the oak species and acacia". Neither in the purpose of this rule, nor in the highlighted categories for the application of the technical intervention methods are not clearly stipulated the forest stands particularities underlying their highlighting and classification, as well as a broad definition of the forest stands „improper culturally" is not given. In one case, this is the „worthiness grade (IV-V)", „the provenience (generative or vegetative)", „the compliance of the station", etc. in other cases this is the „type of forest stands (natural or artificial)", the presence or absence of „the main species (hornbeam and linden)" and so on or „the consistency (0,3 – 0,6)".

V. Leandru (2009) suggested the forest vegetation classification depending on the human influence intensity, in which highlights the natural, artificial and semi-artificial forest vegetation. The natural forest vegetation „managed" depending on the intensity of changes in its composition and structure is divided in the following categories: i) essential forest stands; ii) degraded forest stand; iii) partly derived forest stands; iv) derived forest stands; v) cuttings. The composition, structure and the forest forming species served as the basis for highlighting these categories. This is rather enough for highlighting the forest stands categories with the human influence variation degrees and with the expectation of conscious managing of these forest stands with the application of the ecological reconstruction methods and of the concerned treatments. It remains undefined only the problem of forest stands of vegetative deriving from shoots and branches that prevail in our forests. In case of artificial and semi artificial vegetation V. Leandru has enlightened 13 categories of forest stands with the variation degree regarding only the species and their area, what is, in our opinion, insufficient for the methods of ecological reconstruction and the perspective of many artificial

forest stands is not clear, mainly of those indigenous species of local provenience, which, in time, may pass to the category of natural forest stands if later are naturally regenerated (Vlad I., Chiriță C., Doniţa N., Petrescu L., 1997).

MATERIALS AND METHODS

The research targets are the materials of developing the national forest fund and the analysis of the information about forest stands on the territory, obtained on the itinerary in order to establish the influence degree of human element on forest.

To achieve the objectives set, the research has resorted to office and field methods; bibliographic documentation, planning material analysis, direct observation.

RESULTS AND DISCUSSIONS

As a result of the methodological research in the national forest fund we highlight the natural and artificial vegetation. The natural forest vegetation encloses all the situations in which man, regardless the intensity of his activity has not introduced (by sowing or planting) any new species. The forest natural vegetation reflects, in different cases, different situations of human intervention. From the very beginning we can conclude that in the Republic of Moldova there aren't forest stands which in the course of their development have not suffered human influence, and may be considered „virgin", or have suffered very little, but there have not been performed changes regarding composition and structure and may be considered „quasi-virgin". All the natural forest stands, in a way or another have been influenced and fall into the type of „managed forest stands".

After the intensity of changes in the composition and structure, after the regeneration mode and the presence of species in the main stand, after species composition of the area (indigenous, exotic) and their correspondence to the resort highlight the following types and categories of bushes:

Type N – natural forest stand;

- Subtype F – "fundamental", where the composition and partly the structure of forests stand have been preserved unchanged and are naturally regenerated.

*Category FG – "*generative fundamental" – the best forest stands with a high eco-protective capacity and productivity, they are managed as old forests with natural regeneration from seeds.

Category FV – "fundamental vegetative" – the specific composition has been preserved intact, with an increasing eco-protective and productive capacity, are regenerated naturally vegetativelly (more than 74% oak species forest stands) as young forests and are very vulnerable to climatic changes conditions.

- Subtype D – "degraded" – a low productivity as a result of grazing and/or

extracting of the valuable samples of main species; as well as all the stands with 0,1 – 0,3 consistency.

Category DG - "degraded generative" – stands with reduced consistency under 0,6 with samples from main species naturally derived from seed but with the crown and trunk weakly developed.

Category DV – "degraded vegetative" – stands with a reduced consistency, with a low productivity and eco-protective capacity, with samples of main species vegetativelly derived, very vulnerable to the changing ecological conditions.

- Subtype PD – "partly derived" – stands have lost their productivity and have suffered modifications in the specific composition, in the sense of diminishing the main species' proportion and increasing the supporting species' proportion.

Category PDM – "partly derived with mixed species" – it is significantly changed the composition in favor of mixed species, but the main species is present.

Category PDE – "partly derived with invading exotic species" – essential changes in composition and structure, reduced economic value and low eco-protective potential. The spaces appeared from the foolish extraction of the main species are replaced by the exotic invading species (*Robinia pseudacacia*, *Acer negundo*, *Morus alba*, *Amorpha fruticosa*, etc.) and only partly by the mixed species.

- Subtype TD – "totally derived" – stands with radically transformed composition; reduced productivity and the absence of the main species.

Category TDM – "totally derived with mixed species"–formed exclusively from mixed species (*Carpinus betulus*, genus *Tilia*, *Acer campestre*, etc.) without the participation of the main species – genus *Quercus*.

Category TDI – "totally derived with invading exotic species" – are formed largely from invading exotic species where the main species - genus *Quercus* is totally absent.

Type A – artificial forest stand

- Subtype AI – "artificial from indigenous species" – achieved from indigenous species by direct plantings and/or sowings.

Category AIP – "artificial from indigenous species, permanent" – the composition that corresponds to ecological conditions, achieved by sowings and or plantings (in foresting case), or under the old stand crown. Productive stands with long cycle of production.

Category AIT – "artificial from indigenous species, temporary" – formed from indigenous species non-corresponding to the ecological conditions or with the main species (in this case the oak) from introduced biotopes (in former USSR the acorn was brought from thousands of kilometers).

- Subtype AE – "artificial from exotic species" – realized from pure exotic or mixed species, in very variable number, using more than 25 introduced species.

Category AEP – "artificial from exotic species, permanent" – it is highlighted through increasing productivity, the most of which being pure and even aged with a cycle of production of 50-80 years (*Juglans nigra*, *Quercus rubra*, *Sophora japonica*, *Gleditsia triacanthos*, etc.).

Category AET – "artificial from exotic species, temporary" – pure or compound from more exotic species which are not appropriate for the type of station, low productive and with a reduced eco-protective potential. In this category are included a great part of the pure acacia stands which are in a great degree of drying.

- Subtype AIE – "artificial from indigenous and exotic species" – present in many stations with a large diversity of the composition, with a different productivity and resistance.

Category AIEP – "artificial from indigenous and exotic species, permanent" – possesses a good resistance to the ecological conditions and a increasing productivity. The indigenous species may occur in the main role (*Quercus robur* with *Fraxinus lanceolata* or with *Robinia pseudacacia*, *Sophora japonica*, *Celtis australis*) or secondary (*Juglans nigra* or *Quercus rubra* with genus *Tilia* and *Fraxinus excelsior*) forming stands with a long cycle of production.

Category AIET – "artificial from indigenous and exotic species, temporary" – indigenous and exotic mixtures of the species which are totally non corresponding to the ecological conditions (station) or from species with a different potential of ecological resistance, low productive and with a reduced eco-protective capacity.

CONCLUSIONS

1. The performed researches allowed the highlighting in the national forest fund of 14 categories of forest stands classified into two types and seven subtypes, regarding the changing intensity in composition, the structure, the presence of species/ the main species in stands and their mode of regeneration, generative or vegetative, the areal of species from composition and their compliance to the station.

2. The right classification of the forest stands in these proposed categories allows the highlighting of the surfaces that require ecological reconstruction with application of both the most appropriate management measures and of the appropriate ecological reconstruction methods.

BIBLIOGRAPHY

1. Giurgiu V., Repere pentru un necesar program al reconstrucției ecologice în protejarea şi dezvoltarea durabilă a pădurilor României, Bucureşti, 1995, p. 202-210;

2. Leandru V., Clasificarea vegetației forestiere în funcție de intensitatea influenței omului, Revista de Silvicultură şi Cinegetică, Anul XIV, Nr.25, 2009, p. 19-22;

3. Norme tehnice (privind folosirea, conservarea şi dezvoltarea pădurilor din Republica Moldova), Chişinău, Agenția „Moldsilva", 2012;

4. Palancean A, Gogu V., The argumentation of the expediency of undertaking environmental reconstruction works in the „Codrii" Natural Reserve, Jurnal of Botany, Chişinău, 2013, vol. V, N2(7) p.19-26;

5. Paşcovschi S., Primele rezultate ale cercetării tipurilor de pădure artificiale şi derivate din R.P.R., I.C.E.S., Studii şi Cercetări, vol. XX-XIII, Bucureşti, Ed. Agro-Silvica, 1963;

6. Vlad I., Chiriță C., Doniță N., Petrescu L., Silvicultura pe baze ecosistemice, Ed. Academia Română, Bucureşti, 1997.

MORPHOLOGICAL AND TAXONOMIC DESCRIPTION OF *LUPINUS ANGUSTIFOLIUS L.* SPIKELIKE SAMPLES INTO VIR COLLECTION

E. V. Vlasova

All-Russian Horticultural Institute for Breeding, Agrotechnology and Nursery, Moscow, Russia

Abstract: The morphological descriptions of the taxonomic characteristics of 29 spikelike samples of blue lupine are presented. The additions into intraspecific classification of *L. angustifolius* L. are submitted. The taxonomic position of the studied samples is fixed.

Key words: blue lupin, *Lupinus angustifolius* L., determinate habit, reduced branching, restricted branching, spikelike (spiciform) type, epigonal mutant, intraspecific classification, taxonomy

INTRODUCTION

A spikelike (spiciform) morphotype of blue lupin is the mutant (determinate) form of the wild form, characterized by unlimited branching. A distinctive feature of the spikelike type is the absence of branching in the top part of the main stem and the formation of pods on the main stem both on the cluster and under it - in the leaf axils instead of the lateral branches, whereupon generative sphere becomes similar to the ear of cereals. The authors also use the terms "epigonal mutants" and "highly restricted branching" to describe such morphotypes. [1-4].

In the intraspecific classification Kurlovich BS - Stankevich AK (1990), supplemented by Kurlovich BS (2002) and Kuptsov NS (2006), the type of branching: namely determinant and fasciated morphotypes, isolated in the rank of form. The color and the presence of the anthocyanin on the vegetative organs are the diagnostic features of the subspecies. Both the corolla coloring and the painting on the seed testa characterize the species. The present taxonomy of *Lupinus angustifolius* L. includes 29 species, 16 subspecies, 25 determinant and 2 fasciated forms. [4-6].

Purpose of research: the morphological description and the taxonomic characterization of 29 spikelike samples with the aim of systematization and inventory of the world gene pool of *Lupinus angustifolius L.*

MATERIALS AND METHODS

There are 840 samples of blue lupine in the VIR collection (St.-Peterburg, Russia). The morphological description of 29 spikelike samples of *Lupinus angustifolius L.* from VIR-collection was being conducted in the field conditions of Moscow region according to the list of descriptors [7] annually in 2009-2011. For taxonomic

characterization of the samples used the classification Kurlovich BS - Stankevich AK (1990), supplemented by Kurlovich BS (2002) and Kuptsov NS (2006) [4-6].

RESULTS AND DISCUSSION

As a result of the morphological descriptions and their comparison with the diagnostic features of the species, the subspecies and the forms, we have found that the existing intraspecific classification of *Lupinus angustifolius L*. best reflects and codifies the intraspecific diversity represented into VIR-collection, but needs to include the following amendments:

1) The describing of the color and the pattern on the seeds should be supplemented by information about the presence on the seeds of the triangular spot above the seam and the strip below the seam. Especially this note relates to the description of *Var. albidus Kurl. et Stankev.*, as in the description of the variety listed only the white seed color, but there is no information about the presence on the seed of the colored triangular spot and the strip, while in the diagnostic samples (Typus: k-2096 Unicrop, Australia, k-2687 Lanedeks-1, Belarus) such elements are present.

2) The descriptions of species *Var. corylinus Kurl. et Stankev*. and *Var. albocyringeus Taran* contain the inaccuracy that was found after comparison of the taxonomic characters of these species with the corresponding diagnostic samples (Typus: k-2666 Apendrilon, Greece, and Typus: k-1981 Nemchinovsky 846, Russia, respectively): The green cotyledons were present in the descriptions of the species while the anthocyanin was present on the cotyledons of the diagnostic samples actually.

3) It is desirable that the description of the vegetative organs be supplemented with the information about the presence of the anthocyanin on the inflorescence axis, since this sign, according to our observations, is closely associated with the presence of the anthocyanin on the cotyledons.

We reported previously about some of the listed remarks [8].

The samples were named the species, the subspecies and the forms as a result of the morphological descriptions and as above mentioned amended into intraspecific classification:

1. Eleven samples with the pale violet color of the corolla (k-*2648 Ladny, k-3288 Nemchinovsky 846* x *Mut 1, k-3289 Mut 1* x *Frost, k-3551 Mut 1* x *Frost, k-3695 Denlad, k-3637 Bryansky 1272, k-3723 Nadezda, u-0142672 Vektor from Russia; k-2697 Lanedeks 3, k-3471 E 1 from Belarus; k-3762 Borveta from Germany*) characterized by uniformity of the morphological markers: the white color of the seed without the triangular spot and the stripe, the presence of the anthocyanin on the cotyledons and the inflorescence axis and the absence of the anthocyanin on the tip of the flower's keel; the dark green color of the stem with the presence of the anthocyanin in the weak or medium degree, the dark green leaves with the anthocyanin border. ***Var. albocyringeus*** *Taran* ***subvar. albocyringeus f. kloczkovii Kurl. et Stankev***.

2. Five samples with the white color of the corolla (*k-3627 Dikaf 1, k-3628 Dikaf 11, k-3546 Dikaf 14, k-3368 Determinant 5 from Russia; k-2665 Lanedeks from Belarus*) characterized by the white seeds with the brown spot, both the brown triangular spot above the seam and the strip below the seam; the anthocyanin on the vegetative organs is absent; the leaves are green and light green color. ***Var.albidus Kurl. et Stankev. f.kuptsovii Kurl. et Stankev***.

3. Ten samples with the blue color of the corolla characterized by the dark green color of the stem with the presence of the anthocyanin in the weak or medium degree; the dark green leaves with the anthocyanin border; the presence of the anthocyanin on the cotyledons, the inflorescence axis, the tip of flower's keel; the presence of the four types of the color and the pattern on the seed:

a) the "wild-type": on the gray background are both the mesh brown spots and the large white spots; the triangular spot above the seam and the strip below the seam are black coloured (samples: k-*3367 Determinant 4, k-3696 Line 16, from Russia; k-3502 L-155 from Poland; k-3521 BSHA-270, k-3525 BSHA-405, k-2954 Vika 65 from Belarus*) - ***Var. angustifolius subvar. angustifolius f. angustifolius***

b) the beige background with the brown spots and the small black dots; the triangular spot above the seam is brown coloured; the strip below the seam is black coloured (samples: *k-1354 Melkosemyanny from Latvia; k-2983 Melkosemyanny 63, k-3218 ABR-1 from Belarus*) - ***Var. corylinus Kurl. et Stankev. f. zhukovskii Kurl. et Stankev***.

c) the white spots on the dark brown background; the triangular spot above the seam and the strip below the seam are black coloured (k-*2955 Pershasvet, Belarus*) **- *Var. griseomaculatus Kurl. et Stankev. f. belorussicus Kurl. et Stankev***.

4. One sample -*k-3365 Determinant 2, Russia* - differs from the rest samples with the blue color of the corolla by anthocyanin (purple) color of the vegetative organs (the cotyledons, the stem, the leaves, the inflorescence axis, the tip of flower's keel). The seeds are dark brown with the small white spots; the triangular spot above the seam and the strip below the seam are black coloured. The new form into species ***Var. albopunctatus Kurl. et Stankev.***

5. Two samples with the pink color of the corolla (k-*3639 Bryansky 1298, Russia and k-3501 L-300, Poland).* The samples are characterized by "wild type" of color and pattern on the seed (on the gray background are both the mesh brown spots and the large white spots; the triangular spot above the seam and the strip below the seam are black coloured), the dark green stem with the presence of the anthocyanin in the weak or medium degree; the dark green leaves with the anthocyanin border; the presence of the anthocyanin on the cotyledons, the inflorescence axis, the tip of flower's keel. The samples have been attributed to ***Var. purpureus Kurl. et Stankev***. on the basis of the corolla coloring and the painting on the seed testa. The assignment of the subspecies for this samples is problematic since the presence of the anthocyanin on the cotyledons in combination with the green color of the vegetative organs are characteristic for this samples, but is not inherent for any of the subspecies.

CONCLUSIONS

1. The additions to the descriptions of the diagnostic features of the species and the subspecies have been incorporated into the existing intraspecific classification of *Lupinus angustifolius L*.

2. The new form, which is absent in the existing taxonomy has been discovered in the process of studying of the samples:

Var. albopunctatus Kurl. et Stankev. 1990, l.c.:24. - *L.angustifolius var. maculatus Atab.* l. c.:129, nom. illeg. - Flowers – blue. Seed – almost black with tiny white dots and spots.

Typus: 'Benyakonsky 335', Belarus, k-1477, reproduction of Pushkin`s laboratories of VIR, 10.7.1989, B.S. Kurlovich (WIR).

f. spicatus Vlas. f. nova - Rami lateralii nullum esse vel valde abbreviati. Flores axillaria. Morphotypus spiciformis.

Typus: 'Determinant 2', Bryansk, Russia, к-3365, reproduction of Center of preservation, maintenance and study of gene pool SSI All-Russian Breeding and Agrotechnological Institute of Horticulture and Nursery RAAS, 2010

Side shoots – absent or shortened, flowers – axillary**.**

3. The taxonomic position of 27 samples has been found. There are two samples (*k-3639 Bryansky 1298, Russia and k-3501 L-300, Poland*), which was identified only the species: ***Var. purpureus Kurl. et Stankev.***, but was unable to establish a subspecies. We need to conduct a reconciliation of the descriptions of the subspecies **s*ubvar. purpureus*** and **s*ubvar. rhodanthus Kurl. et Stankev***. with the appropriate diagnostic samples to solve this problem.

BIBLIOGRAPHY

1. Adhikari K.N., Galwey N.W., Dracup M. Adhikari K.N., Galwey N.W., Dracup M. The genetic control of highly restricted branching in narrow-leafed lupin (Lupinus angustifolius L.) // Euphytica.-117.-2001.-P.261-274.

2. Debely G., Derbensky V. Determinant and fasciated forms mutants as a starting material in breeding blue lupine (L. angustifolius L.) // Genetics.-1994.-V. 30 -P.40.

3. Dracup M., Thompson B. Narrow-leafed lupins with restricted branching //Annals of Botany. 85.-2000.-P.29-35.

4. Kuptsov N.S., Takunov I.P. Lupin: genetics, breeding, heterogeneous crops. - Bryansk, Klincy: 2006.-576 p.

5. Kurlovich, B.S. and Stankevich, A.K. Intraspecific diversity of three annual lupine species (Lupinus L.). //Bull.Appl.Bot.Gen. Pl.Breed. -Leningrad: 1990, 135: 19-34.

6. Kurlovich B.S. Lupins. Geography, Classification, Genetic Researches and Breeding.- St. Petersburg: Publicing house "Intan", 2002.-468 p.

7. The International comecon list of descriptors for the genus Lupinus L.-Leningrad: VIR.-1985.-38p.

8. Vlasova E.V. Problems to systematizations of Lupinus angustifolius L. spikelike types into VIR collection. // NI Vavilov's ideas in the modern world. Abstracts. III Vavilov Int. Conf. 6-9 November 2012 - ST. Petersburg: SSI VIR RAAS.-2012.-P.53.

III. INTRODUCTION OF PLANTS AND SUSTAINABLE USE OF PLANT RESOURCES

NATIONAL PLUM VARIETIES OF AZERBAIJAN

D.B.Bayramova

Azerbaijan Genetic Resources Institute of ANAS, Baku, 155 Azadlig Ave., AZ1106, Azerbaijan e-mail: bairamova-dilshad@mail.ru

Abstract: Many researches on plum varieties in Azerbaijan revealed that different plum varieties have been distributed in various regions of Azerbaijan. Local varieties of Azerbaijan plums basically have been widespread in Shirvan region of Gharabagh area, Ordubad region of Nakhchivan Republic also Khachmaz and some other regions. Domestic of varieties have high yield potential, good quality, special taste and some other noticeable characters too. Native people traditionally use plum fruits by various methods, such as: fresh fruit, dried fruit, other types of food (compote, jam, jelly etc.) and also people use them as medicinal plants. Local varieties have various ripening dates and show different and noticeable resistance to some plant diseases.

