ISSN 1512–2123

საქართველო[illegible] მეცნიერებათა აკადემიის

[illegible]ცნე

ბიოლოგიის სერია
Biological series B

2008
No. 1-2
Vol. 6

PROCEEDINGS

of the Georgian Academy of Sciences

ISSN 1512 – 2123

საქართველოს მეცნიერებათა აკადემიის

მაცნე

ბიოლოგიის სერია
Biological series B

2008
No. 1-2
Vol. 6

PROCEEDINGS

of the Georgian Academy of Sciences

Tbilisi. Georgia
2008

To order your copies, please send to:

Georgian Academy of Sciences,
Department of Biology
52, Rustaveli Avenue, Tbilisi, 0108, Georgia
Tel. + 995-32 93-58-92
Fax: + 995-32 93-58-92
E-mail: bio@gw.acnet.ge
www.acnet.ge/matsne/biology

Journal founded in 2001

ISSN 1512 – 2123

Published by NGO "Biomed", 14, L. Gotua st., Tbilisi 0160, Georgia

CONTENTS

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 1-2, 2008.

Biochemistry

DNA MICROSATELLITE ANALYSIS OF GEORGIAN WILD GRAPES

GAMKRELIDZE M.[1], TABIDZE V.[1], GOTSIRIDZE V.[2]

[1]*S.Durmishidze Institute of Biochemistry and Biotechnology,*
[2]*Georgian Institute for Horticulture, Viticulture and Wine-Making.*

(Received March 12, 2008)

Abstract

Seven specimens of wild grape from different geographic zones of Georgia and one from Turkey were analyzed at four polymorphic microsatellite loci (VVMD7, VVMD27, VVS2, ZAG62). As in previously studied Georgian grapevine cultivars, wild population of Georgian wild grapes are also characterized with high level of genetic variability: Genetic polymorphism in wild grape population is slightly higher than that observed in cultural varieties.

Key words: Microsatellite, DNA microsatellite markers, *Vitis vinifera, V. vinifera spp. sylvestris*

Introduction

The Eurasian grape (*V.vinifera L.*) is one of the most widely cultivated and economically important agricultural crops in the world. The cultivated subspecies, *Vitis vinifera* subsp. *vinifera* includes thousands of cultivars (5000 to 7000) and have been domesticated from the wild subspecies - *V. vinifera spp. sylvestris,* which is widespread Eurasian species, occurring as a climbing vine in forests from Spain to Turkmenistan [Alleweldt G., Dettweiler E. 1994; Levadoux L, 1956; Zohary D., Hopf M., 2000]. Subspecies *sylvestris* is dioecious, with female and male flowers occurring in roughly the same proportion in populations and exhibit small, acidic berries relative to cultivated grapes. In the Caucasus, and especially in Georgia, *Vitis vinifera ssp. sylvestris* was very abundant and morphologically variable. Many intermediate wild forms were observed, including those with characters associated with cultivated forms such as white fruits, hermaphroditic flowers, and larger sized seeds. It was proposed that the wild subspecies *sylvestris* has crossed with the cultivated subspecies *vinifera*, producing an array of intermediate forms [Negrul A.M., 1946].

In our earlier studies we analyzed seven Georgian cultivars (Rkatsiteli, Saperavi, Tavkveri, Goruli mtsvane, Aleksandrouli, Chkhaveri and Ojaleshi) at six microsatellite loci: They are characterized by large number of equally frequent alleles, which is typical for the systems with large number of equally frequent alleles [Tabidze V. et al., 2006]. The presented paper is a first attempt to carry out microsatellite analysis of Georgian wild grape.

Materials and Methods

The wild grape samples were collected from different geographic zones of Georgia and one sample was taken from Turkey. Trueness to wild accessions were based on ampelographical analysis of collected grape leaves, which were confirmed by comparing with the morphological descriptions of typical *Vitis vinifera ssp. sylvestris*. In most cases the flowers were also investigated. In the Table 1 the list of investigated wild grapevine samples, with the indication of their geographic locations are given.

Table 1. List of studied wild grapevine samples, with the indication of their geographic locations.

	Vitis vinifera spp. sylvestris specimens	Geographic location of *Vitis vinifera spp. sylvestris* specimens	
1	G-6	Gardabani State protected woodland, Gardabani district	41° 22' 554"N 45° 03' 704"E
2	Q-1	Village Ksovrisi, Mtskheta district	41° 59' 17"N 44° 30' 56"E
3	B-1	Likani, Borjomi district	41° 47' 51,5"N 43° 20' 46,2"E
4	B-3	Chobiskhevi, Borjomi district	41°47'441"N 43°18'149"E
5	S-8	Korugi forest reserve, Sagaredjo district	41°37'48"N 45°27'11"E
6	Qiz-1*	Vashlovani protected woodland, Pantishari gorge, Dedoplistskaro district,	
7	Seva	Village Ruispiri, Telavi district	
8	T-1*	Artvin vilaiet, Turkey	

* The samples were presented by Prof.A.Gegechkori and Dr. N.Lachashvili

Total genomic DNA was isolated from young grape leaves. The leaves were ground in liquid nitrogen. For DNA isolation the CTAB based extraction procedure was used [Lodhi M.A., et al., 1994]. In the case of silica dried leaves DNA was isolated by Plant genomic DNA extraction miniprep system (VIOGENE, USA). Three-step PCR was performed in 25 μl of reaction volumes using Promega PCR Master Mix System: PCR Master Mix solution containing Taq DNA Polymerase, dNTPs, $MgCl_2$ and reaction buffers at optimal concentrations for efficient amplification of DNA template by PCR. 20 pmole primers were used in each reaction (Primers were synthesized by Sigma Genosys Company). Amplification was carried out by using Techne PCR amplifier. PCR conditions are described in our earlier paper [Tabidze V. et al., 2006]. For the electrophoresis 2,5 μl of each PCR product was loaded on 8% polyacrylamide/urea sequencing gel, electrophoresed by 2117 Multiphor II LKB Electrophoretic system and visualized by silver staining technique according to Promega Manual. Allele sizes were determined using defined size markers. As a control, DNA of two French cultivars (Cabernet Sauvignon and Pinot Noire) and our earlier-studied two Georgian cultivars (Rkatsiteli and Saperavi) were used [This P., et al., 2004, Tabidze V. et al., 2006,].