INTRODUCTION

Azerbaijan is the native land of many fruit crops, including cherries, plums, grenade, fig, walnut, filbert, chestnut, etc. Wild-growing plum is known in region of the country since very old times and widespread in woods. Throughout many years the population used the wild forms of these plants growing in wood that further gradually were domesticated and improved in order to obtain local varieties.

From the immemorial times, in Azerbaijan, the domesticated plum indigenous varieties exceed in some qualities, including residing, biological, economic value, productivity, biochemical structure, tolerance to diseases and pests, adaptation to soil-climatic conditions and others in comparison with alien crops.

Among stone fruits plum takes a special place. Dried fruits of plum in the Middle Ages have formed a basis of trade relations of Azerbaijan with foreign countries. Thus, some people which leaved from Azerbaijan to the north, carried with them dried form of cherry-plums and plums, whose seeds have spread from Ukraine to Hungary.

In the Central Europe and Southern Ukraine, the cultivation of plums has expanded only in the Middle Ages. Dried up varieties Albuchara of Shirvan and Ordubad are known in the Near East, in Russia and the Western Europe since old times. [1]

Plums as well as other stone fruits, from the 3rd-4th years enter the period of fructification. Productivity makes up to 200 q. from hectare. Depending on variety,

the fruits are more thin, extended, roundish, the heart-shaped form, colour - green, yellow-red, violet and lilac. The surface of fruits is covered by white, gentle wax. Depending on plum variety, there are stones of fruits separated from pulp and not separated.

Plum fruits are valuable foodstuff and also possess dietary and medical properties. They contain approximately 17% of sugars, organic acids (lemon, apple acid), vitamins - with, B1, B2, P, caratinoids, mineral substances; seeds contain 40% of fat and amigdalin glycoside. Plum fruits are used both in a fresh kind, and in canning manufacture (compotes, jams, jam and other products), the best use – in the form of dried fruits [3,4,5,7].

In ancient times, plum fruits were widely used in medicine, at treatment of illness of kidneys, rheumatism, on increasing appetite and as substances which stop pains in stomach, cut fresh or dried leaves were used for wound treatment. Fresh plums and dried fruits possess light laxative action and diuretic properties.

MATERIALS AND METHODS

Material of research is local, domestic varieties of plum, distributed to different zones of Azerbaijan. Studying of economic-biological indicators was carried out by a technique «The program and a technique of studying fruits, berries and nut-bearing cultures» [6].

RESULTS AND DISCUSSION

Plum (*Prunus domestica* L.) belongs to family *Rosaceae*, subfamily *Prunoideae* and genus *Prunus*. From 10 species of plum widespread in the countries around the world with a temperate climate there are 2 wild species and 1 subspecies and 4 cultivated forms in Azerbaijan. Wild species of plums are extended in the majority areas of Azerbaijan - from a low part to mountain strips, in woods and bushes, and also in valleys of the rivers [2].

In the present paper, the local varieties of native selection distributed in various regions of Azerbaijan are described. These varieties are the following: Ajiqara, Albuxara plum, Apple plum, Abrashi, Xatını, Sarı (Yellow) albuxara, Late ripening yellow albuxara, Black albuxara, Pearch plum, Shakari or Black Kutabi), Vaziri, Early ripening blue plum. Grape-plum, Ordubad albuxarası, Gulaman albuxarası, Nasimi albuxarası (Badam alı). These varieties differ from each other in maturing of fruits, productivity and quality of fruits.

Sarı (Yellow) albuxara. One of the ancient late ripening local varieties of Azerbaijan. The native land of the varieties is Ordubad. Trees are undersized; the crown is roundish with pyramidal form. High-yielding variety. Fructification starts in the 4-5-th year after planting. The variety is propagated with young shoots. The

productivity of 12 year old tree is 80 kg or 22.1 ton per hectare palnted with design of 6x6 m. The shape of a fruit is oval, weight - of 25-35, the colour of the thin peel is light yellow, covered with white wax. At maturing the stone easily separates from pulp. Fruits contain 12% of sugar and 0.8% of acid. Ripening of fruits lasts from July till September. This variety is used in a fresh kind, is favourable for the preparation of compotes, canned food, dried fruits. The variety is resistant to insects-pests. In Azerbaijan there are various forms of this variety with different time of ripening. The local varieties such as Faxri, Guleman, Araz, Ordubad, Nasimi albuxara belong to them.

Black албухара. It is a variety of national selection of Azerbaijan. It is native to Shirvan zone. Trees are rare deciduous and sprouts – downy. Very productive variety. Fruits are egg-shaped with red-cinnamon colour, pulp is sweet and fragrant, and colour is yellow. The fruits contain 10,6% of sugar and 1,21% acid. Stones easily separate from pulp. It is an early, productive and widely cultivated variety. Fruits start to ripen in the middle of June. Along with using them fresh, the fruits are used for preparation of compotes and as dried fruits.

Şaftalı (Peach) plum. is a variety of folk selection in Azerbaijan. The crown of trees is roundish, leaves are dense. Fructification begins in the fourth year after planting. Fruits are large and very beautiful, with red colours on the south side. The fruit pulp is dense; seeds are small and have sweet taste (14%) with a little acid (1.6%). It is drought-tolerant. Productivity of one tree is approximately 150 kg. Fruits ripen in the middle of July.

Shakari or Black Kumabi. A variety of national selection of Azerbaijan. It is native to Shirvan zone. Widespread in all zones of Azerbaijan. Trees are tall with pyramidal form. It is a productive variety. Fruits – of the average size with egg-shaped form. The thin skin is yellow, on the one side is violet - black and is covered by a thick white wax powder. The pulp is firm, fragrant and tasty, of yellow colour. Not juicy, sweet and little bit is sour. It ripens in the beginning of August. It is suitable for drying.

Xatını. An ancient variety of Azerbaijan national selection. It is one of the ancient varieties of Shirvan zone. It is the most widespread and early ripening (from the 10th of June) variety among local varieties of Azerbaijan plums. Trees are tall; the crown is friable in shape of inverted pyramid. Fruits are small, weight of a fruit – 15 g, the colour is black-red and the fruit form is elongated-heart-shaped. Stones are easily separated from pulp, the pulp has green-yellow colour, it is not juicy and fragile, taste - sweet. Stones are very small (0,5 cm), the form - narrow and extended. Therefore this variety still is called a needle - plum. It is suitable for preservation. This variety is not affected by plum-worm.

Vaziri. A variety of national selection of Azerbaijan. It is widely cultivated in

Ordubad area of Nakhchivan Republic. The trees are tall, with roundish crown. It ripens in August. Fruits are egg-shaped, the fruit stem is thick, short, colour of the fruit is light-violet, the peel is dense, the stone is small, does not separate from pulp, the pulp is dense, juicy, fragrant, tasty and sourish-sweet with yellow-green colour.

***Tezyetişən göy gavalı. (Early ripening, blue plum**.)* A variety of national selection of Azerbaijan, it is widespread in Ghuba - Khachmaz region. It is one of the early ripening varieties. Fruits of this variety ripen after gathering of most late ripening apricot varieties. Trees have average height, the crown is wide-sprawling and the sprouts are direct and long. It is observed high annual fructification. Fruits are average in size, elongated with oval form. The colour of fruits' peel is dark blue; the pulp is brown, juicy, very soft and tasty. This variety is affected by the plum-worm.

Üzüm ərik (Grapes plum). A local variety of Azerbaijan, it is widespread in Khachmaz area. Trees have average height and pyramidal crown. Late ripening variety. Fruits ripen in the end of August and in the beginning of September. It is a fruitful variety. Fruits and stones are small, identical in size and form. The colour of a fruit is dark blue, almost black. Fruits are very tasty, sweet. All the sprouts are covered by fruits. It is a unique variety of Khachmaz area which individual fruits are affected only in an initial stage of development by plum worms. Other part of the crop is not affected. Fruits are not affected by decay.

CONCLUSION

Local varieties of plum have been spread in many regions of Azerbaijan, but basically in the Shirvan zone, Khachmaz area and in Nakhchivan.

These varieties differ from each other in terms of maturity, quality of fruits, growth of trees, forms of crowns, productivity, resistance to diseases and pests and exceed alien crops.

In order to receive new, tolerant and qualitative varieties, the use of domestic, local varieties of plum in breeding is purposeful.

BIBLIOGRAPHY

1. A. Rajabli. Fruit plant of Azerbaijan. *State Publishing House,Baku-1966.*

2. A.M. Askerov. *Higher Plants of Azerbaijan.* The outline of Azerbaijan Flora, I-III volumes, Baku, "Elm", 2005-2007.

3. Bayramova D.B. and others. *Information book of gardener. Baku, "Sada", 1997*.

4. V.V.Bespecalnaya, A.M.Vukolova. *Stone fruits.. «Картя Молдовеняске», 1973.*

5. M.A.Pavlova. *Berry crops*. Selxozqiz. Moskow*, 1959*.

6. "The program and method Cultivar fruit, berry and nut crops." Moskow-1973.

7. D.B.Bayramova. *The importance of stone-fruits crops* . "Azerbaijan Aqriculture Science journal" №2, 2013.p.16-20.

THE VEGETATION ON THE LIMESTONE OF THE MEDIUM SARMATIAN UPSTREAM THE NATURAL AREA „FĂUREŞTI"

Eugenia Chiriac, Boris Nedbaliuc
State University of Tiraspol

Abstract. The landscape area "Făureşti" represents a specific region of rocks, which intercalates conditions of different biogeographic sub regions. Fragmented landscape of slopes, difference in altitude, diversity of landscape, characterize the given place as a specific and picturesque area. Seen from east to west or north to south, this place seems detached from a mountain landscape. Plant diversity in this area is specific, but unfortunately poorly known.

INTRODUCTION

Limestone slopes of the valley of the river Nistru have attracted the attention of many botanists. At the beginning of the 19th century, Andrzeijowski (author of Flora of Ukraine, 1869), collected a herbarium based on which Besser (1882) described some new species from the site. Bieberstein, describing Flora Taurico-Caucasica (1808-1809), prepares a supplement in which he describes typical species of Bessarabia. Dates about species on calcareous soils, we can see in the works of Серединский (1872.1873), Lindeman (1882), Zapalowicz (1906). Currently, a special interest is the vegetation on the medium Sarmatian limestone, which is unique. Being included after (P.Pânzaru, 1997) in endemic alliance Genisto-Seselion peucedanifolii, this type of vegetation requires assessment and monitoring activities of the floristic composition and of valuable elements of this land in the vegetable patrimony of our country.

MATERIAL AND METHOD

As investigative practices, the evaluation of the vegetation after the method of Braun-Blanquet (1964) and the analysis of the literature with reference to the investigated area have been used. Field activities were conducted in three sessions: spring (March, April, May 2011-2013), summer (June, July 2011 -2013), and autumn (September, October 2011-2013). Representative growing areas were initially evaluated visually in terms of location and growth characteristics. For the determination and identification of the species, the following papers were used: Postolache Gh. „The vegetation of Republic of Moldova". Chişinău, „Ştiinţa". 1995; Gheideman T. „The determinants of the higher plants of Moldavian Soviet Socialist Republic". Chişinău. 1986; Ciocîrlan V. „Illustrated Flora of Romania" vol. 1,2. Chişinău, „Ştiinţa". 1992; Popovici L. - „The botanic atlas". The didactic publisher. Bucureşti. 1993; "The vegetal world", in 4 volumes (2005 -2007); The Red Book of Republic of Moldova (2001).

Negru A. „The determinants of plants from the flora of the Republic of Moldova". Chişinău 2007.

RESULTS AND DISCUSSIONS

The area under study has a natural complex, located in the Central Region of the Republic of Moldova, on the valley of the river Ikel (right affluent of Nistru). It is a typical rocky place (limestone). As the whole territory of our Republic, the sector had a whole geological evolution throughout the ages and belongs to the Moldavian Platform, whose foundation lies at depths of 500 -1000 m and includes sedimentary deposits of different ages. However, that territory is covered with outcrops of the medium Sarmatian that include clay deposits, sand, limestone and marl belonging to the reef group Goian-Cricova-Făureşti. The River Ikel crosses here a massive limestone reef, forming two steep slopes, having a stony bottom of the valley. The riverbed forms meanders. Reefs do not appear in a continuous section but rather as stacks, separated by oolitic and coquina limestone. The age of the reef group „Făureşti", after the discovered the fauna in the outcrop and in other reefs, in the area of the valley of the River Ikel, shows that it belongs to the lower Bessarabian and the lower part of the upper Bessarabian. The reef group is covered with friable limestone (6-8 m). In this area there are some quarries of limestone that are used in construction. These extractions are carried out by the open method and by the folded method (inside reefs). It is also extracted in the form of rough stone and crushed stone.

Soils are of several types: typical rendzinas (skeletal), which are characteristic of the right side and is formed on the limestone rocks, alluvial floodplain and layered, low salinity – which are developed on the meadow of the River Ikel under the grassy and hydrophilic rug of different species. At the bottom of the right side in the area of springs we have a portion of marshy soils. Gray forest soils are spread between rolled limestone blocks and on them grow shrubs and some herbaceous plants, including plant species included in the Red Book (2001). The right slope is steeper at the top highlighting some stairs resulting from fracturing of limestone blocks that have been gradually sank or have been partially covered by a layer of skeleton soil. It has a Southern position (S - E) and a slope of inclination of about 23-28°. The left side is only mild to the bottom with an altitude of 180 m and has a north position with a slope inclined at an angle ~ - 30-60°. The slopes have a great importance for the distribution of vegetation. Temperate - continental climate with its features has led to the development of a grassy carpet, adapted to these conditions.

Rocky ridges of the right side are developed in spots of a large number of characteristic species: *Thymus marschallianus* Willd.; *T. calcareus* Klok.et Schost; *Sedum acre* L.; S. Telephium L.; *Iris pumila* (L.); *Hyacinthella leucophaea* (C. Koch); *Muscari neglectum* Guss.; *Helianthemum nummularium* (L.) Mill.; *H. canum* (L.) Baumg.; *Trifolium aureum* Poll.; *T. ochroleucon* Huds.; *Lotus corniculatus* L.;

Medicago falcata L.; *M. minima (*L.) Bartalini; *Lotus corniculatus* L.; *Potentilla arenaria* Borkh.; *Berteroa incana* (L.)DC; *Salvia nutans* L.; *Ajuga chia* Scheb.; *Jurinea stoechadifolia* (Bieb.) DC.;

In the eastern part of the slope, in some areas of the slopes, found shelter some species of shrubs: *Crataegus monogyna* Jacq.; *Roşa canina* L.; *Coryllus avellana* L.; *Viburnum opulus* L.; *Swida sanguinea* (L.) Opiz., etc. In spring, here are found a number of species, including ephemerals specific to forests, but have been adapted to the circumstances: *Anemonoides ranunculoides* (L.) Holub.; *Anemone sylvestris* L.; *Fritillaria meleagroides* Patrin ex Schult. et Schult.fil.; *Tulipa biebersteiniana* Schult. et Schult. Fil.; *Polygonatum latifolium* (Jacq). Desf.; *P. multiflorum* (L.) All.; *Viola odorata* L.; *V. collina* Bess.; *V. hirta* L.; *Gagea paczoskii* (Zapal.) Grossh.; *Gagea lutea* (L.) Ker –Gawl.; *Ficaria verna* Hugs.; *Corydalis cava* (L.) Schweigg. Et Korge.; *C. paczoskii* N. Busch.; *Scilla bifolia* L.; *Silene chloranta* (Willd) Ehrh., most of which are protected.

Towards the foot of the slope the depth of soil increases. Depending on the position of the slope and the content of the rock, the floristic composition and the structure of the vegetation is changing. On slopes with south-east and west position with poorly developed soils and with a content of rocks of 35% the grass cover is rarer and consists of: *Agropyron pectinatum* (Bieb.) Beauv.; *Bothrichloa ischaemum* (L.) Keng.; *Koeleria lobata* (Bieb.) Roem. et Schult); *Setaria viridis* (L.) Beauv. ; *Ajuga chia* Scheb.; *Salvia verticillata* L.; *Acinos arvensis* (Lam.) Dandy.; *Alyssum murale* Waldst. et Kit. In middle part of the slope, with a higher soil thickness, grass cover reaches 70-95%. In these places grow: *Achillea collina* J.Becker ex Reichenb. ; *Antemis austriaca* Jacq.; *Jurinea calcarea* Klok.; *Centaurea thirkei* Sch.Bip.; *Artemisia austriaca* Jacq.; *A. absinthium* L.; *Centaurea thirkei* Sch.Bip.; *Cirsium ciliatum* (Murr.) Moench; *Helichrysum arenarium* (L.) Moench.; *Melica transilvanica* Schur.; *Salvia moldavica* Klok.; *Teucrium pannonicum* A. Kerner.; *Galeobdolon luteum* Huds.; *Berteroa incana* (L.)DC.; *Alyssum murale* Waldst. et Kit.; *Filipendula ulmaria* (L.) Maxim.; *Medicago falcata* L.; *Medicago minima (*L.) Bartalini.; *Genista tetragona* Besser; *Chamaecytisus austriacus* (L.) Link.; *Astragalus albidus* (Waldst. et Kit.).; *A. vesicarius* L. var. *pseudoglaucus* (Klokov) Pinzaru comb. et stat. nov.; *Vicia cracca* (L.); *Nigella arvensis* L.; *Myosotis micrantha* Pall. Ex Lehm.; *Echium vulgare* L.; *Linum tenuifolium* (L.); *L. perene* (L.); *Anthericum ramosum* L.; *Poa versicolor* Besser; *Campanula sibirica* L.; *Seseli peucedanifolium* Besser.; *Sedum acre* L., the last being detected sporadically in very small numbers, mostly on rocks and crevices between rocks, where they are protected from negative anthropogenic action (grazing and fire).

CONCLUSIONS

Due to the particularities and exceptional diversity of the relief the vegetation has a unique value. The reported number of species is particularly high compared

to the surface area and compared with the situation of other areas of vegetation in our country. Particular on the Sarmatian limestone is observed a geographical centre forming new species of plants. Among them, in the studied area, we encountered the following species: *Genista tetragona, Poa versicolor*, *Seseli peucedanifolium*, *Thymus calcareus, Astragalus vesicarius* L. var. *pseudoglaucus*. It is also encountered calcific species with discontinuous area such as - *Helianthemum canum*. According to the classification of species of rare plants and in danger of extinction, according to the international classification of endangered species (IUCN, 1994), among the 168 species encountered, four species are assigned to the category CR, 4 species are part of EN, 7 are in the category VU and 5 species of plants are included in LR.

In order to preserve vegetation in the studied area, it is necessary to undertake a series of measures that would include restoring species characteristic of the given place, purifying water resources, rational use of limestone. However, for the next edition of the Red Book of the Republic of Moldova is proposed to include all calcific species in the critically endangered category (CR), endangered category (EN), and in vulnerable (VU) or low risk (LM), only those with a small area of distribution.

BIBLIOGRAPHY

1. Andrzeijowski, Ant., 1869. Flora Ukrainy, czyli opisanie roslin dzico rosnacych w Ukrainy Przed. (după Pânzaru P., Negru A., Izverschi T. „Taxoni rari din flora Republicii Moldova. Chişinău, 2002).

2. Besser, W.S.J.G.,1822. Enumeratio Plantarum hucusque in Volhynia, Podolia, gub. Kiioviensi, Bessarabia, cis-Tyraica. Vilnae:1 -111. (după Pânzaru P., Negru A., Izverschi T. „Taxoni rari din flora Republicii Moldova.Chişinău, 2002).

3. Bieberstein,F.A., 1808/1819. Flora Taurico –Caucasica. Suplimentum 1,2,3. Charkouiae. (după Pânzaru P., Negru A., Izverschi T. „Taxoni rari din flora Republicii Moldova. Chişinău, 2002).

4. IUCN [Species Survival Commission]. IUCN Red List Categories. –IUCN, Gland, 21 p. 1994.

5. Gheideman T.S. Opredeliteli vâsşih rastenii Moldavscoi SSR. Chişiniov « Ştiința ». 1986.

6. Lindemann; E.,1882, Ubersicht der bisher in Bessarabien anfgependenen Spermatophyten. (după Pânzaru P., Negru A., Izverschi T. „Taxoni rari din flora Republicii Moldova. Chişinău, 2002).

7. Negru A. Şabanov G., Cantemir V., Gânju Gh., Ghendov V., Baclanov V. Plantele rare din flora spontană a Republicii Moldova. Chişinău, CE USM, 2002.

8. Pânzaru P., Negru A., Izverschi T. „Taxoni rari din flora Republicii Moldova. Chişinău, 2002.

9. Pânzaru P. a. *Genisto –Seselion peucedanifolii* –aliamța nouă în vegetația calcarelor Sarmațianului Mediu din republica Moldova. Depon. I.C.S.I.T.E. nr.1469-M. 29 p. 1997.

10. Zapalowicz, H. 1906. Krytyczny przeglad roslinnosci Galicyi (conspectus florae Galiciae criticus), I. Krakow. (după Pânzaru P., Negru A., Izverschi T. „Taxoni rari din flora Republicii Moldova. Chişinău, 2002).

11. Серединский, Н.К.,1872 -1873. Материалы для флоры Новороссийского края и Бессарабии. Записки Новороссийского о ва естествоиспыт. 1, 1- 2: 9 -487. (după Pânzaru P., Negru A., Izverschi T. „Taxoni rari din flora Republicii Moldova. Chişinău, 2002).

INTRODUCTION OF CULTIVATED PLANTS AT THE MAIN BOTANICAL GARDEN RAS

A.S.Demidov, S.A.Potapova

The Main Botanical Garden named after N.V.Tsitsin
Russian Academy of sciences Moscow, Russia

Abstract. Plant collection resources of the Main Botanical Garden RAS are the largest ones in Russia and comprise more than 17672 plant taxa, including nearly 10545 species, varieties and forms, and 7127 cultivars. The Garden's collections serve primarily as a basis for a wide range of research work. Its purpose is to create a comprehensive theory of the introduction and acclimatization of plants that would facilitate the determination and selection of the most productive forms of useful plants, indicate means of accelerating their growth, as well as improving their genetic properties.

INTRODUCTION

Important role in expanding the range of relatively poor crop of cultivated plants in non-chernozem zone of Russia are called to play the introduction and acclimatization of varieties, cultivars and species from other geographical areas. Introduction of crops involves search, selection and further study in the new conditions of growing relocated from other geographical zones, a selection from the formed collection funds of the most promising of the complex biological and agronomic characters of plants, development of methods of cultivation and recommendations for their practical implementation. Collections are the basis for research and finding out the most promising species.

MATERIALS AND METHODS

Fundamentals of collection funds of cultivated plants at the Main Botanical Garden of Russian Academy of Sciences (MBG) were laid down in the 50-th years of last century. Species and varieties of apples, pears, plums and cherries were tested during 15 to 30 years with sufficient winter hardiness, shrubs – 6-8 years old, strawberry – 5 years. Sample for the comparative study were received from many institutions that carried out breeding work in the different regions of Russia. Some species were collected in natural habitats and received in an exchange of seeds from botanical gardens and crop farms.

RESULTS AND DISCUSSIONS

The number of tested species and varieties from different regions and continents are following: the greatest number of species used for testing are from Central Asia -36.5%, and then from North America – 18.2%, East Asia – 13.7%, West Asia –

10,5%, Europe – 9.8%, the Far East – 7.4%, the Caucasus – 3.2% and South America – 0.7%. A somewhat different pattern is observed among the studied varieties. The greatest number of them was recieved from Europe – 65.5%, North America – 12.4%, West Asia – 10.7%, Asia –3.3%, Central Asia – 2.8%, the Caucasus – 2.4%, the Far East – 2.0%, Africa – 7% and South America – 0.2%. Collection of cultivated plants currently has 2.389 taxa, including 734 species and varieties of forms and 1655 cultivars. Among them: apple – 16 species, 149 varieties, pear – 11 species, 57 varieties, currants – 13 species, 152 varietes, gooseberries – 62 cultivars, raspberry – 63 cultivars, strawberries – 20 species, 200 cultivars and hybrids, pigeons – 23 cultivars (Gorbunov, 2011).

Over the entire period about 180 species and 800 varieties of fruit plants were tested. Introduction of horticultural crops is of great scientific and practical interest, because in the middle zone of Russia there are not many winter-hardy, productive, resistant to pests and diseases varieties. One of the main reasons for limiting the growth and development of fruit and berry plants in our area is their low frost-resistance. The study of plants in different years, especially in years with extreme weather conditions, makes it possible to identify the most valuable plants for our climate. Thus, the least winter-hardy genera are *Pyrus* L., *Armeniaca* Hill., *Cerasus* Hill., *Cydonia* Hill., *Vitis* L. *Pyrus syriaca* Boiss., *P. nivalis* Jacq., *P. tadshikistanica* V. Zapr., *Armeniaca vulgaris* L. died. The most winter-hardy species: *Malus sylvestris* Mill., *M. baccata* (L.) Borkh., *M. niedzwetzkyana* Dieck, *Pyrus communis* L., *P. ussuriensis* Maxim., *Prunus ussuriensis* Koval. et Kostina.

Leading fruit crops in the central zone is apple. It takes more than 70% of the total area of gardens. So, special attention was paid to the species and varieties of apple trees. It was held introductory study of the varieties to winter hardiness, frost resistance, yield and taste of fruits.

In terms of introduction of the experiment, were studied 20 species and 500 varieties of strawberries produced in breeding centers in former USSR and many foreign countries. Hybrid origin of strawberry *Fragaria ananassa* Duch. from two American species *F.chiloensis* (L.) Duch. and *F.virginiana* Duch. largely determines the broad range of plasticity and adaptability of strawberry varieties. It was established that the adaptability of species depends on the photoperiodic response and climatic conditions of the area. Varieties of northern origin need lower temperatures or a longer day than the varieties from the southern regions.

The best results in the creation of new varieties obtained by crossing of geographically distant origin varieties. These varieties represent a new generation, an aggregate set of self-pollinating lines with different genotypes, in connection with which they have greater adaptability to new environmental conditions. In the crosses of European and North American clones of ecotypes *F. ananassa*, we receive the type of high ecological plasticity.

It is shown that the productivity of cultivars in the introduction is related to their geographic origin. A study of more than 60 varieties from the U.S.A. has shown that the most productive sorts are from the northern and north-eastern states. A little perspective was from Japan, as well as the Far East. Significantly better results were obtained from western varieties, especially the Dutch. The most productive varieties are Crown, Elvira and Bogota. The value of these varieties is determined as stable, high-yielding, large-fruited, with good taste.

Introduction of large-fruited strawberry cultivars is more promising than usual. There is successful introduction of remontant varieties of different origin: United States, Japan and Western Europe. Collection of remontant strawberry of the MBG has 40 sorts. A long term study identified such promising sorts as: Sakhalin, Mount Everest, Red Rich, Ozark Beauty, Arapahoe and Mahern. They are characterized by high and stable yields.

The experiment studied the introduction of wild fruit and berry plants: Schisandra (*Schizandra chinensis* (Turcz.) Baill), Actinidia kolomikta (*Actinidia kolomikta* (Maxim.) Maxim.), Sea buckthorn (*Hippophae rhamnoides* L.), Cherry (*Cerasus tomentosa* (Thunb.) Wall.), Loch (*Elaeagnus multiflora* Thunb.), types of dog rose (*Rosa* L.). The recommendations for farming practices and methods of reproduction were studied. Less common, valuable for their biological and agriculture, sorts of sorbus were also tested: Burka, Titan, Ruby, Grenade, Liqueur. They are characterized by high winter hardiness, good quality fruits, which contain a significant amount of ascorbic acid and some vitamins.

Essential new group introduced into the culture are: cranberry, blueberry, honeysuckle, hawthorn, viburnum, elderberry and others.

There were tested about 150 varieties of grapes in the open field and 40 varieties of culture in the wall. For open field, 5 of the earliest crop were selected, 7 varieties of grapes were selected for the wall of culture.

At present, the collection of aromatic plants is represented by over 300 taxa and cultivars. Thus, through the introductory experiment were passed 70 species and forms of coriander, 84 samples of fennel, 80 samples of marjoram, 50 varieties, hybrids and forms of peppermint. Long-term studies have established that the soil-climatic conditions of the nonchernozem zone of Russia is quite suitable to biological characteristics, the rhythm of growth and development of many aromatic plants, providing a sufficiently high yield and productivity, as the biomass and essential oil, sometimes higher than those found in plants grown in traditional conditions.

In the collection of medicinal plants, of Russia, a number of species is represented by several varieties. In total there are 155 species from 41 families, used in scientific and traditional medical practice. The greatest number of species represented families: Asteraceae Dumort., Ranunculaceae Juss. and Solanaceae Juss. Particular attention was paid to collecting and preserving the gene pool of rare and endangered plants.

The collection of 12 such species, including *Arnica montana* L., *Gentiana lutea* L., *Dioscorea caucasica* Lipsky. In recent years, in our country, homeopathy has become increasingly popular. In homeopathy, a much larger range of plants is used than in the formal allopathic medicine. In this regard, in the collection several new types were introduced: *Cnicus benedictus* L., *Cochlearia officinalis* L., *Nasturtium officinale* R. Br., *Echinacea angustifolia* DC, *E. pallida* (Nutt.) Nutt., *Pulsatilla vulgaris* Mill., *Aconitum napellus* L., *Iris germanica* L. and some others.

For study of introduction selected number of species to create new drugs, such as *Primula veris* L., *Onopordum acanthium* L., *Echinacea angustifolia*, *E. pallida*, *Eupatorium perfoliatum* L.

A long-term comparative study of valerian flora of the former Soviet Union was made. Among the most promising for further research the Caucasian species were included: *Valeriana alliariifolia* Adams, *V. tiliifolia* Troitz. and *V. cardamines* Bieb. All three species are characterized by high productivity of groundwater bodies (59.0-235.0 g per 1 plant). The first two types are distinguished by their high content of valepotriates (up to 8.5% by dry weight), and *V. cardamines* – essential oil (up 5.6%).

In 2005, a monograph "Vitamins in vegetables, fruit and berries in plants of Central Russia" [Bucharin, 2005], which sets out general information about vitamins and their biological role in humans, was published. The contents of vitamins C, B_6, (3-carotene, thiamin, riboflavin, niacin were presented. We consider the vitamin value of vegetables, fruits and berries, depending on variety and climatic conditions of growth.

The most promising species and varieties were found out and we gave recommendations for their cultivation in Central Russia.

BIBLIOGRAPHY

1. Gorbunov, U.N. and others. 2011. Cultivated plants of the Main Botanical Garden named after N.V.Tsitsin Russian Academy of sciencies. KMK Scientific press Ltd., Mooscow. 511.

2. Bucharin, P.D., Shabalink, A.M., Burakova, M.U. 2005. Vitamins in vegetables, fruts and berries in plants of Central Russia. Moscow: Science. 144.

VARIETY OF SUCCULENT MONOCARPIC

M. Gaidarzhy, V. Nikitina

INTRODUCTION

At definition of the term «monocarpic phenomenon», the authors, as a rule, do not indicate which part of the plants is monocarpic. As an example, the annual and biennial plants that blossom once during their life and then die are often cited. Sometimes the authors agree that monocarpic may be such perennial plants as «agaves, palm trees and others» [1, 2].There are distinguished monocyclic (annual plants), dicyclic (biennial plants), pleiocyclic (living 3-5 years) and polycyclic (perennial plants) monocarpic plants. In the botanical literature occurs also the term «monocarpic shoot». This term suppose the shoots of perennial plants to die after fruiting, with preservation of the basal part and buds of regeneration, which give rise to new monocarpic shoots [5].

Our observations of the growth and development of succulents showed that among these plants are monocyclic, bicyclic, pleiocyclic and polycyclic monocarpics and also plants with monocarpic shoots. However, the features of behavior of succulent plants with monocarpic shoots partially contradict the definition given in the literature. Thus, our task was to analyze the succulent monocarpics and clarify the term «plant with monocarpic shoots».

MATERIALS AND METHODS

The collection of succulent plants of the O.V. Fomin Botanical Garden counts more than 2500 species and intraspecific taxa of the more than 300 genera and 38 families. The formation of the collection was started over 100 years ago, and a significant volume was achieved in the late 20th - early 21st century after reconstruction of old greenhouses and new construction. More than 75% of the collection species reached the generative period of development. About 400 species grow in the soil of greenhouses in several expositions, dedicated to certain regions and climatic zones. The age of plants in collection varies from 1-2 up to 90-100 years [3].

RESULTS AND DISCUSSIONS

We found that monocarpic succulents are typical for five families: Aizoaceae, Agavaceae, Asphodelaceae, Crassulaceae, Dracaenaceae (table).

Table

Monocarpic phenomenon within the genera and families of succulents

Family	Genus	Type of monocarpic phenomenon	Species (examples)
Agavaceae	*Agave* L.	polycyclic	*A.ferdinandi-regis* Bgr. *A.parviflora* Torr.
		plant with monocarpic shoot	*A.attenuata* Salm *A.celsii* Hook *A.filifera* Salm *A.stricta* Salm
	Furcraea Vent.	plant with monocarpic shoot	*F.cubensis* Vent
Aizoaceae	*Mesembrianthemum* L.emend.L.Bol.	monocyclic	*M crystallinum* L.
Asphodelaceae	*Bulbine* L.	monocyclic	*B. annua* Willd. *B.semibarbata* Haw.
Crassulaceae	*Aeonium* Webb et Berth.	pleiocyclic	*A.nobile* Praeg.
		plant with monocarpic shoot	*A decorum* Webb *A.arboreum* (L.) Webb et Berth.
	Aichryson Webb et Berth.	dicyclic	*A.bollei* Webb
		polycyclic	*A.domesticum* (Praeg.) Bgr.
	Crassula L.	pleiocyclic	*C.barbata* Thunbg.
		plant with monocarpic shoot	*C.alba* Forsk.
	Greenovia Webb et Berth.	pleiocyclic	*G.aurea* (C.Sm.) Webb et Berth.
	Kalanchoe Adans.	dicyclic or pleiocyclic	*K.daigremontoana* Hamet et Perr.
		plant with monocarpic shoot	*K.velutina* Welw.
	Orostachys Fisch.	dicyclic	*O.cartilaginea* Borissova *O.iwarenge* (Makino) Hara
		polycyclic	*O.spinosus* (L.) Bgr. *O.thyrsiflorus* Fisch.
	Sedum L.	monocyclic or dicyclic	*S.album* L.
	Sempervivum L.	dicyclic	*S.tectorum* L. *S.ruthenicum* Schnittsp. et Lehm
		pleiocyclic	*S.reginae-amaliae* Heldr. et Sart.ex Bois.
Dracenaceae	*Sansevieria* Thunbg.	plant with monocarpic shoot	*S.patens* N.E.Brown *S.trifasciata* Prain

The representatives of the genus Agave are perennial rosette plants with more or less reduced and woody stems. They can be characterized as succulent-leaf low shrubs, polycyclic monocarpics or plants with monocarpic shoots. *Agave* enters into a generative period at the age of 10 to 50 years in the natural conditions, and from 15 to 100 years in conditions of culture [4]. Our observations have shown the diversity of the growth characteristics and development of plants in pre-generative period. Such species as *Agave filifera* Salm, *A.attenuata* Salm, *A.sisalana* Perr., *A.angustifolia* Haw. are characterized by an appearance of several lateral shoots in basal part of plants, and *Agave albicans* Jacobi and *A. yuccaefolia* DC. - lateral rosette of replacement in upper part of the mother rosette. For *Agave stricta* Salm and *A. celsii* Hook. is typical an appearance of lateral shoots after flowering in early senile period. It should be noted that in all cases the peduncle appears from the point of growth, senile period lasts no less than one year and in most species in the axils of flower stalk appear «bourbillies» (vegetative buds), that is a good reproduction strategy for such species. Significantly fewer species of the genus *Agave* (about 1/3 of 220 species) are polycyclic monocarpics. Reaching the generative period, such species die, regenerating only through seeds. In condition of culture, we observed the flowering of such species at the age of over 50. In general the monocarpic phenomenon is typical for all representatives of the genera *Agave*, *Furcraea.*

The family Crassulaceae monocarpic plants are more widespread. The representatives of the genus *Aeonium* as of *Agave* may be both the plants with monocarpic shoots and pleiocyclic monocarpics. Most species are plants with monocarpic shoots. The representatives of the genus *Aeonium* are shrubs or low shrubs, stems become woody with age, succulent leaves are arranged in a basal or apical rosette. When plants are entering into the generative period the peduncle is formed on the central rosette which is older than lateral ones. In the following period the peduncles are formed on the lateral rosettes, which are situated below the first. After flowering the rosette dies, at its basis are forming new shoots. The central rosette, which is the oldest, enters in the generative period. If this species has a great number of rosettes, several rosettes that are located below the first enter in the next generative period. It should be noted that new shoots appear under old rosette which finished blossoming. Thus, with the beginning of flowering, a vertical growth of plant stops. They are getting aerial roots that, finally, promote the root age of lower rosettes, formation of more or less large clone and in distribution of this species in nature. A few numbers of species of this genus are pleiocyclic monocarpics. Probably, the ability of *A.nobile* Praeg., *A.tabulaeforme* (Haw.) Webb et Berth. to enter into the generative period at the age of 3, 5 or more years, that is, in the most favorable for the flowering period of time, and large multifloral peduncles give these species the opportunity to survive in the wild nature.

The widest range of monocarpic phenomenon is typical for representatives of the genus *Kalanchoe.* Within the genus are dicyclic, pleiocyclic plants and plants with monocarpic shoots. *Kalanchoe* is terrestrial, rare epiphytic succulent plant with thick stems and leaves. Shrubs or low shrubs, rare dwarf trees. Phyllotaxis - opposite, in some species the leaves are arranged in verticil. To the plants with monocarpic shoots belong: K.*dixoniana* Hamet, *K.velutina* Welw., *K.zimbabwensis* Rendle and some other species. About 50% of the vegetative shoot of these species die after flowering. The renewal occurs due to the buds of regeneration, which are on the remaining part of the shoot. For *K.gastonis-bonnieri* Hamet et Perr. the remaining after flowering part of vegetative shoot does not exceed 2-3 cm.

A small number of species are dicyclic or pleiocyclic monocarpic plants. After flowering the plants die and the renewal is carried out mainly from seeds. A classic example of such plants for the representatives of the genus *Kalanchoe* is *K.daigremontiana* Hamet et Perr. Under the adverse conditions (low temperatures, insufficient illumination) the plants can grow few years not entering in the generative period. After flowering *K.daigremontiana* dies, easy regenerates vegetatively due to the brood buds, which are formed on leaves and inflorescences.

All species of the genus *Sempervivum* are also monocarpic plants. These are leaf succulents, with a short stem and rosette phyllotaxis. Like plants of the genus *Sansevieria*, in pre-generative period they form the lateral shoots on more or less long stolons and die after flowering. Some species, which according to the old nomenclature belonged to the genus Jovibarba (Sempervivum), are pleiocyclic monocarpics.

The genus *Sansevieria* is represented by grassy, succulent-leaf plants with rosette phyllotaxis. Rosette contains from 1-2 up to 30-40 leaves depending on species, often 5-8. With age *Sansevieria* form clumps due to more or less long underground or overground stolons. In pre-generative period in the basal part of the rosette appear one or more stolons. Entering into the generative period from the point of growth appears the peduncle. The senile period lasts for one or more years and is reflected in the progressive death of leaves rosette. In fact, all the species of the genus are plants with monocarpic shoots.

In general, the monocarpic phenomenon of succulents is typical for both plants of class Magnoliopsida and Liliopsida. But this is not so widespread phenomenon. Among 38 families, that are presented in the collection of the Botanical Garden and include succulents - the monocarpic phenomenon is typical only of five of them. Most species of succulents are polycarpic plants and only a small part is represented by monocarpics. Many monocarpics, according to our observations, are pleiocarpic (blossom at the sufficient moisture, appropriate temperature etc.). But most of them can reproduce not only by seeds, but also vegetatively (viviparism, rooting with leaf cuttings). Such adaptations are especially typical for the genus Kalanchoe. Many

plants with monocarpic shoots are also characterized by their ability to additional vegetative reproduction. This is clearly expressed by the representatives of the family Agavaceae. In many species in the absence of fruits in the axils of stems appear bourbillies. Thus, the reproductive strategy in the succulent monocarpics is almost always ambivalent and involves both seed and vegetative reproduction.