Results and Discussion

Table 2 represents microsatellite profiles of wild grape specimens. Eight studied grape samples at four VVMD7, VVMD27, VVS2, ZAG62 polymorphic microsatellite loci generate 39 alleles and observed heterozygosity (Ho) is ranged from 0.857 to 1.0, with mean value 0.897. Number of alleles varied between 9-12, with a mean value 9.75; an expected heterozygosity is also high: ranged from 0.856 at locus VVMD7 to 0.906 at VVMD27 loci, with mean value 0.874 (Table 3). Mean value of genetic variability of Georgian wild grape is much higher compared to wild grape samples from Tunisia and France [Aradhya M.K., et al., 2003]. The most heterogeneous

locus for Georgian wild grapes is VVMD27. It contains 12 alleles ranged from 175 to 227 bp. 227 bp long allele was outside the size range represented in the literature: In our experiments two such alleles in specimens Q-1 and B-3 were detected [This P., et al., 2004].

Table 2. Genetic profiles of Georgian wild grapevine populations at 4 microsatellite loci. Allele sizes are given in base pairs (bp).

Vitis vinifera spp. sylvestris specimens	*ZAG 62*	*VVMD 27*	*VVS2*	*VVMD 7*
G-6	196 –192	189 – 177	139-131	254 - 235
Q-1	190 –190	227 – 201	145 - 129	259 - 247
B-1	206 – 202	191 – 191	139 - 135	249 - 243
B-3	198 –198	227 – 217	141 - 135	252 - 240
S-8	204 –198	187 – 181	143 - 133	245 - 235
Qiz-1	200 – 188	195 –175	139 - 137	246 - 240
Seva	202 –196	185 –179	143 - 141	-
T-1	-	189 – 185	135 - 133	246 - 246

Table 3. Genetic variability of Georgian wild grapevine populations at 4 microsatellite loci.

	ZAG 62	VVMD 27	VVS2	VVMD7	Mean
Number of alleles	9	12	9	9	9.75
Observed heterozygosity (Ho)	0.857	0.875	1.0	0.857	0.897
Expected heterozygosity (He)	0.867	0.906	0.867	0.856	0.874

Our previous study revealed, that Georgian grape cultivars are characterized with high level of genetic variability: seven studied varieties at six microsatellite loci generates 50 alleles, with 0.833 average observed heterozygosity, which is much higher than already available data of analyzed *Vitis vinifera* accessions of European countries [Tabidze V. et al., 2006, Vouillamoz et al., 2006]. Comparison of genetic variability of cultivated and wild grape revealed high level of genetic diversity in both groups of plant. Extent of genetic polymorphism in wild accessions is only slightly higher, than in cultural varieties, which confirms the idea, that the wild subspecies *sylvestris* has easily crossed with the cultivated subspecies *vinifera*, producing an array of intermediate forms [Negrul A.M., 1946]. Obviously, specific climatic conditions of this region were more favorable for the natural selection of wild variety forms, which gave rise to multitude of new grape varieties adapted to this specific area, where they have been selected by human activities.

References:

Alleweldt G., Dettweiler E. *The genetic resources of Vitis: world list of grapevine Collections*, 2nd edition, 1994.

Aradhya M.K., Dangl G.S., Prins B.H., Boursiquot J-M, Walker M.A., Meredith C.P., Simon C.J. *Genetic structure and differentiation in cultivated grape, Vitis vinifera.* L.Genet.Res.Camb., **81**, 179-192, 2003.

Levadoux, L., *Les populations sauvages de Vitis vinifera. L.* Anales d'amelioration des plantes, **6**, 59-118, 1956.

Lodhi M.A., Ye G-N., Weeden N.F., Reish B.I. *A simple and efficient method for DNA extraction from grapevine cultivars, vitis species and ampelopsis.* Plant Mol.Biol. Reports, **12**, 6-13, 1994.

Negrul A.M. Ampelography of USSR, vol. I, Moscow, "Pischeprom", 1946 (Russian).

Tabidze V., Gamkrelidze M., Gotsiridze V., Glonti T., Beridze T. *Microsatellite analysis of Vitis vinifera L. Georgian cultivars.* Bulletin of the Georgian Academy of Sciences. 174, 333-336, 2006.

This P., Jung A., Boccacci P., Borrego J., Botta R., et al. *Development of a standard set of microsatellite reference alleles for identification of grape cultivars.* Theor.Appl.Genet., **109**, 1448-1458, 2004.

Vouillamoz J.F. et al. *Genetic characterization and relationships of traditional grape cultivars from Transcaucasia and Anatolia.* Plant Genetic Resources, **4**, 144-158, 2006.

Zohary D., Hopf M. *Domestication of Plants in the Old World.* Oxford, 2000.

ქართული ველური ვაზის მიკროსატელიტური ანალიზი

გამყრელიძე მ.[1], ტაბიძე ვ.[1], გოცირიძე ვ.[2]

[1]ს.დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი
[2]მებაღეობის, მევენახეობის და მეღვინეობის ინსტიტუტი

(მიღებულია 12.03.2008)

რეზიუმე

შესწავლილია ველური ვაზის სხვადასხვა ნიმუშთა მიკროსატელიტური პოლიმორფიზმი, საიდანაც შვიდი მათგანი საქართველოს სხვადასხვა გეოგრაფიული ზონებიდან, ხოლო ერთი თურქეთიდან იქნა აღებული. აღნიშნულ ნიმუშთა ოთხი მიკროსატელიტური ლოკუსი შესწავლილია VVMD7, VVMD27, VVS2, და ZAG62 პრაიმერების გამოყენებით. ჩვენს მიერ ადრე შესწავლილ კულტურული ვაზის ქართული ჯიშების მსგავსად ქართული ველური ვაზის პოპულაცია ასევე გენეტიკური ვარიაბელობის მაღალი ხარისხით ხასიათდება: ველური ვაზის პოპულაციის გენეტიკური პოლიმორფიზმი მცირედ აჭარბებს შესწავლილ კულტურულ ჯიშთა ვარიაბელობის ხარისხს.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 1-2, 2008.

Biotechnology

TWO –STAGE ANAEROBIC PROCESS FOR COMBINED PRODUCTION OF BIOHYDROGEN AND BIOMETHANE FROM ORGANIC FRACTION OF MUNICIPAL SOLID WASTES

DUDAURI T.[1], CHACHKHIANI M.[1], TSIKLAURI L.[1], METREVELI B.[2], IASHVILI T.[3], ZAKARIASHVILI N.[3], UGREKHELIDZE V.[1]

[1] *National High Technology Center of Georgia,*
[2] *Batumi State University,*
[3] *Durmishidze Institute of Biochemistry and Biotechnology*

(Received March 24, 2008)

Abstract

To develop two-stage anaerobic fermentation process with hydrogen and methane combined production from organic wastes the following R&D studies have been performed: (i) development of cost effective method for splitting of anaerobic process in hydrogen and methane fermentations; (ii) isolation, study and cultivation of hydrogen producing anaerobic bacteria; (iii) development of optimum conditions for biohydrogen enhanced and sustainable production from organic fraction of municipal solid wastes (OFMSW) and (iv) estimation of energy output from organic wastes under two-stage anaerobic process with hydrogen and methane combined production. As a result of these studies splitting of anaerobic hydrogen and methane fermentation was achieved in the economical way through the use of 10% NaOH solution. In this case activity of spore forming methanogens are practically fully depressed and methane content in obtained biohydrogen does not exceed 0,5-0,6 %. Fermentation of pretreated OFMSW by using co-cultures of thermophilic anaerobic cellulolitic and saccharolytic bacteria of *Clostridia sp.* provides significant increase of hydrogen cumulative production. Methane yield from the metabolic products of dark hydrogen fermentation such as volatile fatty acids and low atomic alcohols at thermophilic conditions made 471 l per kg of dry organic matter. Comparing energy data for two-stage anaerobic process with those for one-stage methane fermentation it can be concluded: 1. In terms of electricity production energy output from OFMSW increases by 26% under two-stage anaerobic process with hydrogen and methane combined production. 2. In terms of heat production energy output from OFMSW increases by 23% under two-stage anaerobic process. Two-stage anaerobic fermentation technology providing hydrogen and methane combined production from organic wastes can be implemented at large scale biogas plants improving process economy. It should be underlined, that introduction of such technology at the existing biogas plants will need low investments.

Key words: Biohydrogen, Biomethane, Anaerobic fermentation, Organic fraction of municipal solid wastes (OFMSW).

Introduction

Nowadays, due to the commercialization of low cost and "CO_2 neutral" hydrogen fuel cells that convert chemical energy of hydrogen directly to electricity with 50-60% electric

efficiency at all system scales, development of sustainable biological hydrogen production from organic residues becomes the important scientific and technological point [Dunn, 2001].

Currently known biological hydrogen production processes are classified as direct biophotolysis, photo-fermentations, indirect biophotolysis, water-gas shift reaction, and so called dark fermentation. A lot of publications have reported on the groundwork for creating renewable hydrogen production systems through photobiological processes performed by photoautotrophic and photoheterotrophic microorganisms. The main drawback of the process is that oxygen produced simultaneously with H_2 inhibits hydrogenase that is extremely sensitive to oxygen. This fact sets a limit to the photochemical efficiency that referring to literature data does not exceed 1.5%. Another group of phototrophic microorganisms such as photoheterotrophic bacteria also use captured solar energy and produce hydrogen from organic substrates like fatty acids. This process is catalyzed by the nitrogenase enzyme which like hydrogenase is highly sensitive to oxygen and in addition is inhibited by ammonium ions. So this process must be conducted under strict anaerobic conditions free of N_2 with limited concentrations of nitrogen sources. The maximum photochemical efficiency of photoheterotrophic hydrogen production can be calculated theoretically, and is approximately 10% on a full solar spectrum basis. In contrast to phototrophic microorganisms, anaerobic bacteria convert a large variety of carbohydrate sources (frequently obtained as refuse or waste products) to hydrogen, CO_2 and metabolites such as volatile fatty acids and alcohols under anaerobic conditions. These bacteria use organic substances as the sole source of electrons and energy converting them into hydrogen [Nadi & Sengupta, 1998]. Since the light is not required to provide additional energy, this process is termed to "dark" hydrogen fermentation. However, dark hydrogen fermentation is an incomplete oxidation. This means that organic matter is oxidized to intermediates like volatile fatty acids, alcohol etc. In natural environments hydrogen producing bacteria species coexist with methanogens which consume the above metabolites including hydrogen and produce their own end products like methane and CO_2. So the most difficulty is to obtain hydrogen as the final product of dark fermentation when mixed microbial cultures readily available in nature such as compost, anaerobic digester sludge, soil etc. are employed for bioconversion of organic wastes [Ueno et al, 2001]. To overcome this drawback some methods like heat treatment of organic wastes and methods based on application of chemicals such as bromethanesulfonate have been successfully tested for the inhibition of methanogenesis at the lab-scale, but it is evident that such approach can not be used for large scale systems.

As for hydrogen producing bacteria, thermophilic and hyperthermophylic anaerobes like *Clostridia sp* show highest process performance and thus these bacteria are the microorganisms of choice for dark hydrogen fermentation [US DOE Nat. Biofuels Program].