In general, out of 14 genera that have monocarpic plants more often occur plants with monocarpic shoots (6 genera); at the second place are dicyclic and pleiocyiclic monocarpics (5 genera) and least of all monocyclic monocarpics. Despite the fact that dicyclic monocarpics are presented in many genera, some monocyclic monocarpics can act as dicyclic, although the number of such species is very small. Such are certain species of *Sedum*, *Orostachys*, *Mesembrianthemum*, *Bulbine*, *Aichryson*, the most closely related species which are polycarpics or polycyclic monocarpics. We can assume that in the process of development of these genera, the individual species were in conditions less favourable to growth and development and thus, their life cycle has been reduced only to one vegetation period. It should be noted that only 6 genera of 14, italics, are presented exclusively with monocarpics plants of different type. They are representatives of the genera *Agave* and Furcraea (Agavaceae), *Sansevieria* (Dracenaceae) and *Aeonium*, *Aichryson* and *Sempervivum* (Crassulaceae). In the composition of other 8 genera are monocarpic and, in most, polycarpic.

Concerning the plants with monocarpic shoots, only for members of the genus *Kalanchoe* we find the ability of resumption from the buds in the basal part of the shoot. The resumption of growth of buds begins after the end of the generative period. In most cases, when preparing for blooming in the basal part of the plants appear substitution shoots that by the time of the generative period may reach the size of the mother rosette. And for some members of the family Crassulaceae (*Aeonium*, *Aichryson*) the buds of regeneration may be located in the central part of the crown of low shrubs or tree.

In connection with our observations, we consider necessary to extend the concept of «plants with monocarpic shoots». The plants with monocarpic shoots are perennial plants, which shoot, aged the generative period, dies after fruiting and the plant resumes due to preservation of the basal part of the shoot with buds of regeneration or lateral shoots, which appeared in pregenerative or postgenerative period.

CONCLUSIONS

Thus, for ecological-morphological groups of plants - succulent is a typical monocarpic phenomenon. The representatives of 14 genera of five families have in their compositions monocarpic plants. These are leaf succulents, often with rosette phyllotaxis. The most widely and variously this phenomenon is represented in the family Crassulaceae, which is a part of the class Magnoliopsida. In the composition

of class Liliopsida the number of species of monocarpic is much larger and the plants with monocarpic shoots are the most typical. We can assume that this phenomenon is inherently connected with the adaptation of certain representatives of the genera and families to less favourable environmental conditions in the process of its development.

We also propose to expand the notion of «a plant with monocarpic shoots» in connection with the observed peculiarities of growth and development of a significant number of representatives of succulent plants.

BIBLIOGRAPHY

1. Biological vocabulary /Біологічний словник / під ред. І.Г.Підоплічка, К.М.Ситника, Р.В. Чаговця. – 1974. – 551с.

2. Biomorphological of the plants / Жмылев П.Ю., Алексеев Ю.Е., Карпухина Е.А., Баландин С.А. Биоморфлогия растений. – 2005. – 256 с.

3. Succulent plants / Гайдаржи М.М., Нікітіна В.В., Баглай К.М. Суккулентні рослини. – 2011. – 175 с.

4. Gaidarzhy M. The monocarpic of plants on the Agavaceae family / Гайдаржи М.М. Монокарпічні рослини родини Agavaceae / Збереження біорізноманфття тропічних та субтропічних рослин. – 2009. – С. 51-54.

5. Vocabulary of the botanical termini /Словарь ботанических терминов/ под ред И.А.Дудки. – 1984. – 308 с.

INFLUENCE OF NECTARIFEROUS PLANTS ON MAINTAINING THE NATURAL BALANCE IN TOMATO FIELD AGROCENOSES

Gladcaia A., Cheptinari V., Nastas T.

Institutul de Genetică, Fiziologie şi Protecţie a Plantelor al AŞM, Chişinău

Abstract. The results of our observation indicate that the sequence of flowering periods of selected nectariferous plants f. *Apiaceae* (*Coriandrum sativum* L.; *Anethum graveolens* L. and *Foeniculum vulgare* Mill) is a precondition for creation of a blossoming conveyer for the purpose of supporting the vital activity of entomophages from early June to late August. The bloom of *A. graveolens* had a greatest impact on attracting of entomophagous f. *Coccinellidae*. Field observations in 2013 indicated that the optimal correlation of the predator-prey (*Coccinellidae: Aphididae*) was registered in June, within the limits of 50 m of the strip of nectariferous plants (1:66,4), which coincided with the period of *A. graveolens* full flowering and the beginning of *C. sativum* flowering. It has been found that the population density of the pest *Heliothis armigera* was higher on the edge of tomato field near the corn fields, while in the vicinity of the strip of nectariferous plants the population density of the pest was significantly reduced.

INTRODUCTION

Mixed cultivation - is an environmental approach to increase biodiversity in agrocenosis of beneficial organisms. Many populations of pests can be controlled by improving the efficiency of local diversity, existing community of natural enemies by changing the concept of environment, called “biological control”. The modern system of plant protection is determined by using the rational capacity of individual cultures. In this connection, there is a growing interest in studying the effect of several crop species cocultivation on population density of the economically significant pests.

Overseeding of nectariferous plants considerably increases the number of useful fauna in agrocenosis. The maximum density of *Coccinellidae* is marked at a distance of 50 m from the edge of the field and shelterbelts [1].

The entomophilous crops should be placed in the form of strips of 250 m wide, alternating with agricultural crops, for increasing the number and effectiveness of entomophages and pollinators, which allows to cancel pesticide treatments against pests and to increase the yield of these crops [2]. A considerable deterrent effect, which reduces the intensity of the cutworm oviposition, is observed after the treatment with an extract of dill. There was obtained a significant antifeedant effect in which the mortality of hatching boll worm caterpillars was the highest (60%, while in the control it was 14.4%) [3]. South American researchers have experimentally proved that the quantity and the variety of predatory arthropods were higher on tomato plants in polyculture with coriander [4].

The aim of our study was the selection of the most effective nectariferous

plants f. *Apiaceae* for attracting entomophages in a tomato field and enhancing the natural defences against pests. Objectives of our investigations were: determining the influence of the nectariferous plants on attracting and accumulation of entomophages; establishing the number of the phytophages on tomato plants; identifying the correlation between the number of phytophagous and entomophages in a tomato field; studying of the impact of the strip of nectariferous plants on the population size of cutworms *H. armigera*.

MATERIAL AND METHOD

The sowing of the nectariferous plants strip (280 m^2), was held on the edge of the field of tomatoes. The total area of the tomato field was 1 ha. Monitoring of the entomophages was conducted according to the standard methods [5]. The species composition of entomophages was determined using «Определителя насекомых европейской части СССР» [6].

The number of phytophages on tomato plants was determined at different distances from nectariferous crop strips. The first was carried out at the field edge and at the strip of nectariferous plants, the next ones - after each 50 m, in 4 replicates. Samplings were carried out weekly. There were placed 12 pheromone traps for determining the spatial distribution of *H. armigera* imago in tomato field by the randomized method. As a result, the scheme of the spatial distribution of *H.armigera* imago for the period from May 27 to September 3, 2013 was constructed by using the program "BioClass".

Phenological observations for the development and flowering nectariferous crops were carried out by counting the number of plants on the account areas. 10% of flowering blossom plants in the crop were accepted as early blossoming; more than 80% of the plants were accepted for mass blossoming; 10% of bloomed plants were accepted as the end of blossoming.

There were carried out the insects' samplings while determining of the nectariferous plants agro-ecological singularities. The degree of occupation of shoots by aphids was determined by viewing the shoots front of a white paper. The density of aphids per 1 m^2 was determined by the density of plants in crops. Samplings were carried out weekly.

RESULTS AND DISCUSSIONS

There were used plants f. *Apiaceae* in the experiment, which combined several factors for attracting of entomophages, i.e.:

a) different blossoming time, for the nutrition time of entomophages extending;

b) presence of essential substances, which deter pests;

c) the economic value of nectariferous plants themselves.

There was held soil preparation and nectariferous plants *f. Apiaceae* - coriander (*Coriandrum sativum* L.); dill (*Anethum graveolens* L.), fennel (*Foeniculum vulgare* Mill.) - were seeded as strip along the edge of a field of tomatoes according to the standard agricultural technology. It has been found out that the sequence of periods of nectariferous cultures flowering was a precondition for the creation of a blossoming conveyor which supported the vital activity of entomophages from early June to late August (Table 1).

Table 1

Blossoming conveyor of nectariferous plants f. *Apiaceae*

Plant species	June			July			August		
	ten-day period			ten-day period			ten-day period		
	1	2	3	1	2	3	1	2	3
Anethum graveolens L.	*	*	*	*	-	-	-	-	-
Coriandrum sativum L.	-	-	*	*	*	-	-	-	-
Foeniculum vulgare Mill.	-	-	-	-	-	*	*	*	*

Legend: - vegetation; * flowering.

The ratio of phytophages (*Aphidoidea*) and entomophages f. *Coccinellidae* in a tomato field reflected in the table 2.

Table 2

Correlation between the *Aphidoidea* and *Coccinellidae* number on the field of tomatoes according to the distance …. from the strip of nectariferous plants

denotation	Correlation between the number of Aphidoidea and Coccinellidae on the average individuals/m^3								
	50 m away from the strip of nectariferous plants			100 m away from the strip of nectariferous plants			150 m away from the strip of nectariferous plants		
	June	July	August	June	July	August	June	July	August
f. *Aphidoidea*	79,7	78,1	361,2	127,5	111,8	555,1	182,7	150,1	513,5
f. *Coccinellidae*	1,2	0,5	0,4	0,8	0,3	0,1	0,3	0,1	0
Coccinellidae : *Aphidoidea*	1: 66,4	1 : 156,2	1: 903	1: 159,3	1 : 372,7	1 : 5551	1: 609,1	1: 1051	0

The population of aphids on tomato field was distributed unevenly during vegetation and flowering periods of nectariferous plants. Thus, the size of the aphid population was minimal at a distance of 50-80 m away from the strip of nectariferous plants. Entomophages are not able to exist fully in agroecosystems without nectariferous flora, as well as they cannot do without their hosts and victims. Close

positive correlation between the presence in the fields of entomophilic cultures and effectiveness of pests entomophages on vegetable crops is observed in the publications of Asyakin [7]. According to these data the high concentration of entomophages is created in crops of entomophilous plants, and their larvae significantly reduce the number of pests. Nutrition of entomophages females on plants-nectariferous promotes maturation of sexual products, which is a prerequisite for the realization of their high potential for fertility and efficiency. The mass reproduction of aphids doesn't occur, when the ratio of predator and prey in the colonies of aphids constitutes 1:40 and 1:60. We have fixed the optimal ratio of predator-prey in June, within 50 meters of strip of nectariferous plants (1:66,4) in our observations. This coincides with the period of full flowering of dill and the beginning of flowering of coriander (table 2).

For identifying of outbreaks distribution the spatial - temporal model of pests distribution in the tomato field was constructed with the help of the programme BioClass according to the results of trapping of *H. armigera* males on the pheromone traps (Figure 2).

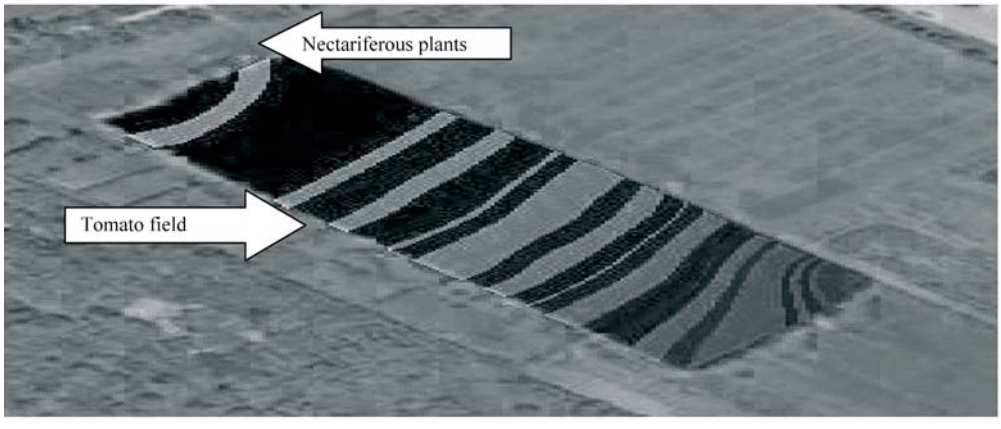

Figure 2. The spatial - temporal model of pests distribution in tomato field, constructed with the help of programme BioClass.

Outbreaks of distribution of the *H. armigera* pest in the space of tomato field during the development of the second generation are marked on the map. Green colour indicates the absence of the pest, but different intensity of the yellow colour indicates areas of cutworm accumulation.

Thus, according to the above figure, the smallest trapping of males in pheromone traps (140 males) was observed in close proximity to the strip of nectariferous plants (10 m), in contrast to the opposite edge, adjacent to the corn field, where catch was at in 2 times more (254) .

CONCLUSIONS

1. The results of our observation indicate that the sequence of flowering periods of selected nectariferous plants fam. *Apiaceae* (*Coriandrum sativum L.; Anethum graveolens L. and Foeniculum vulgare Mill*) is a precondition for the creation of a blossoming conveyer for the purpose of supporting the vital activity of entomophages from early June to late August. The bloom of *A. graveolens* had the greatest impact on attracting of entomophagous f. *Coccinellidae.*

2 Field observations of the 2013 year indicated that the optimal correlation of the predator-prey *(Coccinellidae: Aphididae)* was registered in June, within the limits of 50 m of the strip of nectariferous plants (1:66,4), which coincided with the period of full flowering of *A. graveolens* and the beginning of flowering *C. sativum.*

3. It has been found out that the population density of the pest *Heliothis armigera* was higher on the edge of tomato field near the corn fields, while in the vicinity of the strip of nectariferous plants the population density of the pest was significantly reduced.

BIBLIOGRAPHY

1. Мохрин А.А., Видовой состав и эколого-биоценотические связи кокцинеллид в агробиоценозах ставропольской возвышенности, автореферат диссертации, Санкт-Петербург, Пушкин, 2009 г.

2. Чаплыгин М.П. Эколого-биоценотическое значение посевов энтомофильных культур в фитосанитарной стабилизации агроландшафтов. Диссертация, Краснодар, 2006 г.

3. Исмаил Абдель-Хале, Применение экстрактов растений для борьбы с хлопковой совкой, ВНИИ защиты растений, автореферат диссертации, Санкт-Петербург, Пушкин,1991.

4. Мария Медейрус I, III, Эдисон R Sujii II; Елены C Мораиш III); Efeito da diversificação de Plantas HC abundância da traça-Do-tomateiro predadores et Dois Sistemas de cultivo, III departamente de ecologia, Brasilia-DF, morais@unb.br

5. Îndrumări metodice pentru testarea produselor chimice şi biologice de protecție a plantelor de dăunători, boli şi buruieni în Republica Moldova" Sub redacția generală a doctorului în ştiințe Ion Lazari. (Chişinău, 2002).

6. Заславский В.А. Сем. *Coccinellidae* – Божьи коровки// Определитель насекомых Европейской части СССР. Т. 2. Жесткокрылые и веерокрылые. – М.-Л.: Наука, 1965. – С. 319-326.

7. Асякин, Б. П. Растения нектароносы в биологической защите овощных культур от вредителей / Б. П. Асякин, Jl. П. Красавина, Н. А. Белякова. — СПб, 2003.-44 с.

INDOOR PLANTS CULTIVATED IN BOTANICAL GARDEN IASSY USED IN TRADITIONAL MEDICINE

Ifrim C.

„Alexandru Ioan Cuza" University of Iaşi - Romania, „Anastasie Fătu" Botanical Garden

Abstract: The botanical gardens are institutions that initially have had at their basis collections of medicinal plants. The Botanical Garden from Iaşi has a collection of exotic plants used in the traditional medicine with a number of 97 taxa belonging to a number of 51 botanical families. The symptoms for which are used are quite various and different in the areas where the plants are cultivated as compared to the plants from their original areas. These plants represent a valuable didactic material for the researchers in the area, for the botanists, students in biology and pharmacy. A number of taxa present a special conservative value and are protected by a series of official documents of national or international importance.

Key words: greenhouses plants, traditional medicine, Botanical Garden Iassy

Rezumat: Grădinile botanice sunt instituţii care iniţial au avut ca punct de plecare colecţii de plante medicinale. Grădina Botanică din Iaşi deţine o colecţie de plante exotice utilizate în medicina tradiţională ce numără 97 de taxoni ce aparţin la 51 de familii botanice. Simptomele care sunt tratate sunt foarte variate şi diferite în zonele în care plantele se cultivă faţă de zonele de origine. Aceste plante reprezintă un material didactic valoros pentru cercetătorii în domeniu, pentru botanişti, studenţi biologi şi farmacişti. Un număr de taxoni prezintă valoare conservativă deosebită şi sunt protejaţi prin diferite documente oficiale de importanţă naţională sau internaţională.

Cuvinte cheie: plante de seră, medicină tradiţională, categorii sozologice, Grădina Botanică Iaşi

INTRODUCTION

The botanical gardens are cultivating diverse plant collections, but from a historical point of view the collections of medicinal plants represent the cradle of all similar institutions. There was permanently a connection between the study of the plants from the botanical gardens and the research activity from the medical and the pharmaceutical area [1]. The founder of the Botanical Garden from Iaşi, Anastasie Fătu, was a doctor and a naturalist and had understood in that period (1856) the importance of such a specific space destined for the studious young people.

The plants with medicinal use represent a very valuable and accessible material base for the specialists and the students from the pharmaceutical domain. Each plant from this category represents a strategic reserve which can prove to be useful in any moment; thus its improvement with diverse species, well-informed and accurately identified, is a permanent preoccupation. For the wide public the gardens must adopt an adequate presentation of this collection, promoting the knowledge and the interest for the medicinal plants aiming for a better understanding of the relations man-nature.

Thus the botanical garden can become a local leader in the discovery, explanation and dissemination of all information about the diverse and dynamic relations between the population and the plants all over the world. The effective use of this collection for the preservation depends by the implementation of a series of professional standards involving continuous activities for documentation and genetic management.

MATERIALS AND METHODS

The material is represented by the plant collections from the Greenhouse Complex of the Botanical Garden „Anastasie Fătu" from Iaşi. These collections comprise in total approximately 2000 taxa, originating from the Mediterranean, tropical and Equatorial areas from the world. According to the information of the specific literature there were identified 97 taxa used in the traditional medicine. The botanical classification is due to the current works in the field and the medicinal use of the plants is drawn from the classical and the most recent works [9, 10, 11, 13, 16].

Some of the identified species have a high preservative value, being mentioned on the International Red List, The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 1973) or any other documents on national interest concerning the protection of the biodiversity [8, 12, 15]. The sozological mentioned categories are according to the documents published by IUCN (International Union for Conservation of Nature 2003).

RESULTS AND DISCUSSIONS

The plants were used for treatments since the Middle Paleolithic, approximately 60,000 years ago, but real data regarding the traditional medicine (ethno-medicine) can be discovered in the documents reminding about their usage. The medicinal plants were traded for centuries and this sector is developing very fast, growing with 12-15% per year in the very developed countries. During the colonization of the Africa, the traditional medicine was intentionally suppressed or restricted in almost all countries of the black continent [6], the western medicine being a part of the civilized colonial mission. The same happened in India with the ayurvedic medicine.

The demand for medicinal plants is continuously growing in the industrial and developed countries due to an increase of their population and to an incessant awareness for the benefits of the natural products as an effective, economic and sure alternative to the industrial synthesized medicines with high costs. It is considered that the tropics are a unique source of chemicals with potential in obtaining modern medicines and the future investigation upon the species of the tropical forest can provide solutions for different diseases for which there are not yet discovered satisfying treatments.

In general the plants with medicinal usage are exposed in the botanical gardens outdoors, amidst the group of useful plants, situation encountered also in the Botanical

Garden from Iaşi. Most often are emphasized the species recognized as valuable after comprehensive pharmacological investigations, but here are also numerous other plants used in the traditional medicine from different areas of the world, which are potentially new sources of drugs [3].