Materials and Methods

The works on biohydrogen and methane combined production from OFMSF included the following research: splitting of anaerobic process in hydrogen and methane fermentation; isolation, study and cultivation of hydrogen producing anaerobic bacteria of *Clostridia sp*; development of optimum conditions for biohydrogen enhanced and sustainable production; study of optimal conditions for methane intensive and stable generation from dark hydrogen fermentation by-products like volatile fatty acids and alcohol and estimation of energy efficiency for both: single-stage anaerobic treatment of OFMSW with solely methane production and for two-stage anaerobic processing of OFMSW with hydrogen and methane combined production.

1. Splitting of anaerobic process in hydrogen and methane fermentations. Series of batch experiments aiming at the depression of methanogens under mixed culture fermentation were dedicated to studies of pH influence on the splitting of dark hydrogen and methane production in two discrete stages. For this purpose 10% solution of NaOH was added to the substrate in such

amount that pH was in the range of 9 -10. Monitoring parameters were gas composition and productivity. Gas composition was measured by using gas chromatographs (Perkin Elmer) first equipped with flame ionization detector (for methane measuring) and the second chromatograph with thermal detector for determining hydrogen concentration in obtained biogas. Gas-flow meter coupled with biofermentor where fermentation of OFMSW was performed provided measurement of amount of obtained biogas.

2. Isolation, study and cultivation of hydrogen producing anaerobic bacteria. Mixed culture of hydrogen producing bacteria species was isolated from the soil and water samples of the hot springs in Tbilisi. In order to depress activity of non-spore forming anaerobic bacteria, initially the above samples have been pretreated at 90°C during 15 min. Spore forming hydrogen producing bacteria of *Clostridia sp* were isolated and cultivated by using the following nutrient media (g/l): NH_4Cl -2.0; $KHPO_4 \times 3H_2O$ – 4.0; KH_2PO_4 - 2.0; $MgCl_2 \times 6H_2O$ – 0.42; $CaCl_2$ – 0.05; solution of microelements – 1 ml; yeast extract – 0.3; $Na_2S \times 9H_2O$ -0,5. Cultivation was performed at 55°C in the temperature controlled anaerobic box with inert gas inlet and outlet systems. Cultivation took 10 days. Obtained bacteria cultures were purified by using agar-agar solid nutrient media. For study of bacteria colonies isolated from swampy soil, forest soil, municipal landfill and cattle dung the solid and liquid nutrient media of the same composition were used. Bacteria growth rate in liquid media was determined by the optical density change. Perkin Elmer UV-VIZ spectrophotometer - Lambda EZ- was used for these purposes. Morphological characteristics of the stained sections of bacteria after 3, 5 and 10 days of cultivation were studied by using light microscope. Metabolic products such as: methyl and ethyl alcohol, formic acid, acetic acid, propionic acid, valeric acid, isovaleric acid butyric acid, isobutiric acid, valeric and isovaleric acids were measured on Perkin Elmer gas chromatograph AutoSystem XL with flame ionization detector. Carbon dioxide was determined by using Perkin Elmer IR spectrometer - Spectrum RX. Hydrogen was measured on gas chromatograph with thermal detector.

3. Development of optimum conditions for biohydrogen enhanced and sustainable production. Here influence of pretreatment of OFMSW (by using freeze explosion and mechanical milling of frozen organics) on biohydrogen yield was studied. For this CO_2 based "freeze explosion" method (similar to ammonia "freeze explosion" process) was used. The idea is based on the contact of cellulosic wastes with pressurized CO_2 followed by rapid release of carbon dioxide pressure that results freezing of organic matter. After a given contact time, when the carbon dioxide pressure is released the liquid carbon dioxide evaporates. The temperature decrease associated with evaporation of the volatile liquid tends embrittle cellulose fiber and enhance the effect of the pressure release on overall fiber disruption. During the experiments contact of liquid CO_2 and cellulosic wastes was carried out in high pressure autoclave vessel. Organic fraction of MSW containing cellulosic materials like filter paper, newsprint, wood chips and cardboard were first impregnated with water and charged to the vessel. 1, 7 kg CO_2 was introduced in the above vessel and pressurized under 65 atm. After 1 hour, pressure of liquid carbon dioxide was gradually released. By the end of the process the vessel was opened and the frozen wastes were milled in the crusher. Enzymatic hydrolysis (performed by using the enzyme complex of *Trichoderma reesi*) was used to monitor the digestibility of above wastes. In order to develop optimum conditions for bio-hydrogen sustainable production hydrogen production was studied as the function of following parameters: concentration of organic solids, pH, T and hydraulic retention time.

4. Development of optimal conditions for methane intensive and stable generation from by-products of dark hydrogen fermentation. Studies included optimization of bio-methane production from the metabolites of dark hydrogen fermentation such as volatile fatty acids and alcohols. Experiments were conducted both in mesophilic and thermophilic conditions using the plug flow bio-reactor. Monitored parameters were gas production and composition and concentration of total fatty acids in effluent. Analyses of volatile fatty acids have been performed

using Perkin Elmer gas chromatograph with flame ionization detector. Total fatty acids were measured by using HPLC (Gilson).

Based on results obtained energy output for both single stage anaerobic treatment of OFMSW with solely methane production and for two-stage anaerobic processing of OFMSW with hydrogen and methane production at two discrete steps was estimated.

Results and Discussions

When using 10% solution of sodium hydroxide for inhibition of methanogenic activity, non-spore forming methanogens were fully inactivated. As for hydrogen producers their activity was not diminished. Under such conditions at 55°C hydrogen cumulative production came to 40.6 l/kg dry organic matter. This fact could be explained in such a way that in contrast to hydrogen producers, methanogenic bacteria are active only in the limited range of pH (6-7) and under alkali or acid environment they become fully depressed. Under conditions mentioned above, methane production practically stops. Alkali environment at the same time promotes substrate's solubilization and hydrolysis that in its turn provides enhanced yield of hydrogen.