The taxa cultivated in the greenhouses of the Botanical Garden from Iaşi with applications in the traditional medicine are originating from different regions of the world and belong to a number of 51 botanical families. Most of the species are angiosperms (78%), and only 4 taxa belong to the group of pteridophytes (*Adiantum capillus-veneris* L., *Asplenium nidus* L., *Cyrtomium falcatum* (L. fil.) C. Presl, *Pteris cretica* L.), and 4 taxa are gymnosperms (*Cycas circinalis* L., *Cycas revoluta* Thunb., *Stangeria eriopus* (Kunze) Baill., *Cupressus sempervirens* L.). Among the angiosperm the family with the most numerous representatives is Liliaceae - 10 taxa, followed, in decreased order by Rutaceae - 5 taxa, Araceae, Crassulaceae, Moraceae - 4 taxa, Amarillydaceae, Apocynaceae, Fabaceae, Geraniaceae, Lauraceae - 3 taxa, Cycadaceae, Acanthaceae, Agavaceae, Bromeliaceae, Euphorbiaceae, Lamiaceae, Malvaceae, Myrsinaceae, Myrtaceae, Poaceae, Rosaceae, Rubiaceae, Urticaceae - 2 taxa, most of the families being represented by only one taxa: Anacardiaceae, Arecaceae, Begoniaceae, Berberidaceae, Cactaceae, Casuarinaceae, Commelinaceae, Droseraceae, Ericaceae, Lythraceae, Malpighiaceae, Marantaceae, Oleaceae, Passifloraceae, Piperaceae, Plumbaginaceae, Punicaceae, Sarraceniaceae, Saururaceae, Solanaceae, Vitaceae, Zingiberaceae. According to the provenance area, most of species are originated from Asia (29%), from Africa are coming 24%, and from the Central and South America 18%. A reduced number of species have a Mediterranean dissemination (10), and 6 of them are distributed in the Eastern area of Asia up to Australia. *Drosera rotundifolia* L. are widely spread circumboreal species.

The plant parts used as remedies are the roots, the rhizomes, the bulbs, the bark, the stem and/or the leaves sap, fruits or even the whole plant (*Adiantum capillus-veneris, Drosera rotundifolia, Houttuynia cordata, Kalanchoe laciniata [5]*, *Pilea microphylla, Xanthosoma violaceum*). Much rarely are used the inflorescences or the solitary flowers (ex. *Ananas comosus, Cordyline terminalis*, *Ficus religiosa, Gardenia jasminoides, Phoenix dactylifera*) or the ovules (*Cycas revoluta*). The plants are used fresh or dry, single or together with parts from other plants. It is well known by the ecologists the fact that the medicinal plants collected for their roots or bark are the most endangered due to their over-exploitation.

The symptoms treated by using plants are numerous, from simple colds, coughs, headaches or toothaches, inflammations to some more severe illnesses such as cancer, diabetes, dysentery, tuberculosis. There are numerous remedies specific to the areas with critical economic development: plants used against the snake bites (*Clivia miniata, Gloriosa superba, Ficus religiosa, Mimosa pudica*), elephantiasis (*Asplenium nidus, Coleus blumei*), replacement for the mouthwash (*Barleria prionitis*), pre/postpartum

impairment (*Agapanthus africanus, Bowiea volubilis, Citrus sinensis, Clivia miniata, Oryza sativa*).

Most of the species have numerous applications, sometimes extremely diverse. The lemon (*Citrus limon*) is a relevant example: the leaves, bark and its fruits are used for the treatment of the troubles of the following apparatus: respiratory, digestive and cardiovascular. It is interesting to observe the completely different way of employment of the same plant in different areas, in the original area as opposed to the area in which it is cultivated. Thus from the plant *Mangifera indica* from Bangladesh the people are using the seeds in the treatment for the diabetes; in Zimbabwe the bark is used for cases of diarrhea and dysentery; in Tanzania against the toothaches and in Brazil the leaves are used for several respiratory troubles. The proceedings in the usage of the plants are diverse, from the preparation to the infusion, decoct, cataplasms and to chewing the fragments for a therapeutic effect or inhaling the smoke emanated from the burned leaves of *Asplenium nidus* or the vapors generated after the burning of some plant parts.

In Romania [2], from almost 100 registered species, there were cultivated along time quite a few (approximately 15), most of them with Mediterranean origin [7]. Some plants are recognized as ornamental species (*Begonia rex)*, other as condiments (*Laurus nobilis, Rosmarinus officinalis*) or food plants (*Citrus reticulata*).

There is a real rebirth, currently, for the reintroduction of the practices and the materials used in a traditional way with ecological and economical consequences. There is a real pressure on the ethno-botanists as for the salvation of the traditional knowledge, but also for the education of the younger generations about the plants importance and their cultivation with reference to the urbanization degree. The actual demand for medicinal plants has a growing tendency with real consequences upon the environment, especially if the savage material is not supplied with cultivated matter. In economically disadvantaged areas the trade with medicinal plants is the only source of income, and one of the most eloquent examples is South Africa. The statistics are showing that for the trade purpose there are collected over 700 species. After an attentive monitoring activity of the species *Clivia miniata* (Lindl.) Bosse, *Haworthia limifolia* Marloth and *Bowiea volubilis* Harv. it was determined a decrease of the populations with almost 40%, among the main causes being the collection for medicinal purposes, the decline period being 100 years, respectively 60 and 30 years. All the three taxa are mentioned on the Red List of South East Africa in the category vulnerable (VU), *Begonia dregei* Otto & A. Dietr. in the category endangered species (EN), and *Aloe ferox* Mill**.,** *Crassula multicava* Lem. subsp. *multicava*, *Haemanthus albiflos* Jacq. and *Myrsine africana* L. in the category least concern (LC).

Some of the collected species are very important as they are mentioned in the official documents concerning the preservations of the biodiversity, and some 15 of them are specifically recommended by the BGCI [5] to be monitored for the next 5

years: *Adianthum capillus-veneris* L., *Bowiea volubilis* Harv., *Catharanthus roseus* (L.) G. Don, *Cinnamomum spp*. (*C. camphora* (L.) Sieb.), *Drosera rotundifolia* L., *Gloriosa superba* L., *Haworthia limifolia* Marloth, *Laurus nobilis* L., *Myrsine africana* L., *Obregonia denegrii* Frič, *Pelargonium spp.* (*P. radens* H. E. Moore, *P. zonale* (L.) L'Hér. ex Aiton), *Ruscus aculeatus* L., *Stapelia gigantea* N. E. Br. On the Red List IUCN we can discover *Cycas circinalis* L. – endangered (EN), *Cycas revoluta* Thunb. - least concern (LC) and *Stangeria eriopus* (Kunze) Baill. - vulnerable (VU). *Aloe ferox*, *Bowiea volubilis*, *Obregonia denegrii* and *Euphorbia tirucallii* are present in the CITES annexes. The species registered in one of the sozological mentioned categories have a decrease in population due to an abusive collection from the nature for the purpose of their exploitation and/or because of the destruction of their natural habitats.

The plants of the collection are constantly observed; there are studied the peculiarities of their growth and the development and reproduction during the period of their vegetation. As for the capacity of propagation, there is the species self-spreader (*Adiantum capillus-veneris*), most of the plants are successfully propagated by means of seeds or on the vegetative way, but some taxa require special care. The representatives of the families Cycadaceae, Zamiaceae and Arecaceae are quite difficult to be multiplied, either due to their biological particularities, or due to the fact that they do not form seeds or the seeds do not germinate.

There are numerous international projects following the sustainable exploitation of the medicinal plants and of their traditional knowledge and all these missions are mentioning the important role of the botanical gardens. These institutions represent the ideal place for the knowledge dissemination towards the public [14], being the place where can be completed studies concerning the germination and the adequate agro-cultural conditions for a widespread cultivation, as well as for other activities that can lead finally to a pressure decrease from the savage plant populations. By the international exchanges of botanic material made annually with approximately 500 similar institutions, the Botanical Garden from Iaşi is aiming to obtain new taxa which can enrich the existing collections. All these activities are registered in the conservation effort "ex situ", of the biodiversity in general.

CONCLUSIONS

The Botanical Garden from Iaşi is housing in its greenhouses 87 species of plants used in the traditional medicine, from very divergent areas of the world. Some of these plants are threatened due to the massive collection, therefore are protected by national or international regulations. Within the conservation programs "ex situ" there are continuously monitoring the evolution of these taxa in protected conditions, there are performed different studies upon the germination and also as regards to the agro-techniques aiming to a widespread cultivation.

BIBLIOGRAPHY

1. Beffa, M. T. Della, Cravotto G. Officinal plants in botanical gardens: from hortus simplicium to the research of new medicines. *În* Anca Sârbu (coord.). *Diversitatea plantelor în contextul strategiei europene de conservare a biodiversității. Sinteze*. Edit. "alo, Bucureşti!" , 2001, pp. 166-169.

2. Butură V. Enciclopedie de etnobotanică românească. Edit. Ştiinț. şi Enciclop., Bucureşti, 1979, 271 pp.

3. Fabricant D. S., N. R. FARNSWORTH. The Value of Plants Used in Traditional Medicine for Drug Discovery. Environ. Health Perspect., 2001, **109**(suppl 1):69–75.

4. Hawkins, B. Plants for life: Medicinal plant conservation and botanic gardens. Botanic Gardens Conservation International, Richmond, U.K., 2008, 50 pp.

5. Jhuma D., G. K. Dash. *Kalanchoe laciniata* (L.) DC: A lesser known indian medicinal plant. IJSIT, 2013, **2**(2): 158-162.

6. Owuor B. O., Kamoga D., Kung/u J., Njoroge G. N. *Some medicinal trees and shrubs of eastern Africa for sustainable utilisation and commercialisation*. 2006.

7. Roche, de la, F. A. Les plantes bienfaisantes. Gautier et Languereau Ed., 1924, pp. 377.

8. Sanbi (South African National Biodiversity Institute). *Red List of South African Plants* version 2012.1.

9. Schmelzer, G., H. & Gurib-Fakim, A.. Plantes medicinales. PROTA, 2000.

10. Tilford G. L. *Edible and Medicinal Plants of the West*, Mountain Press Publishing Company, 1997*,* 239 pp.

11. Vandebroek I. & all. The importance of *botellas* and other plant mixtures in Dominican traditional medicine. J Ethnopharmacol., 2010, **128**(1): 20–41.

12. Walter K. S., Gillet J. H. (eds.). 1998. *IUCN Red List of Threatened Plants*, Compiled by the World Conservation Monitoring Centre. IUCN - The World Conservation Union, Gland and Cambridge.

13. Wiart C. *Medicinal Plants of Asia and the Pacific,*CRC Press, 2006.*,* 288 pp.

14. Wyse Jackson, P.S. & Sutherland, L.A. *International Agenda for Botanic Gardens in Conservation.* Botanic Gardens Conservation International, U.K., 2000.

15. *** Convention on International Trade in Endangered Species of Wild Fauna and Flora. Washington, D.C., 1973 and Bonn, 1979.

16. *** Plants For A Future, http://www.pfaf.org

USING VIRUS TO RESTORE AND CONSTRUCT STABLE FOREST ECOSYSTEM FOR CONTROL OF THE SPECIES *HYPHANTRIA CUNEA DRURY*

Stingaci Aurelia

The Institute of Genetics, Physiology and Plant Protection
Academy of Sciences of Republic Moldova
e-mail:aurelia.stingaci@gmail.com

Abstract. Using the viruses to control sustainably the species *H. cunea*, which can damage forest and decorative plants, was reviewed in the paper. And the status of forest pest insects and bio-control technique in Republic of Moldova were reintroduced. The criterion of stable ecological system and the methods of restoration and construction were also analyzed. Meanwhile, the experiment in which species of insect viruses were used and successful control of the species *H.cunea* persistently indicate that the insect viruses play an important role in restoring and constructing stable forest ecological system.

Key words: *Hyphantria cunea*, forest ecological system, biological control, baculoviral preparation, VG, VPN

INTRODUCTION

Numerous investigations on the development of biopesticides have been initiated as legislation and government policy have demanded less reliance on chemical pesticides and greater adoption of biological products. In Europe, some countries have set goals of reducing pesticide use by 50%. Public demand for safer, environmentally-benign alternatives to synthetic chemical pesticides and more stringent barriers applied by regulatory agencies worldwide has led to increased interest in microbial pest control agents (MPCA) based on viruses, bacteria, fungi, protozoa and nematodes as the active ingredient. The MPCA market has recently experienced an increase of 47% between 2004/2007 with sales worth $396 million (CPL Consultants, 2010). Despite this increase, the microbial biopesticide market still only represents about 1% of the sales of chemical pesticides (CPL Consultants, 2010; Marrone, 2007). Factors impeding the establishment of strong MPCA markets are complex (Chandler et al., 2011; Ravensberg, 2011) but include the burdensome costs associated with the registration of commercial products that are aimed at relatively small niche markets (Chandler et al., 2011; Ehlers, 2011).

Therefore, the forest pest control in Moldova in the 21st century must follow the sustainable forest protection strategy. The forest pest control technology which is reasonable, based on ecological principle, and harmonious with the environmental protection is very important to implement the strategy.

The present paper gives information of baculovirus preparations; we discuss

how baculovirus evolution, host range determination and pathogenesis have contributed to their inherent safety for non-target organisms including humans. The virus also can accumulate itself in the environment and the host population, and it can control the pest insects for a long term by forming epidemic disease in the pest insect population through the external environment stimulation. The quite stable food chain relation of plant-pest insect-natural enemy can be gradually established. Thus it can reduce the risk of the pest insect continuous outbreak and realize the persistent control of pest insects.

MATERIALS AND METHODS

The researches have been realised on the caterpillars of 2-3 ages of the *H. cunea.* In the study, we used the Nuclear Polyhedrosis Virus, selected and identified in the laboratory of the insect viruses.

For the contamination of the laboratory insects, we used the dosed feeding, which contains 10 polyhedrons for each caterpillar. The monitoring of the insects lot and the estimation of the dead caterpillars has been carried out daily, beginning with the 3rd day of the contamination. The caterpillars *H. cunea* were kept under laboratory conditions at 27˚C.

For infection of larvae there was necessary a preliminary preparation of viral suspensions, using for that purpose pure or initial suspensions and applying dosed infection of insects according to the Vago C. procedure (1972) and its different modifications (Chukhrii et al., 1990, 1991).

During the process of identification and determination of biological activity of baculoviruses there was necessary its purification. At initial phases purification of VPN and VG does not differ substantially. Dead larvae were soaked with the help of a mixer, and the biological mass was mixed with sterile filtered bidistilate through an apron screen.

For purification of VPN were used several methods, for which we have used the modifications of our institute, consisting of the following phases. Filtered viral suspension is centrifuged within 30 min at 1000 rpm in ȚLN-2 centrifuge. The obtained deposition is washed three times with water. The obtained suspension is centrifuged in the gradient of sucrose concentration (70-20%) and is centrifuged at 3000 rpm within 10 min. Zones with concentration of 40-50% were put together and layered in the gradient of 50-60% and after 15 min of centrifugation there was obtained the fraction of SPVC.

For determination of concentration of baculoviral suspensions there were used different methods, especially electronic microscope (Chukhrii et al., 1990). Titration of baculoviruses with the help of quantum microscopy depends on the kind of virus. Thus, if VPN may be examined with all kinds of optical microscopes, because they have relatively big size (0,5-10 mcm), then VG having much smaller size (0,01-0,5

mcm), is at the edge of optical microscope resolution, that's why they were mostly treated with the help of electronic microscopes.

For the determination of baculoviral concentration there are used different methods, especially of electronic and optical microscopy (Chukhrii et al., 1990). Titration is carried out with the help of Goreaiev chamber or in the fixed and colored preparations. There were elaborated different methods of determination of biological activity of baculoviruses. At the initial phase viral suspension is titrated, determining its concentration. Then there is prepared a series of successive dilutions with the help of which are infected larvae of the second age (it is rational to use 40 larvae of the same physiological state). After the third day there is determined the mortality of larvae by options, and is being prepared the diagram of "dose-effect" relation. For that reason there is applied the method of sample analysis. Then some additional calculations are made, which allow transformation of axis for obtaining of the "dose-effect" relation in the form of straight line and not in the form of asymmetrical curve. Construction of diagram allows us to determine the logarithm of the viral suspension dose, which ensures the death of 50% of the experimental larvae. Knowing the virus concentration and volume of viral suspension it is easy to determine lethal concentration (CL_{50}).

The mathematical treatment was registered on the 15th day after contamination; the statistical treatment was made according to Dospehov, 1985, Gar. 1963

RESULTS AND DISCUSSIONS

The baculovirus can accumulate itself in the environment and the host population, and it can control the pest insects for a long term by forming epidemic disease in the pest insect population through the external environment stimulation. The quite stable food chain relation of plant-pest insect-natural enemy can be gradually established. Thus it can reduce the risk of the pest insect continuous outbreak and realize the persistent control of pest insects. Thus, there were registered substantial results at the examination of biological activity of viral biological mass obtained from larvae which died on different days after infection with viral suspension (Table 1).

Results placed in the above table show the difference between the parameters of biological activity of biological mass obtained on the different days from the infection with baculoviruses. There are not noticed any substantial differences of biological activity in the case of viral suspension with the same concentration (10^7 pol./ml). Good results were registered at the analysis of lethal time necessary for obtaining a death rate of 50% of larvae (TL_{50}). That parameter has minimal value in the first 5 days from infection. In the terms of that aspect, biological mass obtained from dead larvae after these days is characterized by parameters specific to wild strains obtained from natural conditions, that aspect induces the difference of biological activity of biological mass obtained from dead larvae on different days of infection and denotes the possibility of application of that measure in the process of improving baculoviral

strains applied for elaboration of viral insecticides (Voloşciuc, 2009). Other authors also have confirmed the results of the investigations in that field, (Adams et al., 1991; Denoth et al, 2002; Ilienih, 2007).

Table 1

Biological activity of viral suspension obtained from dead larvae on different days from infection with baculoviruses.

Options	Concentration, Pol./ml	Number of larvae	Biological activity, %	TL_{50}, days
Day 3	10^7	100	91.4	5.3 ± 0.57
Day 4	10^7	100	9.8	5.0 ± 0.41
Day 5	10^7	100	96.4	7.8 ± 0.75
Day 6	10^7	100	92.4	5.6 ± 0.63
Day 7	10^7	100	100.0	6.7 ± 0.72
Day 8	10^7	100	93.5	6.6 ± 0.65
Day 9	10^7	100	97.2	5.5 ± 0.52
Day 10	10^7	100	98.3	7.9 ± 0.73

Biological efficacy combat emerges more obviously, if comparing the situation of deposition density of the surface treated with the witness. This comparison results in the conclusion that the treated surface virologic deposition of the low density of about 151 times, whereas in the control it was increased approximately 4 times. The researches point out *H. cunea* the critical stage and will be very useful for the baculovirus treatments management. The results of the experiments are presented in the table 2.

Table 2

The effectiveness of the biological treatment with virus preparation after egg deposition density

Plant species	No. Trees review	Nr.oviposition		Biologic efficacy of treatments
		before treatments	aftes treatments	
Oak (*Quercus robur*)	50	0,51	-	100,0
Maple (*Acer negundo* L)	60	3,73	0,1	97,31
European privet (*Ligustrum vulgare*)	35	4,50	-	100,0
Common walnut (*Juglans regia* L)	50	5,01	0,05	99,00
Field Elm (*Ulmus campestris*)	40	2,10	-	100,0
Lime (*Tilia cordata*)	30	2,34	-	100,0
Media	-	3,03	0,02	99,33
Control	100	0,5	2,1	-

Analysing the biological effectiveness of the baculoviruses we noticed a value of 97-100%. Baculoviruses were used to prepare Virin ABB-3. The material was used to prepare the biological preparation Virin-ABB-3.

CONCLUSIONS

The insect virus pesticide is only one method to construct and restore the stable ecosystem, other methods can also be applied as the effective methods as long as they are useful to the control of target pest insects and do not cause destruction of the environment.