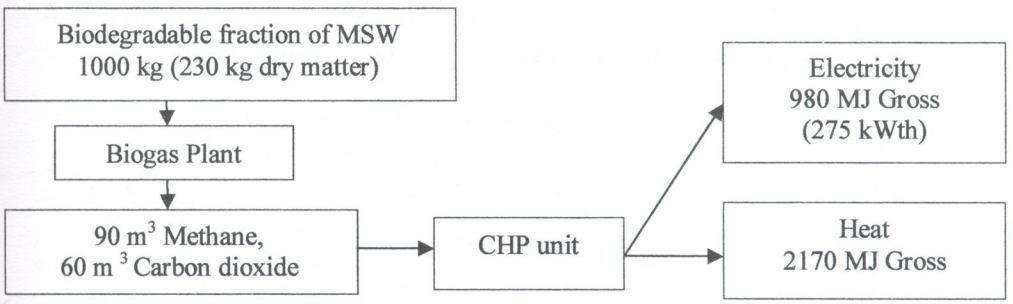
Morphological and biochemical studies of mixed cultures of anaerobic thermophilic hydrogen and methane producing bacteria isolated from swampy soil, forest soil, municipal landfill and cattle dung showed that three main microorganisms dominated in the above samples: *Clostridium thermocellum:* The bacterial cells of isolated culture have a rod like shape. The young cells are Gram positive. The round spores with terminal arrangement were not observed. These bacteria are strict anaerobes with an optimal growth temperature of 55-58°C and pH of 7.0. However the strain of *Cl. thermocellum* was also active at pH 9, but activity was significantly decreased in 8 hours. *Cl. Sacharoliticum:* In the liquid nutrient media isolated and purified bacteria grow by making the medium equally muddy. Formation of granules is not observed. Colonies grown on the solid media mostly are of R form, diameter – 1-2 mm. Colonies are of brown colour, dry and with clogged edges. The cells of these bacteria have a rod-like shape. Their spores are round with terminal arrangement. During the spore formation the sporangium swells. During the bacteria growth in liquid media pH drops because of fatty acids formation. The young cells are Gram-positive. The bacterium is a strict anaerobic thermopile. Optimal growth temperature is 50-55^{0}C and optimal growth value of pH is 7.0. These bacteria are active in sugars fermentation. **Three types of Methane forming bacteria were observed:** 1.Thin, slightly curved rods of cylindrical form, non-spore forming, and the ends are rounded. Cells are single or in chains of 2-4 cells. 2. Rods with flat end both long and hair like form with sizes 1-1.5x2-2.5 μm. Cells are both Gram positive and Gram negative. Spores and capsules are not observed. Cells of hair-like rods are placed in shells. Inside the shell they are divided by partitions. Single cells having shells can be met too. 3. Spherical-like shape cells with diameter of around 1.5-2.5 μm, form chains. By comparing obtained results with data published in: "The Bergey's manual of determinative bacteriology" we suppose that bacteria isolated on mineral nutrient media described above, belong to *Methanobacterium thermoformicicum Methanotrix and Methanococcus.*

Cumulative biohydrogen production from OFMSW in thermophilic conditions under pH 9 by using co-cultures of *Cl. thermocellum* and *Cl. Sacharoliticum* reaches 41 l/kg dry organic matter. Hydrogen production started in 5-6 hours and was finished in 8 hours. Methane content in obtained biogas didn't exceed 0.1-0.2%. The highest cumulative yield of hydrogen - 52 l/kg dry organic matter- was achieved when fermenting pretreated OFMSW with above co-cultures. Studies on optimization of bio-hydrogen sustainable production in continuous flow system showed that optimum hydrogen production can be reached when percentage of organic solids and carbohydrates

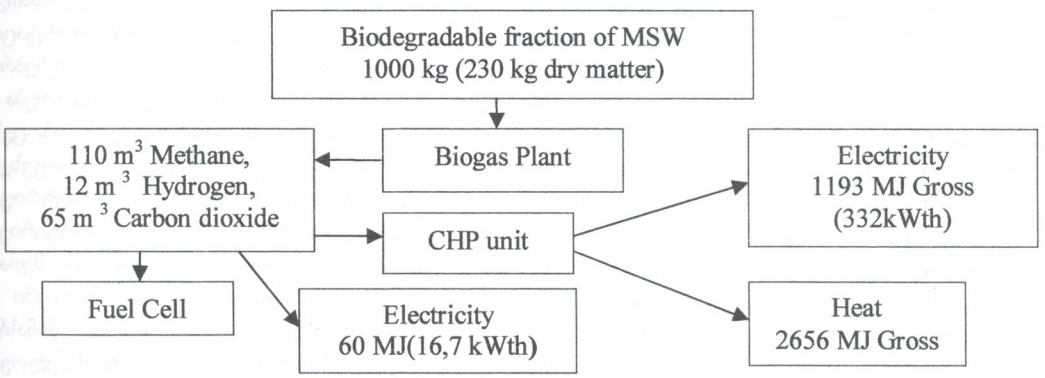
do not exceed 6% and 5000 γ/ml correspondingly. Percentage of total fatty acids which are metabolite products of anaerobic hydrogen fermentation reached 7 g/l.

Studies related to optimization of bio-methane production from the metabolites of dark hydrogen fermentation such as volatile fatty acids and alcohols show that under thermophilic conditions more organics could be processed in shorter time than under mesophilic process. The optimum organic loading rate is 2.3 g/l of total fatty acids. HRT was defined as 5 days. Using the lab scale upflow anaerobic reactor built for bio-methane production in thermophilic conditions the cumulative production of biomethane was determined experimentally. When using cattle manure as inoculum (source of methanogenic bacteria), methane cumulative production in thermophilic conditions makes 478 l/kg dry organic matter.

Energy output from OFMSW with solely methane production:



Energy output from OFMSW through two-stage anaerobic fermentation:



Comparing energy data for two-stage anaerobic process with those for one-stage methane fermentation it can be concluded the following: 1. In terms of electricity production energy output from OFMSW increases by 26% under two-stage anaerobic process with hydrogen and methane combined production. 2. In terms of heat production energy output from OFMSW increases by 23% under two-stage anaerobic process. Thus, two-stage anaerobic fermentation technology providing hydrogen and methane combined production from OFMSW can be implemented at large scale biogas plants that will significantly improve process economy.

Acknowledgments: The work was financially supported by the ISTC, under the project G-891.

References:

Dunn, S. *Hydrogen futures: Toward a Sustainable Energy System.* Paper 157, Worldwatch Institute, Washington, DC, 2001.

Nandi R., Sengupta S. *Microbial production of hydrogen: an overview.* Crit Rev Microbiol., **24**, 61-84, 1998.

Ueno Y.U., Haruta S., Ishii M., Igarashi Y. *Microbial community in anaerobic hydrogen-producing microflora enriched from sludge compost.* Appl Microbiol Biotechnol., **57**, 555-562, 2001.

US DOE National Biofuels Program: www.ott.doe.gov/biofuels/

წყალბადისა და მეთანის კომბინირებული მიღება მუნიციპალური მყარი ნარჩენების ორსაფეხურიანი ანაერობული ფერმენტაციით

დუდაური თ.[1], ჩაჩხიანი მ.[1], წიკლაური ლ.[1], მეტრეველი ბ.[2], იაშვილი თ.[3], ზაქარიაშვილი ნ.[3], უგრეხელიძე ვ.[1]

[1] საქართველოს მაღალი ტექნოლოგიების ეროვნული ცენტრი;
[2] შ. რუსთაველის სახ. ბათუმის სახელმწიფო უნივერსიტეტი
[3] დურმიშიძის სახ. ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 24.03.2008)

დამუშავებულია ორსაფეხურიანი ანაერობული ფერმენტაციის ტექნოლოგიის საფუძვლები ორგანული ნარჩენებიდან წყალბადისა და მეთანის კომბინირებული მიღებისათვის. ამისათვის ჩატარებული იქნა შემდეგი კვლევები: (i) მეთანური და წყალბადური ფერმენტაციის პროცესების განცალკევება ეკონომიკურად ეფექტური მეთოდის გამოყენებით; (ii) ბუნებრივი წყაროებიდან წყალბადის პროდუცენტი ბაქტერიული კულტურების გამოყოფა, კულტივირება და შესწავლა; (iii) წყალბადის მიღების ოპტიმალური პირობების დამუშავება და (iv) პროცესის ენერგეტიკული ეფექტურობის შეფასება. მეთანური და წყალბადის ფერმენტაციის პროცესების განცალკევება მიღწეულ იქნა ისეთი იაფი რეაქტივის გამოყენებით როგორიცაა ნატრიუმის ჰიდროქსიდი (10%-იანი ხსნარი). აღნიშნულ პირობებში მეთანწარმომქმნელი ბაქტერიების აქტივობა პრაქტიკულად სრულად ითრგუნება და შესაბამისად მიღებულ ბიოწყალბადში მეთანის შემცველობა არ აღემატება 0.5-0.6%-ს. კლოსტრიდიუმის გვარის ცელულოლიტიკური და შაქრების ამთვისებელი ანაერობული თერმოფილური ბაქტერიების გამოყენებით წინასწარ დამუშავებული სუბსტრატის ფერმენტაციისას, წყალბადის ჯამური გამოსავალი მნიშვნელოვნად იზრდება და შეადგენს 52 ლ/კგ მშრალი ბიომასა. წყალბადური ფერმენტაციის თანაური პროდუქტების, ცხიმოვანი მჟავებისა და დაბალმოლეკულური სპირტების შემდგომი ანერობული ფერმენტაციით (სადაც საქონლის ნაკელი გამოიყენება მეთანწარმომქმნელი ბაქტერიების წყაროდ) მეთანის ჯამური გამოსავალი შეაგენს 471 ლ მეთანი/კგ მშრალი ორგანული მასა. ორსაფეხურიანი და ერთსაფეხურიანი (მხოლოდ მეთანის წარმოქმნა) ანაერობული ფერმენტაციის პროცესების ენერგეტიკული მაჩვენებლების შედარებამ აჩვენა, რომ ორსაფეხურიანი პროცესის შედეგად, სადაც კომბინირებულად მიიღება მეთანი და წყალბადი, ელექტროენერგიის წარმოება შესაძლებელია გაიზარდოს 26%-ით, ხოლო თბური ენერგიის წარმადობა – 23%-ით. ამრიგად, მუნიციპალური მყარი ნარჩენების ორგანული ფრაქციის გადამუშავების ორსაფეხურიანი ანაერობული ფერმენტაციის დანერგვით დიდი წარმადობის ბიოგაზის საწარმოო ქარხნებში მნიშვნელოვნად გაიზრდება პროცესის ენერგეტიკული და ეკონომიკური მაჩვენებლები.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 1-2, 2008.

Biotechnology

SELECTION OF MICROSCOPIC FUNGI - XYLANASES PRODUCERS

*KVESITADZE E., KUTATELADZE L., JOBAVA M., ZAKARIASHVILI N., KHOKHASHVILI I., DZALAMIDZE I., LASKHISHVILI E.

Durmisnidze Institute of Biocemistry and Biotechnology, David Agmasheneblis Kheivani, 10km. Tbilisi 0159, Georgia
E-mail: kvesitadze@hotmail.com

(Received March 24, 2008)