From the results it can be concluded that this method of fighting, in addition doses viral preparation, special importance has choosing the most favorable treatment times, about larval development and overgrowth of foliage trees. Treatments should be applied only during the period when larvae are the first two age and leafy trees. The method put forward in this paper is the preliminary result of the experiments, within the framework of which, we have been trying to control the foliage feeding insects for many years. And this method, taking the virus as the main measure to restore and construct the stable ecosystem, were the pest insects had occurred.

BIBIOGRAPHY

1. Adams J & Bonami J. R. 1991. *Atlas of invertebrate Viruses.* Edit: CRC Press, 1991., Boca Raton FL. 480 pp.

2. CPL Business Consultants. *The 2010 Worldwide Biopesticides Market Summary*, 2010. Volume 1, June 5, 2010 - Pub ID: BGEQ2703518. MarketResearch.com, Rockville, MD, USA.

3. Ciuhri M., Volosciuc L. T, CatanaV. D, Mencer E. M. *Sistema metodov identificatii virusov iadernogo poliedroza*. Edit. Ştiința. Chisinev: 1990, 44 pp. [In Russian].

4. Chandler, D., Bailey, A.S., Tatchell, G.M., Davidson, G., Greaves, J., & Grant, W.P. *The development, regulation and use of biopesticides for integrated pest management*. Philosophical Transactions of the Royal Society of London. Series B, Biological, 366 : 1987-1998.

5. Denoth M., Frid L., Judith H. Mzers. 2002. *Multiple agents in biological control: improving the odds?*. J.Biological Control. Edit: Elsevier Ltd, London UK. **24**: 20-30.

6. Ehlers, R.-U. *Regulation of biological control agents and the EU policy support action REBECA*. In: *Regulation of Biological Control Agents,* R.-U. Ehlers (Ed.), 2011, pp. 3-23, Springer Dordrecht, ISBN 978-90-481-3663-6, Heidelberg, Germany

7. Marrone, P.G. *Barriers to adoption of biological control agents and biological pesticides.* CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 2007 2, No. 051. doi: 10.1079/PAVSNNR20072051 ISSN 1749-8848.

8. Ravensberg, W.J.. *Progress in Biological Control 10. A Roadmap to the Successful Development and Commercialization of Microbial Pest Control Products for the Control of Arthropods*. 2011, ISBN 978-94-007-0436-7, Springer Science + Business Media B.V.

9. Voloşciuc L. T. *Biotehnologia producerii şi aplicării preparatelor baculovirale în protecția plantelor*. Mediul ambiant. Edit . 2009, Î.E.P. Ştiința.Chişinău.: 262.

10. Volosciuc L. T.. *Problemele identificării şi ameliorării baculovirusurilor*. Buletinul AŞM, Ştiințele vieții. 2010, **1**: 96.

BIOLOGICAL PECULIARITIES AND NUTRITIONAL VALUE OF *ASTRAGALUS GALEGIFORMIS* L. AND *GALEGA ORIENTALIS* LAM. SPECIES IN MOLDOVA

Teleuță Alexandru [1], Țîței Victor [1], Coşman Sergiu [2]

[1]Botanical Garden (Institute) of the Academy of Sciences of Moldova, Chişinău, 18 Padurii str., MD 2002 Republic Moldova.
Phone: +373.22.52.38.98 Fax: + 373.22.55.04.43
[2]Institute of Biotechnology in Animal Husbandry and Veterinary Medicine of M.A.F.I., Chişinău, Maximovca,
MD 6525 Republic Moldova Phone: +373.22.35.92.95
Corresponding author email: director@gb.asm.md

Abstract. We investigated the biological peculiarities, chemical composition and nutritional value of the new perennial leguminous plants native to Caucasus: *Astragalus galegiformis* L. and *Galega orientalis* Lam. in Moldova's conditions. *Medicago sativa* L. was used as control. *Astragalus galegiformis* and *Galega orientalis* achieves at the first harvest, a productivity of 6.00 kg/m^2 – 7.12 kg/m^2 natural forage, second harvest – 1.68 - 2.04 kg/m^2 natural forage. The natural forage of this species is characterized by a high content of raw protein, fat, nitrogen-free extractive substances and lower content of cellulose and minerals; contains a normal amount of protein which corresponds to the zootechnical standards, so, to a nutritional unit, correspond 127 - 147 grams of digestible protein and the highest content is found in the forage at the species *Galega orientalis*.

Keywords: biological peculiarities, fodder leguminous plants, *Astragalus galegiformis, Galega orientalis*, chemical composition, nutritional value, yield.

INTRODUCTION

Agriculture plays an important role in providing the population of the Republic of Moldova with food products. Livestock productivity enhancement contributes to sustainable development of agro-food sector and rural areas in terms of the need to establish a balance between economic development and environmental impacts of agriculture. The growth prospects of agro-food market depend on provision of livestock with protein. Production enhancement of forage protein can be achieved by increasing the diversity of leguminous crops, expanding their cultivation areas by creating new varieties with high genetic potential for productivity, quality and high resistance to biotic and abiotic stresses. It is important to mention that on the roots of leguminous plants are formed nodules containing nitrogen-fixing bacteria, which effectively exploit the soil, contribute to the normal development of microbiological processes in the soil and increase its fertility. Therefore, they play an important role in environmental protection and as improvers of the ecological situation since there is no need to apply fertilizers [1, 2, 3, 4, 14].

For over six decades of scientific research on mobilization of plant resources, the collection of non-traditional fodder plants of the Botanical Garden (Institute) of the ASM was founded. It contains over 300 species and cultivars, including 70 leguminous fodder grasses, mobilized from different areas of Central and South Europe, Caucasus, Asia and America [5, 6, 7, 13].

High or low yield of grass fodder significantly determines cost price and competitiveness of production. Traditional legume crops, such as alfalfa and clover tend to exhaust with the lapse of time, and after 3-4 years their productivity gets considerably lower. A vital solution of forage protein problem in the Republic of Moldova is introduction of new perennial leguminous plants *Astragalus galegiformis* L. and *Galega orientalis* Lam., natives of Caucasus.

Astragalus galegiformis L., (syn. *Astragalus galegifolius*, *Tragacantha galegiformis*) is herbaceous perennial up to 1.2-2.5 m in height. Leaves grow up to 20 cm long, with 13-16 pairs of leaflets, oblong-ovate, 12-25 mm long, rounded. The pea-shaped flowers are arranged in racemes up to 30 cm long. Calyx – 5-6 mm long, weak and short with linear-subulate teeth. Corolla – yellow or yellowish-white flag 14 to 15 mm in length. Pods – 12-15 mm, plano-convex, laterally compressed, glabrous, long-stipitate, mucronate, 4-6 seeds. 2n = 16. *Astragalus galegiformis* is an excellent source of nectar and pollen for honeybees. [8, 14].

Galega orientalis Lam. is herbaceous perennial, forms a solid shrub of 10 to 18 leafy stems, 0.8-2.0 m. Alternate, odd-pinnate 15-30 cm long leaves have a good feature to stay unscrambled during drying hay with pinnate. Tap-root system is composed of combined lateral rhizomes. At a depth of 7 cm the main roots produce 2-18 lateral offspring – rhizomes. They grow horizontally over 30 cm in length, and form buds, which are sprouting shoots. The main mass of roots is located at a depth of 50-80 cm, at a maximum of 2 m. From 2 to 4 x 1.0 to 4.5 cm nodules formed on lateral roots. Root nodules contain endophytic *Rhizobia galegae* which perform nitrogen fixation and thus foster its accumulation in rhizosphere and increasing soil fertility. Noteworthy, metabolic products emerged during endosymbiosis are exchanged between bacteroids and host cells and thus support host plant productivity and protein increase. Tap-rooted and rhizomatous with overwintered rhizomes emerging in spring to initiate new shoots which eventually take root and become independent plants. Mellifluous inflorescences are comprised of bright lilac clusters with 25-70 florets. 2 to 4 cm long pods contain 5 to 8 kidney-shaped seeds, yellowish green in colour but later light brown. Seed size is 2.5-4.0 mm long, l.7 – 2.0 mm wide. 2n = 16. Cross-pollinated by bees [1, 8, 15].

The objective of this research was to evaluate biological peculiarities, yield, fodder value of new species *Astragalus galegiformis* and *Galega orientalis* in Moldova's conditions.

MATERIALS AND METHODS

The species *Astragalus galegiformis* L. and *Galega orientalis* Lam., of the collection of non-traditional fodder plants Botanical Garden (Institute) of the ASM served as object of study, control variant – alfalfa *Medicago sativa*. The experiments were performed on non-irrigated experimental land in the Botanical Garden (Institute) of the ASM, on usual chernozem, they started in spring, when the soil had reached the physical maturity. The seeds were sown at a depth of 1.5-2.0 cm with soil compaction before and after sowing. The evidence area of the plot was of 10 m^2. The number of repetitions – 4. The scientific researches on growth and development, yield and nutritional value of fodder were carried out according to the methodical indications [9, 11, 12, 16].

RESULTS AND DISCUSSIONS

As a result of the research, it has been found that the degree of seed germination and the period of seedling emergence of the studied species differ significantly. The species *Astragalus galegiformis* and *Galega orientalis* have a longer period of germination due to the high content of hard seeds that require a greater amount of moisture and higher temperatures of the soil (16 °C). Thus, the seedlings of *Astragalus galegiformis* emerge on the soil surface after 33 days, that is 17 days later than the seedlings of *Medicago sativa* and 5 days later than the seedlings of *Galega orientalis*. In the first year of vegetation, the growth and development of the aerial part of *Astragalus galegiformis* and *Galega orientalis* is slow and the development of their root system is intense. *Astragalus galegiformis* plants develop a 35-43 cm tall stem and *Galega orientalis* 1/3 of plants at the flowering stage, reach a height of 45-48 cm. *Medicago sativa* plants go through all development phases and, in the first year, form seeds and natural fodder can be harvested twice.

In the following years, in spring, when temperatures above 5 °C are established, *Galega orientalis* plants resume their vegetation 8-12 days earlier than *Medicago sativa* plants. *Astragalus galegiformis* resume their vegetation the latest – 3-5 days later than *Medicago sativa* plants, but have a faster growth and development rate, they need a shorter period until the formation and ripening of seeds. Analyzing the results from Table 1, we note that due to the favorable weather conditions from the spring of 2014, *Galega orientalis* plants resumed vegetation at the beginning of March, and those of *Medicago sativa* and *Astragalus galegiformis* – 7-10 days later. The species *Galega orientalis* and *Astragalus galegiformis* formed flower buds in the middle of May, but *Medicago sativa* plants – at the beginning of June. It was found that the flowering stage of *Galega orientalis* and *Astragalus galegiformis* species starts at the end of May and the seed ripening – during July, occurring 20-32 days earlier as compared with the control. The leguminous forage crops differ also according to the

growth rate. Thus, by the end of April 2014, the shoots of *Astragalus galegiformis* plants reached 63.0 cm tall, but *Galega orientalis* – 57.2 cm, exceeding the *Medicago sativa* plants with 21.9 cm and 16.1 cm, respectively. This rhythm is maintained until flowering when *Astragalus galegiformis* plants reach a height of 190.6 cm and those of *Galega orientalis* – 146.7 cm, respectively.

Table 1

Biological peculiarities of the species of the family *Fabaceae 2014 y.*

Indicators	***Medicago sativa* L.**	***Galega orientalis* Lam.**	***Astragalus galegiformis* L.**
Beginning of vegetation	12.03	5.03	15.03
The period, days from the beginning of vegetation up to:			
-budding	86	68	61
- flowering	102	80	70
- seed ripening	153	133	121
Plant height, cm			
- end of April	40.1	57.2	63.0
- at flowering	85.2	146.7	190.6

It is known that the growth and development rate of plants reflects on natural forage productivity and accumulation of dry matter. The yield of natural forage at the 1st harvest (Table 2) of the introduced species is much higher as compared with the control, reaching 6.00 kg/m^2 at *Galega orientalis* and 7.12 kg/m^2 at *Astragalus galegiformis*. The natural forage of *Galega orientalis* is distinguished by high leaf content (56%). Also, we mention that in all studied species, the dry matter content in natural forage from the first harvest (15.52 - 16.71%) is lower in comparison with the forage obtained from subsequent harvests.

Growth revival of plants after the first harvest is slower and influences the formation of natural forage yield and the accumulation of dry matter [12, 13, 16]. After the first harvest, at *Astragalus galegiformis*, unlike *Galega orientalis*, secondary shoots are formed from large buds, which are located just above the collar and can be harvested one more time. *Galega orientalis* regenerates from the buds, situated on the stem that remains above the ground after harvest and partially from underground buds, which usually form generative shoots. *Galega orientalis* plants in this period form the highest natural forage yield of 2.04 kg/m^2, exceeding the control by 10%, but their dry matter content is lower. We note that *Astragalus galegiformis* plants have lower productivity of both: natural forage (1.68 kg/m^2) and dry matter (0.35 kg/m^2). The slow revival after mowing of *Astragalus galegiformis* plants is also mentioned by

other researchers [8, 14]. However, the forage at the second harvest, as compared to the first harvest, varies by the leaf content. The leaf content of *Galega orientalis* and *Astragalus galegiformis* increases while that of alfalfa decreases, constituting 42%.

Table 2

Productivity of the species of the family *Fabaceae,* 2014y.

Indicators	***Medicago sativa* L.**	***Galega orientalis* Lam.**	***Astragalus galegiformis* L.**
The yield first harvest:			
- fresh mass, kg/m^2	3.65	6.00	7.12
- dry matter, kg/m^2	0.61	0.96	1.10
- leaf share of the fodder,%	50	56	49
The yield second harvest:			
- fresh mass, kg/m^2	1.85	2.04	1.68
- dry matter, kg/m^2	0.43	0.41	0.35
- leaf share of the fodder,%	42	65	57

Table 3

Biochemical composition of the dry matter and nutritional value of the natural fodder, first harvest 2014 y.

Indicators	***Medicago sativa* L.**	***Galega orientalis* Lam.**	***Astragalus galegiformis* L.**
raw protein, *%*	16.16	17.80	17.64
raw fats, *%*	1.88	3.55	3.69
raw cellulose, *%*	34.74	30.50	23.21
nitrogen free extractive substances,*%*	37.22	39.47	48.47
mineral substances, *%*	10.0	8.69	7.00
1 kg of natural forage contains:			
nutritive units	0.14	0.15	0.16
metabolizable energy for cattle, MJ/kg	1.49	1.51	1.61
dry matter, g	167.1	160.4	155.2
raw protein, g	27.0	28.6	27.4
digestible protein, g	20.3	22.0	20.3
raw fats, g	3.1	5.7	5.7
raw cellulose, g	58.1	48.9	36.0
nitrogen free extractive substances, g	62.2	63.3	85.0
mineral substances, g	16.7	13.9	10.9
Digestible protein, *g/ nutritive unit*	145	147	127

In order to maintain vital functions and give different productions, animals need continually an exogenous contribution of nutrients they receive from forage. Biochemical composition of the dry matter from forage influences the digestibility

and nutritional value, health and productivity of animals. We might mention that natural forage from the first harvest of 2014, compared with the subsequent harvests, is distinguished by a low content of dry matter. Analyzing the biochemical composition of the dry matter from forage, Table 3, we find that introduced species are characterized by a high content of raw protein in comparison with alfalfa (17.64-17.80%). The increased protein content in the fodder obtained from these species is also mentioned by other authors [1, 8, 11, 14].

Fats from forage are the main source of energy for animal organism and are necessary for the normal course of vital processes; contribute to the accumulation of fat in milk. It was found that the fat content of *Astragalus galegiformis* reaches 3.69 % and of *Galega orientalis* – 3.55 % dry matter, exceeding about 2 times alfalfa. We might mention that compared with the control, the natural forage of the introduced species *Astragalus galegiformis* and *Galega orientalis* has lower content of cellulose and minerals.

Table 4

The content of amino acids (mg/100 mg dry matter) in the natural fodder, first harvest 2014 y.

Amino acids	*Medicago sativa* L.	*Galega orientalis* Lam.	*Astragalus galegiformis* L.
asparagine	2.646	2.662	1.799
threonine	0.640	1.051	0.687
serine	0.736	1.202	0.843
glutamine	1.408	1.296	1.397
proline	0.894	1.670	0.514
glycine	0.634	0.757	0.745
alanine	0.597	1.310	0.627
valine	0.654	0.371	0.559
methionine	0.103	0.114	0.163
isoleucine	0.526	0.404	0.492
leucine	0.881	1.749	1.777
tyrosine	0.358	0.784	0.422
phenylalanine	0.589	0.958	0.595
histidine	0.301	0.511	0.370
lysine	0.695	1.185	0.673
arginine	0.252	1.019	0.548

Astragalus galegiformis is characterized by a high content of nitrogen-free extractive substances, which has positively influenced the nutritive and energy value of natural forage. So, 100 kg of natural forage of *Astragalus galegiformis* contain 16 nutritive units with 161 MJ metablolizable energy, that is 2 nutritive units and 12 MJ more than *Medicago sativa*, 1 nutritive unit and 12 MJ more than *Galega orientalis*. The forage of *Galega orientalis* has a higher content of digestible protein

(147 g/nutritive unit) in comparison with alfalfa and *Astragalus galegiformis.* Forage quality is determined by the biological value of protein, represented by essential amino acid content. The dry matter of *Astragalus galegiformis* as compared with *Medicago sativa*, Table 4, is characterized by a higher content of threonine, serine, glycine, alanine, methionine, leucine, tyrosine, phenylalanine, arginine. This essential amino acid content is higher, especially leucine (200%) and methionine (58%). The fodder of *Astragalus ponticus* is characterized by a higher content of amino acids: threonine, serine, proline, glycine, alanine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, but the content of glutamine, valine, isoleucine is lower compared with *Medicago sativa.*

CONCLUSIONS

The slow growth and development of plants in the first year of development, their acceleration in the second year and full development in the third year of vegetation are specific characteristics of *Galega orientalis* and *Astragalus galegiformis. Astragalus galegiformis* achieves, at the first harvest, a productivity of 7.12 kg/m^2 natural forage with nutritional value of 0.16 nutritive units/kg and a digestible protein content of 127 g/nutritive unit that complies with the zootechnical standards.

Galega orientalis achieves, at the first harvest, a productivity of 6.00 kg/m^2 natural forage with nutritional value of 0.15 nutritive units/kg and a digestible protein content of 147g/nutritive unit that complies with the zootechnical standards. These species can serve as initial material for improving and implementing new varieties of leguminous species for fodder production.

BIBLIOGRAPHY

1. Balezentiene L. 2011. Introduction and agro economical value of *Galega orientalis* Lam**.** 3rd International Symposium on Weeds and Invasive Plants. October 2-7, 2011 in Ascona, Switzerland. www.wsl.ch/.../7-193-final-Extend_abstr193.

2. Duke J.A., 1981. Handbook of legumes of world economic importance. Edit. Plenum Press, New York. 345 p.

3. Lock J. M., and Schrire B. D., 2005, Galegeae. In: Lewis G. Schrire, B. Mackinder B., Lock J. M. (Eds.) *Legumes of the World*. Surrey, Richmond, p. 475-487.

4. Lüscher A., Mueller-Harvey I., Soussana J.F., Rees R.M., Peyraud J.L., 2013, Potential of legume-based grassland-livestock systems in Europe. *Grassland Science in Europe*, 18: p. 3-29.

5. Teleuță A., Țîței V. 2012. Species of *Galega orientalis*, *Polygonum sachalinense*, *Silphium perfoliatum* and their agrobiological pecularities in Republic Moldova's conditions. *Acta Horti Botanici Bucurestiensis* Nr. 39. p.95-100.

6. Teleuță A., Țîței V. 2014. Biological peculiarities of species of the genus *Medicago* L. from Botanical Garden of the Academy of Sciences of Moldova. Journal of Botany, Vol. VI, Nr. 2(9), Chişinău, 2014. p.68-74.

7. Teleuță A., Țîței V. 2014 Some biological peculiarities and value of the forage of *Astragalus ponticus* Pall. *Contribuții Botanice* XLIX, p.67-73.

8. Бораева З. Б., Бекузарова С. А. 2010. Интродукция астрагала козлятниковидного в Республике Северная Осетия-Алания. *Аграрный вестник Урала*, 9-10 (75-76) с.8-11.

9. Ермаков А.И. (ред). 1987. Методы биохимического исследования растений. Агропроиздат. Л., 430 с.

10. Иванов А.И. 1985. Изучение коллекций многолетних кормовых растений (методические указания). Л. 48 с.

11. Капсамун А.Д. 2011. Производство и использование кормов из козлятника восточного в рационах молочных коров. Автореф. диссертации на соискание ученой степени доктора сельскохозяйственных наук. М., 45 с.

12. Новоселов Ю.К. (ред). 1983. Методические указания по проведению полевых опытов с кормовыми культурами. ВНИИК. М. 197 с.

13. Телеуцэ А., Цыцей В. 2012. Нетрадиционные растения семейство бобовых - кормовые достойнство и продуктивность в условиях Республики Молдова. *Таврійський науковий вісник № 80 частина 2.* с.338-342.

14. Чибис С. П., Степанов А. Ф., Чибис В. В. 2011. Питательная ценность астрагала галеговидного. Вестник Алтайского государственного аграрного университета № 1 (75), с.65-68.

15. Утеуш Ю.А. 1991. Новые перспективные кормовые культуры. К. 192 с.

ROLE OF NECTARIFEROUS PLANTS IN ENTOMOPHAGES DINAMICS

P. Vition

The Institute of Genetics, Physiology and Plant Protection of ASM

Abstract. The phytocenotic role of nectariferous plants in trophic link of entomophages is estimated.

INTRODUCTION

Formation of the entomofauna zoocomplex is in a tight link with the phytocenosis and depends at a great rate on crop rotation of the plants which are to be cultivated [1].

MATERIALS AND METHODS

Investigation of the entomophages *Tachidae, Syrphidae, Chrisopidae* was carried out on nectariferous plants from fam. *Apiaceae* 1 dill, 2 coriander, 3 fennel [2, 3] and on tomato field as well.

RESULTS AND DISCUSSION

The dill (*Anethum graveolens* L.), coriander (*Coriandrum sativum* L.), fennel (*Foeniculum vulgare* Mill) are condiment – aromatic, nectariferous plants of *Apiaceae* fam. At the phase of apple tree flowering the qualitative dynamics structure of *Chrysopidae* fam. is formed of: *Chrysopa carnea Steph, Chrysopa perla L., Chrysopa Formosa Br.,* wich constitute 50%, coriander *Chrysopa Formosa Br.,Chrisopa carnea Steph,* -33%, fennel - 16,7%,*Chrysopa carnea Steph*. On apple trees the following species were registered: fam. *Syrphidae, Diptera*,: *Sphaerophoria scripta Linne, Syrphus ribesii Linne, Syrphus corollae Fabricius, Paragus tibialis Fallen*., wich consist 40%, coriander - *Paragus bicolor F., Syrphus corollae Fabricius, Sphaerophoria scripta Linne, Syrphus ribesii Linne* , - 43%, fennel: *Syrphus corollae, Fabricius, Ceriana conopsoides Linne*, - 20%. Among fam. *Tachindae.Diptera* on dill: *Compsylura consinnata Mg.- Ichn., Tachina grossa Lim., Tachina larvarum L*., wich constitute - 50%., coriander : *Tachina grossa Lim., Compsylura consinnata Mg.- Ichn*, - 32%., fennel: *Tachina grossa Lim*. - 18%. Faunistic structure in tomato field constitues 16% of *Chrysopidae*, 32% - *Syrphidae*, 12% - *Tachindae*. The density of *Chrysopidae* was 19%, 18 individuals / 100 threads, *Syrphidae* 30%, 29 individuals / 100 threads, *Tachindae* 11%, 13 individuals / 100 threads.

On nectariferous plants numeric efective constitute: *Chrysopidae* 33%, *Syrphidae* 40%, *Tachindae* 27%.

Table 1

***Tachindae, Syrphidae* and *Chrysopidae* dynamics from different species of nectariferous plants**

N/O	Taxonomic groups of entomophages	Dill	Coriander	Fennel
I	*Chrysopidae*	**53%**	**33%**	**17%**
1	Number of individuals/100 threads	**6**	**4**	**2**
II	*Syrphidae*	**56%**	**31%**	**13%**
2	Number of individuals/100 threads	**9**	**5**	**2**
3	*Tachindae*	**47**	**30%**	**20%**
III	Number of individuals/100 threads	**5**	**3**	**2**

Qantitative dynamics structure is: *Tachindae* **47%,** *Syrphidae* **56%,***Chrysopidae* **53%, coriander** *Tachindae* **30%,** *Syrphidae* **31%,** *Chrysopidae* **33%, fennel** *Tachindae* **20%,** *Syrphidae* **13%,** *Chrysopidae* **17%.**

Table 2

The radius of spread in dependence on distance (m) of *Tachindae, Syrphida* and *Chrysopidae* from the nectariferous plants in agrocenoses of tomato field

N/O	Taxonomic group of entomophages	Strip of nectariferous plants in flowers from fam. Apiaceae	Tomato field at 50 m distance from nectariferous cultures	Tomato field at 100 m distance from nectariferous cultures	Tomato field at 200 m distance from nectariferous cultures	Tomato field at 300 m distance from nectariferous cultures
I	*Chrysopidae*	100%	58%	41%	33%	16%
1	Number of individuals/ 100 threads	12 ind.	7 ind.	5 ind.	4 ind.	2 ind.
II	*Syrphidae*	100%	62%	50%	43%	25%
2	Number of individuals/ 100 threads	16 ind.	10 ind.	8 ind.	7 ind.	4 ind.
3	*Tachindae*	100%	50%	40%	30%	11%
III	Number of individuals/ 100 threads	10 ind.	5 ind.	4 ind.	3 ind.	1 ind.

Maximal dynamics of entomophages was registered on the tomato field at a 50 m distance at flowering phase. Constitues: the majority of entomophages and pollinating insects is concentrated on aromatic cultures of Apiaceae, Asteraceae Lamiaceae, Rosaceae, and from spontaneous flora - Papilionaceae, Violaceae, Polygonaceae, Ranunculaceae, Brassicaceae, Apiaceae, Asteraceae Lamiaceae, Rosaceae.

CONCLUSIONS

The phytocenotic role of cultivated aromatic plants and from spontaneous flora in trophic nutrition link of the entomophages and pollinating insects was evaluated.

BIBLIOGRAPHY

1. П.Г Витион Структура энтомофагов вредителей люцерны в централъной зоне РЕСПУБЛИКИ МОЛДОВА Российская Академия Селъскохозяйственных наук Оделение защиты и биотехнологии Растений Сибирское Регионалъное оделение Росселъхозакадемии Сибирский научно- исследователъский Институт Земледелия и Химизации Селъского Хозяства Материалы Международной Научно – Практической Конференции П. Краснообск, 24 –26 июля 2013 г. Защита Растений в Современных Технологиях Возделывания Селъскохозяственных Кулътур стр. 76 –79.

2. Лебедев В.В. О роли нектароносов в привлечении насекомых – энтомофагов в агробиоценозы. // IX Съезд всесоюзного энтомологического общества частъ: 2. Тезисы докладов. Киев. Октябръ. 1984. Стр. 9.

3. Коренев А.А Структура энтомо - комплекса нектароносных трав в яблоневом саду // Тезисы докладов республиканской научной конференции “ Проблемы управления и конструирования фаунистического комплекса в антропогенном ” Кишинев. 14 –15 ноября. 1989, стр. 55 –56.

IV. LANDSCAPE ARCHITECTURE

MANAGEMENT MEASURES FOR SUSTAINABLE AGRICULTURAL LANDSCAPES OF MOLDOVA

Tamara Leah
"Nicolae Dimo" Institute of Soil Science, Agrochemistry and Soil Protection,
tamaraleah09@gmail.com

Abstract. Features of the contemporary landscape and proposed measures to keep its sustainability for the long term have been presented. Actual agricultural landscapes and the near future are in the last analysis a type of ecological construction engineering. Developing, maintaining and improving it in the future both for food and aesthetic purposes will require engineers, agronomists, horticulturists, zootechnicians, veterinarians, phytopathologists, pedologists, orchardists and other specialists in the field of agricultural sciences having a serious agro-ecological training. Increasing production efficiency of agricultural landscapes and transformation of agro-ecosystems into a structure with multiple functions of protecting the environment is impossible without deepening the branches applied ecological variants.

INTRODUCTION

The first to intuit the existence of the natural system that we call today "landscape" was the German naturalist Alexander von Humboldt (1769-1859), who after searching from the point of view of natural history more regions of the globe, made a great idea about biogeographical formations of the world [1]. Unlike natural landscapes, the structure of agricultural landscapes is simpler, resulting in a distribution of species organized by humans, usually in a single or very few seek to maximize energy flow in populations of a single species crop varieties selected for various habitats.

Landscape management is an action, in terms of sustainable development, which ensures the maintenance of landscapes in such way as to guide and harmonize the changes which are brought by social, economic and environmental activities. The lack of a coherent strategy, short and long-term administrative and spatial organization of agricultural lands, make the agricultural landscape look like as a dysfunctional system, based largely on the exclusive influence of anthropogenic and natural zonal factors.

Any strategy or orientation direction of agricultural policies should take into account the structure and potential productivity of agroecosystems. Up to now there is no national strategy on optimizing crop structures, oriented not only to the current requirements of the market, but also to increase the quality of population and animal food and to maintain the soil fertility status.

From this point of view, the actual structure of the crop in Republic of Moldova (68.5 percent of cereals and maize crops, 20.5 percent of technical crops, 9.2 percent of forage plants, and only 1.7 percent of vegetable crops) can be considered a grain country that has retained this status the last two decades [2]. Changing the current structure of culture by increasing the proportion of protein legumes at least 20 percent and decreasing the arable surfaces cultivated with cereals (especially maize) to more than 40 percent of the arable land, would streamline the entire country's agricultural landscape both the optimization of cultivation technologies and the growth of agricultural production [3].

MATERIALS AND METHODS

In order to motivate sustainable management measures of agricultural landscapes were selected and analyzed the available environment friendly measures mentioned in the elaborated national programs regarding the improvement of the fertility of degraded soils with low productivity and its action plans of implementation [4, 5].

RESULTS AND DISCUSSIONS

Territorial organization and location of sowing crops should be made based on the evaluation of land ecological utility, further creating forest and erosion belts, constructing basins regulators, recultivating eroded soil, improving radical land damaged by ravines. Priority should be given to the reconstruction and restoration of existing systems, soil protection from damage by flood waters and heavy rains etc. This will allow the formation of harmonized, highly productive agricultural landscapes, increase soil fertility and create optimal systems of erosion protection, the overall improvement of the entire ecological situations, including health.

Based on the system (complex) measures on soil and environment protection for each farm should be established the set of management activity limits aimed at ensuring environmental sustainability of agrolandscape [4]:

- maximum allowable share of arable land;
- the maximum allowable rate of afforestation and protection planning;
- reorientation of the structure of the sown areas in order to increase the share of crops which protect the soil;
- strictness in determining the components and the realization of technological operations during the cultivation of agricultural crops;
- limits in the use of fertilizers and pesticides on eroded soils;
- soil recovery limits with erosion hazard if they pass in the stage of improvement (grassing).

Under the influence and with increasing of human impact on natural complexes and continuous infringement of nature protection functions it is necessary to compensate

these destruction by creating more efficient anthropogenic biocomplexes or technical engineering systems. But it is much easier to prevent errors at the stage of granting land recovery goals than to liquidate the consequences of unexpected errors. This has been the safest for planning agricultural land, using the principles of landscape.

In this planning must be included all the management schemes for directing the biosphere regimes approved in time and over the life: using adaptive nature, biological farming and livestock under grazing; agriculture phyto-amelioration; reasonable measures, systems of water management, of irrigation, to solve social problems. The conditions of application and use of each of these depends on many factors: climate, relief, hydrological regime, the territory state, presence of management objects, roads, high voltage lines, etc. These conditions are achieved as best for organization of agricultural landscape using the planning principles.

In all cases, are determined the state and local management purposes for a concrete territory. These goals fall within the concept of using nature based on knowledge of the state territory, resources and recovery opportunities, taking into consideration ecological criteria (standards and limits). Exactly at this stage it is necessary to determine the rational correlation of arable land, perennial plantations, pastures, meadows, forests, water sources, which will be further developed in landscape planning.

On the plane territories the denominated organizational elements are aquatic objects, forest protection belts and perennial plantations. On the steppe territories the most evident and, at the same time, organizational elements are aquatic objects, forest protection belts, perennial plantations. On the eroded terrain, at these facilities, hydraulic installations will be added: furrows, canals, terraces.

Landscape construction and reconstruction. The major objectives of environmental policy in maintenance and development of the natural potential capacity to ensure the environmental safety of the population are expanding forests, grasslands, wetlands, urban and rural green spaces. Achieving these objectives is problematic, given that the majority of land is privately owned. In the process of completing the projects of landscape reconstruction it is necessary that the use of the land to be assessed by ecological criteria and the status of the various recovery stages [6].

Protective forest plantations. These are the artificial ecosystems, which possess considerable biological mass and energy, and in the arid and semi-arid zones have their phytoproductivity that contributes to growth for agriculture. They disturb (fragment) the monotony of large open spaces, form the much needed diversity to the long functioning of the agrobiological system components. And finally, the trees and shrubs give new anthropogenic landscape a more vivid aspect, aesthetic expressiveness and dynamism. Today may be afforested about 74 thousand hectares of unproductive land (gullies, landslides, slope with inclination 30°), [6].

Landscape of agricultural land correctly organized is a composition of different elements (components) of structure linked with a green housing with a system of forest plantations. This system includes forest strips and bush scenes on irrigated and non-irrigated field, anti-erosion forest plantations and protective water basins, wooded pastures, plantations surrounding farms, plantations for rehabilitation, plantations on slopes and within river systems and other forest plantations.

Protection plantations on the arable land. Arable lands with erosion terrain avoid linear straight field boundaries. Roads and forest strips must be located under the contour. This will cause soil processing and performing the other works on the slopes as contours. Creating forest plantations on the irrigated lands should be an indispensable condition for entry into exploitation. Aquatic objects and forest plantations are the expressive elements of the anthropogenic landscape, which possess tremendous energy capacity. Improving water resources and forest plantations reconstruction gives the greatest economic, environmental and social effect.

Forest plantations on the grasslands. Ecological reconstruction of herbaceous vegetation is required on the area of 500 thousand ha, occupied by pastures that are damaged as a result of overgrazing. Organizing on the landscape principles of pastures territories provides the establishment of forest plantations, planting trees and shrubs in the form of strips and slow clusters, forming the so-called green shadows, feeding functions - improvement, planting trees around sheepfolds, farms around watering places for cattle [3, 6].

A special form of forest plantations on grasslands of saline soils are shaped shrub band plantations intended for improvement - fodder purpose, drought-resistant, able to restore multiple times vegetal mass (regenerate) after regular use as fodder to cattle. Such plantations are an added feed for animals and also fulfill the role of soil protection and snow retention. As a result, improve the growth conditions of pastures' vegetation.

On pastures, it is necessary to allow an optimal or moderate number of animals, to implement the grazing rotation and the grazing system on parcels in order to avoid complete destruction of vegetation cover from the ground surface. For parceling the pastures are used artificial and natural fences. The natural demarcation of parcels is done by planting forest belts from one or two rows of trees. They are cheaper than artificial ones, more resistant, more durable and more vivid (in case of thorny bushes and trees).

Expanding wetlands (swamps). To restore the ecological balance in river valleys, are required expansion works of wetlands on 10-20 thousand ha. In the small river valleys, through special projects, it is necessary to create a cascade of wetlands that serve as areas of biological self-purification of river waters and contribute to biodiversity conservation.

Recreation. Problem solving to recreation have a multilateral aspect of ensuring resort to creating more favorable conditions for the rest of the population, for practicing sports, hunting and fishing. This includes the creation of green areas around cities, sanitary protective plantations around industrial enterprises, national parks, special areas of recreation with strips of forests and water bodies etc. As a rule, the local entertainment capabilities may have different degree of planning, but are more attractive if they have water bodies (lake, river) and groves of trees and bushes. Such lands (surfaces) are designed according to the planning principles of landscape planning. According to the principles of landscape design, the recreational areas are provided with territories of rehabilitation, road and transport network.

Protected natural areas. The organization of natural protected areas is facing common obstacles related to land redistribution, setting new regime for use. These areas cannot be small inclusions in a big massive of intensively exploited agricultural land (when the protected areas are small, the most significant island effect, sponge effect, etc. occur). Therefore, they must be considerable territories (enough) by size in order to function safely as a protected object.

A scientific importance, have the standards of conservation of natural and natural-anthropogenic ecosystems, as well as landscapes, with scientific - informational purposes, for studying the dynamics of evolution of the situation, development of methods of biological circuit control and conservation of gene pool. They must be centers with an abundance of rare plants and animals, habitat (refuge) for endangered organisms under the pressure of civilization, which are specific to the given landscape and ecosystem [7].

The protected natural areas in addition to protect the territory of certain landscapes, can support, and in some cases, can create a favorable ecological balance on rather large territories around them, if there is a considerate anthropogenic support of these territories by creating forest protection belts, water supply, irrigation performance for the enlarged reproduction of the landscape resources, which, currently, do not meet the growing demands of society in terms of volume and quality.

Agriculture is a complex ecological system, which contains a lot of subsystems. All these subsystems must be considered in strict correlation with their importance for nature and economy. Reconstruction and widening of existing protected areas system should be based on the concept of a National Ecological Network in that the most valuable natural areas surrounded by buffer zones and connected by strips of forest and natural vegetation oases. This will help to reduce the adverse impact of fragmentation of natural habitats, maintain wildlife migration corridors, in stabilizing the ecosystems and sustainable use of biological diversity at the landscape level.

CONCLUSIONS

Agriculture organized on the landscape principles must be strictly adapted to the environmental conditions and progressively oriented to opportunities of using real biological productivity of nature. In this case the agrophyto- and zoophyto-ameliorative plantations meet the long-term stabilizing role for agriculture which in reality may be more favorable compared to open areas (without forest plantations).

Protective forest plantations and their influence on the environment play a polyfunctional role. In this sense they are of great significance for biosphere. Such conception of the creation of protective forest plantations incontestably convinces us about its importance not only for agricultural production, but also for a number of other branches of the national economy: water management, fisheries, industry, transport, health, etc.

In this regard, a correct landscape, having influence on the hydrological and thermal regime of the territory, including the land subject to erosion, salinization, positively influences the quality of water, soil, atmosphere, flora, fauna, socio-demographic conditions. So then, creating agricultural correct landscapes are at the rank with other parts of the state environmental strategy.

BIBLIOGRAPHY

1. www.creeaza.com/referate/geografie/.php. The concept of landscape or landshaft.

2. Activitatea agricolaă a micilor producători agricoli in Republica Moldova. Biroul Național de Statistică al Republicii Moldova. Chishinau, 2013, p.17.

3. *Programul Național Complex de Sporire a Fertilității Solului*. Ch.: Pontos, 2001, 118 p.

4. *Programul Complex de Valorificare a Terenurilor Degradate şi sporire a fertilității solurilor*. Partea I. Ameliorarea terenurilor degradate. Ch.:, Pontos, 2004, 212 p.

5. *Programul Complex de Valorificare a Terenurilor Degradate şi sporire a fertilității solurilor*. Partea II. Sporirea fertilității solurilor. Ch.:, Pontos, 2004, 128 p.

6. *Лях Т.Г., Лях. К.Н.* Структуризация и реконструкция ландшафтов и их значение в борьбе с засухой и для охраны почв Молдовы. *Современные проблемы почвоведения и природопользования в Сибири.* Всероссийская молодежная конференция, 25-27 июля 2012, Томск, Россия. Томский госуниверситет, 2012. Ч.II. Современные проблемы прородоплльзования. с.417-426. ББК 40.3; УДК 631.4; 504. № 12.741.11.0189.

7. Leah T. Dezvoltarea resurselor forestiere - principiu de bază pentru stoparea eroziunii şi restituirea terenurilor degradate. *Dezvoltarea durabilă a sectorului forestier - noi obiective şi priorități.* Mater. Simpozionului Internațional, 17-19 noiembrie 2011. Chişinău, ”Print-Caro” SRL, 2011, p.197-199. ISBN 978-9975-56-012-2.