Abstract

The collection of microscopic fungi isolated from different ecological niches of the South Caucasus and accounting more than 2000 cultures has been created. Some strains of fungi including several extremophiles actively producing xylanase under submerge conditions of cultivation have been selected. In the collection among xylanase producers dominate the representatives of the following genera: *Aspergillus, Penicillium* and *Trichoderma.* Among active producers of xylanase should be noted *Penicillium canescens* 41 (mesophile), *Aspergillus niger* A 7-5 (extreme halophile, thermotolerant), *Trichoderma viride* X1-6 (alkaliphile). These strains are distinguished by having cellulase activities at zero level at high activity of xylanase. Such strains are rare exception in nature. As a result of myco-toxicological studies it was stated that the selected strains are nontoxic and nonpathogenic. The physiology and some biochemical characteristics of the selected strains have been investigated, the nutrient media for each particular strain was optimized and conditions of growth were established. The strain *Penicillium canescens* 41 reveals the highest xylanase activity at 27°C and pH 4.0; *Aspergillus niger* A 7-5 at 40°C, pH 6.0; *Trichoderma viride* X1-6 at 30°C, pH 7.5. As a result of optimization of the nutrient media the activities of xylanase are increased by 100, 75 and 60 %, respectively. The preparation of xylanase has been obtained and the temperature and pH optimum for xylanase action has been revealed. For the xylanase produced by the strain *Penicillium canescens* 41 the optimal conditions for enzyme action are at temperature 45-50°C and pH 4.4; for *Aspergillus niger* A 7-5 - at 65°C and pH 6.5 and that of *Trichoderma viride* X1-6 - at 50-55°C and pH 7.8-8.5.

Key words: xylanase, extreme halophile, thermotolerant, alkaliphile, microscopic fungi.

Introduction

Microorganisms are considered to be the most prospective source for enzymes in both small and large scale production [Wipapat et al., 2002; Kvesitadze, 1999]. In addition to differing stabilities, especially in case of extremophilic microorganisms, enzymes from microorganisms have a number of advantages: unlimited wide spectrum of enzymes variety; short period of the time needed for the production of enzymes; possibility to increase the level of any particular enzyme

biosynthesis via the selection of nutrient media and cultivation conditions; wide possibilities of gene cloning.

In recent years the interest towards the stable to different conditions enzymes and organisms of their producers has exstremely increased due to the wast potential of their application in enzyme production. Xylanase hydrolizing hemicellulose and actively participaiting in wooden material degradetion, atracts attention due to its application in a number of biotechnologies. stable forms of this enzyme having increased resistance to different extreme conditions are especially interesting.

Materials and Methods

The selection of microscopic fungi was carried out from strains isolated from different ecological niches of the south Caucasus. The action of temperature, pH and salt concentration (NaCl) on growth, development and production of xylanase by *Penicillium canescens* 41, *Aspergillus niger* A 7-5, and *Trichoderma viride* X1-6 has been investigated.

The microscopic fungi were cultivated in the temperature range of 5-55°C, with the temperature interval of 5°C, and at pH in the range from 2.0 to 10.0 with the 0.5 pH interval of nutrient medium. The optimal values of temperature and pH provided the maximal fungi colony growth is determined according to the colonies diameters and growth rate.

For the revelation of galophylicity of strains NaCI was added to nutrient medium in concentrations from 0.5M to 4.0M.

The active enzymes producers screening was conducted by submerged cultivation of strains. 10-days conidia culture suspensions served as sowing material. The submerged cultivation of microscopic fungi strains was performed in 750 ml conical Erlenmeyer flasks on the thermostatic shaker at 180-200 rpm, at different temperatures, during 72 hours.

For the production of xylanases submerged cultivation was carried out in a liquid medium of following composition, %: soy bean flour – 3.0; $NaHPO_4$ – 1.5; $(NH_4)_2SO_4$ – 0.2; KCl – 0.05; $MgSO_4$ – 0.015. In case of cellulase production, cultivation was carried out in a liquid medium, %: microcrystalline cellulose-1.0, $NaNO_3$-0.3, KH_2PO_4-0.2; $MgSO_4 \times 7H_2O$-0.05; maize extract-1.5.

The activities of the enzymes were determined in the cultural filtrates of the strains.

For the detection of xylanase activity the Michael method was used [Bailey et al., 1992]. Total cellulase activity was determined by the method of Ghose, based on the potential of cellulase to perform hydrolysis of insoluble substrates (filter paper) to reducible mono- and oligosaccharides [Ghose, 1987]. Reducing sugars were estimated according the Somogyi-Nelson method [Nelson, 1944; Somogyi, 1952].

Zoopathogenicity of the studied cultures was investigated by intravenous injection of the fungal suspension to rabbits [Ohga et al., 1966]. The method of Berestetsky (1969) was used to determine phytopathogenicity. Toxicity was studied by Diekman technique [Diekman, 1992].

The xylanase crude preparations have been obtained via the common methodology. Culture liquid was filtered, than cooled up to 4°C, and added acetone, or ethyl alcohol, or isopropanol in various quantities.

The temperature optimum of the action of xylanase was measured at temperature range from 20 to 80°C.

pH optimum of the action of xylanase has been determined in a range from 2.0 to 10.0.

Results and Discussion

Since the natural conditions play a significant role in growth, development and physiological functions of microscopic fungi determining their variety [Wipapat et al., 2002; Kvesitadze, 1986], the strains were isolated from different soil-climatic zones to obtain diverse experimental material. The collection of microscopic fungi isolated from different ecological niches of the South Caucasus and accounting of more than 2000 cultures has been created.

As a result of carried out studies among the various genera of microscopic fungi the excistance of active producers of xylanases and cellulases have been detected. As it has been determined some of them belong to fungi-extremophiles group.

The strains of active producers of xylanase and cellulases were selected through screening of microscopic fungi cultures under submerged cultivation.

Among the cellulases and xylanases producers prevail the representatives of *Aspergillus*, *Trichoderma*, *Sporotrichum* and *Penicillium* genera. As it is seen (Fig. 1), among the producers of xylaneses and cellulases there are a number of thermophilic, alkaliphilic, acidophilic and halophilic microscopic fungi strains. As expected, most of xylanase producers simultaneously synthesized cellulases. Especially high xylanase and cellulase activities were exposed by two thermophilic cultures: *Sporotrichum pulverulentum* 43 and *Chaetomium thermophile* I1.