INTRODUCED CONIFERS IN DENDRARY NAS OF AZERBAIJAN AND USE IN LANDSCAPE DESIGN

Mammedov T.S., Abbasova Z.G.
Mardakan Arboretum National Academy of Sciences of Azerbaijan

INTRODUCTION

The urgency of a problem of gardening in large and industrial cities of Azerbaijan increases every year. Urban population growth, quantity of transport, building of new inhabited objects and bridges, creation of new parks and gardens demand wide scale greenery work. Expansion has transformed gardening sets of our cities into a green oasis, has changed the city microclimate, has reduced the force of influence of north and has relieved the city of summer storms.

Last years, within the limits of the program «Strategy of preservation of biodiversity of flora and enrichment by new species» was carried out a variety of scientific research works on introduction.

In the arboretum has been developed a new assortment of decorative plants for green building of republic which is distinguished for high-decorative qualities, hardiness in our conditions. By means of decorative plants, it is possible to create various landscape compositions, as leaf-bearing decorative and coniferous. The coniferous trees and bushes get an increasing popularity in modern greenery design (1).

MATERIALS AND METHODS

For many years, more than 60 species and 15 forms of coniferous plants which belong to 14 genera and 9 families are introduced in dendrary. The basic volume is made up by species *Pinus* L. (17,5 %), *Juniperus* L. (16 %), *Chamaecyparis* Spach. (14,5 %), *Cupressus* L. (9,3 %). We study bio-ecological, decorative features and ways of adaptation of these species in order to establish their perspectivity in the conditions of Absheron on purpose to use them in landscape design (2,3).

RESULTS AND DISCUSSION

Long-term researches have shown that, basically, coniferous plants grow well and develop in dry subtropical conditions of Absheron. These plants have different high decorative qualities.

In dendrary, on the central avenue, durable and decorative species are *Cupressus arizonica* Greene, *C.sempervirens* L. *f. horizontalic* (Mill.) Gord., *C.sempervirens* L.*f.pyramidalis* Targ., *C.lusitanica* Mill. In collections are met specimens of species *Cupressus* L. at the age of 70-90 years, 10-15 m tall, with different forms of crown and have good external shape.

The basic volume is made up by representatives of genus *Pinus* L. - *Pinus eldarica* Medw., *P.halepensis* Mill.*, P.pithsunda* Stev., *P.canariensis* C.Smith., *P.nigra* Arn.*,* which are used for creation of perennial evergreen compositions. In «Old park», *P.longifolia* Roxb. and *P.pinea* L., which were planted in 1924-26, occupied especial place. They have found in here very favorable conditions for growth and development.

In collections, there are 12 species and forms of juniper. *Juniperus virginiana* L., has been grown since 1934 in dendrary and has 6 m of height and diameter of crown – 3 m. After 80-years collections increased with new species and forms: *Juniperus virginian*a L.*f.glauca*, *J.depressa* Stev., *J.chinensis* L., *J.chinensis* L.*f.glauca*.

Photo 1. Compositions from species and forms of *Cupressus* L. and *Juniperus* L.

In park landscapes, the species and forms of *Thuja* L. with spherical, vertical, cone-shaped or weeping crowns - *Thuja orientalis* (L.) Endl., *Th.pilicata* Don., *Th.occidentalis* L.*f.aureo-variegata*, *Th.occidentalis* L.*f.compacta* play a special role. They also differ in colour of needles - motley, golden, bluish. For the creation of border are used clipped forms of *Thuja orientalis* (L.) Endl. The species of *Thuja* L. are durable, frost and heat tolerant, undemanding to soil and moisture.

Picea pungens Eng. and *P.orientalis* Link. *aureo-spicaba* are characterized by weakened growth on Absheron. The annual growth makes up 3-5 cm.

The separate attention is paid to those species which were introduced from our dendroflora - *Juniperus foetidissima* Willd*., J.polycarpos* C.Koch*, Pinus eldarica* Medv., *Taxus cuspidata* Sieb.et Zuss. They are rare and vanishing species, which have been included in the «Red Book». These species normally pass all phenological phases and give qualitative seeds in dendrary. The annual growth makes up 10-15 cm.

The species and forms of *Chamaecyparis* Spach. have found application in various compositions - *Ch.lawsoniana* Parl., *Ch.nootkatensis* Spach., *Ch.pisifera* Sieb.et Zuss., *Ch.optusa* Sieb.et Zuss. *f. "aurea"* etc. They differ in the shades of needles and forms of branches, they look attractively enough.

We continue research works in order to increase the gene pool of dendrary with new species and forms of coniferous tree-bushes.

In dendrary, in the last years, new species and forms were introduced: *Araucariya araucana* C.Koch., *Cunninghamia lanceolata* Lamb*., Juniperus depressa* Stev*., Microbiota decussata* Kom*., Picea orientalis* Link*. f. "aureo-spicaba", Taxus baccata* L*. f. "festigiata", Cycas revoluta* Thunb*. etc.*

We study propagation, growth and development, frost-heat-resistance, agricultural techniques of cultivation and decorative features and their perspectivity. These plants are possible to be used in different objects of green building.

Photo 2. Compositions of coniferous plants

CONCLUSIONS

Decorative properties of introduced conifers, also as the size and the crown form, character of branching, needle colouring are rather various and give the chance to create highly decorative, durable, expressive all the year round compositions.

The results of research give us possibilities to use in greenery of Absheron high-decorative species and forms of coniferous plants – *Pinus* L., *Chamaecyparis* Spach., *Thuja occidentalis* L., *Cycas revoluta* Thunb., *Cupressus arizonica* Greene, *Cryptomeria japonica* Don., *Juniperus polycarpos* C.Koch.,) etc.

BIBLIOGRAPHY

1. *Mammadov T.S. Dendroflora of Azerbaijan.* I t. Elm, Baku, 2011, 312 p.

2. *Mammadov T.S. Tree-bushes family of Absheron*. Elm, Baku, 2010, 468 p.

3..*Lapin P.I., Sidneva S.V. Estimation perspectivity introducted tree plants on visual supervision. In art.: Experiment introducted tree plants*. MBG, Moscow, 1973, 7-67 p.

APPLICATION OF PROCEDURES FOR PESTS' DENSITY REGLATION IN THE PUBLIC GARDENS

Gavrilița Lidia[1], Nastas Tudor[1], Gorban Victor[1], Nețoiu Constantin[2], Corneanu Mihaela [3]

[1]Institute of Genetics, Physiology and Plant Protection ASM
[2]Forest Research and Management Institute, Craiova, Romania
[3]University of Agricultural Sciences and Veterinary Medicine Timisoara, Romania

Abstract. During the year 2013 under the bilateral project of the Institute of Genetics and Physiology and the Plant Protection Institute of ASM, together with scientists from Romania from Forest Research and Management Institute, and partners from Craiova University of Agricultural Sciences and Veterinary Medicine from Timisoara (Romania) were conducted a series of studies. We monitored and captured mass *Lepidoptera* insect species using pheromone traps in public Yards of Rep. Moldova and Romania. Launches with *Trichogramma* against the pests were carried out. The biological efficacy of *T. embryophagum* Htg. and *T. evanescens* Westw, varied in different plant species (oak, mulberry, chestnut, plum, apple, flowers) from 64.5 to 73.3% in Moldova and from 36.3 to 75.0 % of Romania.

Keywords: protection, efficacy, pests, pheromone, monitoring.

INTRODUCTION

Scientists involved in the IMPACT project are investigating new natural methods for pest control in forests and public parks which are subject to climate changes. In a changing world, strongly affected by recent anthropogenic impact and climate change, international cooperation is a key element of a joint effort aimed at reducing and halting biodiversity loss. This situation is reflected in the book "The impact of environmental factors on biodiversity". Also in this source it is indicated that 15589 species of plants and animals are threatened with extinction, 45% of the Earth's forests have disappeared, 10% of coral reefs in warm areas are already dead (MAICAN, 2010) [3]. This project is an imperative of the time, influenced by the necessity of environmental protection with alternative methods to chemical ones (biologically active substances and entomophague GAVRILIȚA, BUTNARU, 2005) [1]. This problem was discussed in the bilateral cooperation in scientific research, technological development and innovation Program between the National Authority for Scientific Research of Romania and the Academy of Sciences from Moldova. During the 2013 year within the bilateral program Institute of Genetics, Physiology and Plant Protection ASM, together with scientists from Forest Research and Management Institute (Craiova) and partners from University of Agricultural Sciences and Veterinary Medicine from Timisoara (Romania), conducted a series of researches in this specific area.

Chemical use in the parks from Moldova and Romania leads to environmental conditions worsening. The processes and methods of protection proposed in this current project are an imperative of time and meet current requirements to improve the environmental conditions. *Trichogramma* species are polyphagous, parasitizing different hosts of Lepidoptera Diptera, and Coleoptera. According to the author LENTEREN, 2000 [2], *Trichogramma* is being launched annually on a surface area of 45 million hectares against pests on different crops.

It is known that a vast complex of pests attack plants from public parks [4]. The consequences can be felt upon the decorative plants from the parks which lose their attractiveness for the visitors. It is also to consider that chemical treatments are prohibited in the public areas because of the negative influence on human health. The solution is described here below.

Research goal: Develop and implement procedures for controlling the density of populations of harmful organisms by applying biologically active substances and *Trichogramma* spp for integrated pest control in public parks from Moldova and Romania.

MATERIALS AND METHODS

Pheromone traps were used for highlighting, recording, monitoring and mass capture of harmful species of insects, which threaten forests and ornamental cultures in parks from Moldova and Romania. During the 2-3 generations development of pests, enthmophagous have had a very important role in reducing pests' density. This is the reason for pheromone traps have been used at adult phase. On the other hand, at egg phase *Trichogramma* launches were performed with a norm of 200.000-300.000 individuals per hectare. After each release, records and analyses were taken from the field to determine the number of parasitized pest eggs.

The objects of investigation are the different species of *Trichogramma* (*T. evanesces, T. embryophagum*) reared on host eggs in laboratory conditions of *Sitotroga cereallela*. Research objects though, in the Botanical Garden in Chisinau, Craiova, Timisoara are the pests *Hyphantria cunea, Lymantria dispar, Tortrix viridana, Grapholitha funebrana, Grapholita molesta, Laspeyresia pomonela, Cameraria ohridella, Helicoverpa armigera* which cause great damage to trees and ornamental plants in parks and botanical gardens, which were monitored with pheromone traps.

RESULTS AND DISCUSSIONS

To monitor harmful insect species across the Botanical Garden (Institute of Botany ASM), pheromone traps were placed at a number of cultures (mulberry - *Morus alba*, Oak - *Quercus pedunculiflora*, Plum - *Prunus cerasifera*, Apple - *Malus domestica*, Chestnut - *Aesculus species* hipocastanum and a collection of decorative

flowers). For the experiments, there were mounted a total of 37 pheromone traps for monitoring the following pest species: *T. viridana* L - green oak moth; *H. armigera* Hb.- corn earworm; *G. funebrana* Tr. - Plum Fruit Moth; *G. molesta* Br. - Oriental Fruit Moth; *L. pomonela* - codling moth; *L. dispar* L. - European gypsy moth. Records on the number of males captured in pheromone traps were taken every 7 days, during the period from 20th of May till 3rd of September in 2013.

During the vegetation period, at the Botanical Garden (Institute of Botany of the Academy of Sciences) pheromone traps were also used to decrease pest population density (with mass male capturing method). Data showed that the number of captured pest *L. disapar* (at Oak) ranged from 1 to 23 males/trap with an average of 2.2 males/trap. The pest *T. viridana* (at Oak) ranged from 1 to 38 males/trap with an average of 2.0 males/trap. The pest *G. funebrana* (plum) ranged from 26 to 170 males/trap with an average of 30.5 males/trap. Capture of the pest *G. molesta* (plum) ranged from 19 to 177 males/trap with the average of 31.1 males/trap. *L. pomonella* (apple) ranged from 7 to 69 male/trap (average - 31.6 Males/trap). Capturing the pest *C. ohridella* (chestnut) ranged from 95 to 22.261 males/trap (average - 515.8 males/trap). *H. armigera* (decorative flower species) ranged from 2 to 75 males/trap (average – 9.6 males/trap). Table 1, Fig. 1.

Table 1.

Number of males captured in pheromone traps during the research based on the crop species and pest species (Botanical Garden, Chisinau, 2013)

Pest species / Cultures	Total number of males captured with pheromone traps	Average male number captured per trap
Lymantria dispar Linnaeus,1758 (Oak)	105±4,5	2.2±0.2
Tortrix viridana Linnaeus,1758 (Oak)	99±3,8	2.0±0.1
Grapholitha funebrana Treitschke,1835 (plum)	1467±5,2	30.5±2.2
Grapholitha molesta Busck,1916 (plum)	1492±5,9	31.1±1.9
Laspeyresia pomonella Linnaeus,1758 (apple)	657±5,2	31.6±1.2
*Cameraria ohridella Deschka & Dimic,*1986 (chestnut)	24762±7,2	515.8±3.2
Helicoverpa armigera Hiibner,1809 (decorative flowers)	461±4.2	9.6±1.2

In the parks from Craiova and Timisoara (Romania), monitoring and mass capturing of the main pest species was conducted from 24th of July till 2nd of August 2013. Experiences have taken place in several areas of Craiova, such as: Botanical

Garden, Alley ICAS, Romanescu Park and Youth Park, as well as Timisoara: ICAS and USAB Park.

Pheromone traps were placed in plots on 6 different species of trees: mulberry - *Morus alba*, Oak - *Quercus pedunculiflora*, Plum - *Prunus cerasifera*, Apple - *Malus domestica*, Chestnut - *Aesculus hipocastanum*, Plane - *Platanus acerifolia* and decorative flower species. In total 35 pheromone traps were placed to monitor the following pest species: *H. cunea, G. funebrana, C. ohridella, H. armigera, L. dispar*. Pheromone traps were placed in plots on 6 different species of trees: mulberry - *Morus alba*, Oak - *Quercus pedunculiflora*, Plum - *Prunus cerasifera*, Apple - *Malus domestica*, Chestnut - *Aesculus hipocastanum*, Plane - *Platanus acerifolia* and decorative flower species. In total 35 pheromone traps were placed to monitor the following pest species: *H. cunea, G. funebrana, C. ohridella, H. armigera, L. dispar*.

As a result of the research's data analysis it has been demonstrated that most males number during the research period were captured in pheromone traps placed on chestnut trees. This way, for the species *C. ohridella*, on average, 93 males per trap were captured in the USAB Park from Timisoara and 54 males at ICAS Alley from Craiova. At plum culture the *G. funebrana* species was captured, on average, 43.0 males/trap in the Botanical Garden from Craiova and 19.0 males/trap in Romanescu park. Pest species *C. ohridella* (chestnut) was captured on an average of 19.0 males/ trap in Romanescu park from Craiova. In the parks of Timisoara and Craiova, *G. funebrana* pest species (plum) was captured on an average of 14.0 to 17.0 males/trap (USAB Park, Youth Park). At chestnut culture there were captured an average of 12.0 males per trap of pest *C. ohridella* in ICAS, Timişoara and 12 males/trap in Youth Park, Craiova. Pest species *L. dispar* (Oak) were captured on average number of 8.0 males/trap in Youth Park from Craiova. The pest *H. armigera* (decorative flowers) was captured on average of 7.0 males/trap in ICAS, Timişoara. *L. dispar* (Oak) has been captured on average number - 6.0 males/trap in ICAS, Timisoara and at plane culture - 5.0 males/trap in the Botanical Garden, Craiova. Pest species *L. dispar* (Oak) has been captured on an average of 4.0 males/trap (Botanical Garden, Craiova) and the species *H. cunea* (mulberry) – around 1 male/trap.

This way it was found that monitoring of pests using pheromone traps allows us to obtain very useful information for plant protection through biological means. It has been shown that both in Romania and Moldova, chestnut species were the most attacked species by the pest *C. ohridella*. In the Botanical Garden from Moldova, against pests there were conducted four treatments with *Trichogramma.* After each treatment, it was kept the track of the number of eggs parasitized and percentage of parasitized eggs. The percentage of parasitized eggs at plum cultures in combating the pest *G. funebrana* with *T. embryophagum*, ranged from 27.0% to 68.6%, in combating the pest *G. molesta* the percentage ranged from 25.0% to 64.5%. At flowers instead, in

combating the pest *H. armigera*, biological effectiveness of *T. evanescens* ranged from 27.0% to 73.3%. At apple cultures, against the pest *L. pomonella, T. embryophagum's* effectiveness after four treatments ranged from 28.0% to 65.4%. In control (no launches), parasitized eggs of the below cultures ranged from 0.4% to 7.8%.

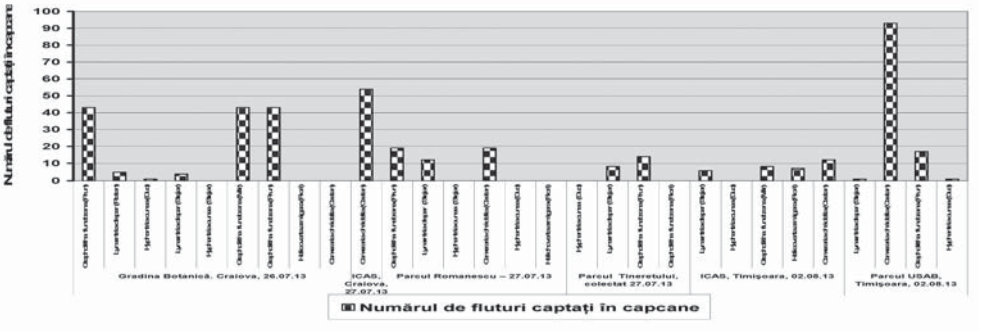

Figure 2. The number of males captured in traps in Botanical Gardens and parks (Craiova, Timisoara), Romania, 2013.

In Romania, against the pests, there were conducted treatments with *Trichogramma* at the Botanical Garden and two parks. The biological efficacy of *T. embryophagum* HTG. and *T. evanescens* Westw., varied at different plant species (oak, mulberry, chestnut, plum, apple, flowers) differently, from 36.3 to 75.0%. Different species of *Trichogramma* were collected from various plant species, for conducting further researches. The species collected from Romania and Moldova formed a stock of individuals which are in diapause at the Institute of Genetics, Physiology and Plant Protection from Rep. Moldova to fulfill bilateral joint project conditions. In control (where no releases were made), the percentage of parasitized eggs in Romania during the research reached 0.7-7.2%.

CONCLUSIONS

1. As result of the researches, the harmful species of insects, such as *T. viridana, H. armigera, G. funebrana, G. molesta, C. pomonella, C. ohridella, L. dispar*, were monitored, highlighted and identified outbreaks (which represent a great danger), with the help of pheromone traps in the Botanic Gardens and Parks from R. Moldova and Romania.

2. Methods for monitoring and mass capturing have been developed to regulate pest population density by applying biologically active substances (sex pheromones) and signaling dynamics of generating development pests.

3. The biological efficacy of *T. embryophagum* HTG. and *T. evanescens* Westw., varied at different plant species (oak, mulberry, chestnut, plum, apple, flowers) from 64.5 to 73.3% in Rep. Moldova, and from 36.3 to 75.0% in Romania.

4. The species collected from Romania and Moldova formed a stock of individuals which are in diapause at the Institute of Genetics, Physiology and Plant Protection from Rep. Moldova to fulfill bilateral joint project conditions.

REFERENCES

1. Gavrilița, L. & Butnaru, 2005. Asupra populațiilor locale de *Trichogramma* din zona Timişoara, *Trichogramma* şi combaterea biologică. Redactori: Dr. K. Fabritius L. Roman, Timişoara: p.4-7.

2. Lenteren J. 2000. How Can Entomology Contribute to Sustainable Crop Production. Course reader, 3rd International Course on Agroecology "Biodiversity, food and sustainable development". Perugia. Italy: 1-10.

3. Maican, S. 2010. Impactul shimbărilor climatice asupra insectelor . cartea „The impact of evironmental factor son biodiversity" editura Academiei Române, Institutul de biologie Bucureşti: p.51-67.

4. Mihailov, I. 2005. Plantele ornamenpale din oraşul Chişinău şi consecințele atacului insectelor dăunătoare. Materialele Conferinței Internaționale, consacrate aniversării a 60 de ani de la fondarea Universității de Stat „Alecu Ruso", Presa Universitară Bălțană. Bălți: p.25-27.