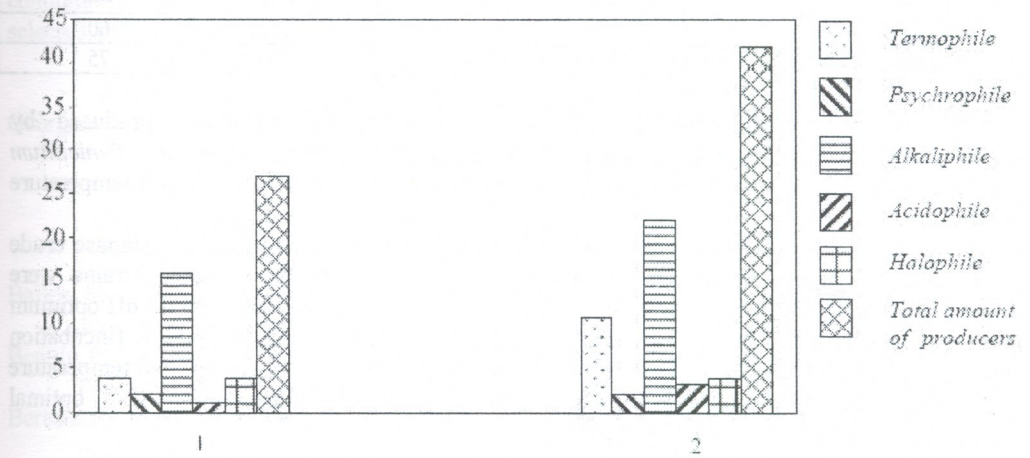


Fig. 1. Extremophilic microscopic fungi producers of enzymes; 1. Producers of xylanases; 2. Producers of cellulases.

It should be also underlined that the existence of several strains, producers of cellulases free xylanases has been detected. Finally, for further experiments three physiologically differing strains were selected: *Penicillium canescens* B41 (mesophile), *Aspergillus Niger* A7-5 (extreme halophile/thermotolerant), and *Trichoderma viride* X1-6 (alkaliphile).

According to myco-toxicological studies it was stated that the selected strains are neither toxic nor pathogenic and maybe successfully used in a various industrial and agricultural processes.

The metabolic potential of fungi greatly depends upon the selection of the appropriate nutrient medium and the carbon source plays a special role in enzyme biosynthesis by the mechanisms of repression or induction in some enzymes synthesis [Duarte, 1994; Beguin, 1994].

The temperature is extremely important factor for microorganism's growth and physiological activity, firstly the effect of temperature on the enzyme production was studied. Taking into consideration that the temperature optimum of growth of selected strains while their submerged cultivation was determined within 30-55°C, *Penicillium canescens* B41 revealed

maximum of xylanase activity at 27°C, *Aspergillus niger* A7-5 – at 40°C, and *Trichoderma viride* X1-6 – at 30°C.

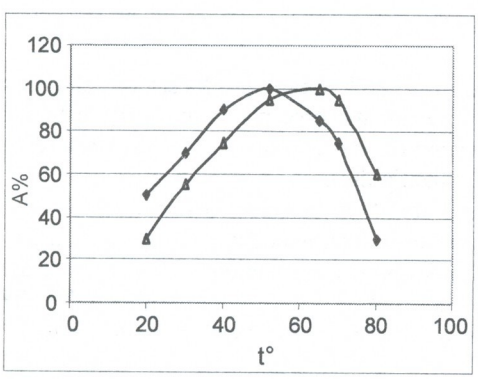
The optimal pH for the growth of these strains has also been determined. Since the strain *Trichoderma viride* X1-6 is alkaliphile, the submerged cultivation of this strain was performed in pH ranges from 6.5 to10.0. In spite of good growth of this strain in all range of pH the highest xylanase activity was determined at cultivation of the strain at pH 7.5, in more alkaline medium the xylanase activity was correspondingly reduced, while at pH 6.0 only 25% of the activity was retained. For strains *Penicillium canescens* B41 and *Aspergillus niger* A7-5 the optimal pH for growth and xylanase production was correspondingly 4.0 and 6.0. Further optimization of growth conditions and nutrient media composition, increased the activities of extracellular xylanase above 60% (Table 1).

Table 1. Microscopic fungi strains, active producers of xylanases

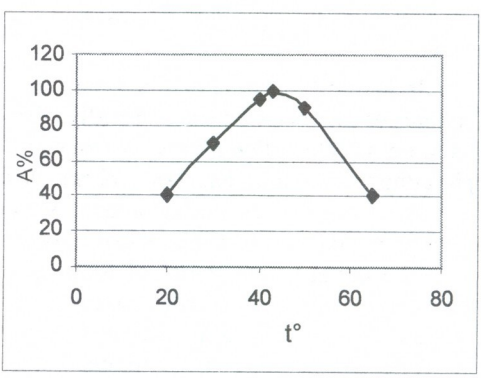
#	Strain	Initial activities, unit/ml	ctivities after optimization of nutrient edia, unit/ml,	Activities after estimation of optimal conditions, unit/ml,	Increased activities, %.
1	*Penicillium canescens* B 41	12.0	18.0	24.0	100
2	*Aspergillus niger* A 7-5	10.0	12.0	16.0	60
3	*Trichoderma viride* X 1-6	16.0	20.0	28.0	75

The comparison of physical-chemical characteristics of xylanases produced by extremophes – *Aspergillus niger* A 7-5 and *Trichoderma viride* X 1-6 and mesophile – *Penicillium canescens* 41 exposed the differences in enzymes stabilities under different pH and temperature conditions.

Ethyl alcohol was found as the best organic solvent for the isolation of xylanase crude preparations The temperature and pH optimums of xylanases of the selected strains were determined (Fig. 2a, b; Fig.3 a, b). As is seen from these figures the temperature and pH optimum of xylanase action from the strain *Penicillium canescens* B41 corresponds to 45-50°C (incubation time 60min) and pH 4.4. For the xylanase from *Trichoderma viride* X1-6 the optimal temperature and pH equal to 50°-55°C and pH 7.8-8.5. For xylanase of *Aspergillus niger* A7-5, optimal conditions of enzyme action are 65°C and pH 6.5.



a)



b)

Fig. 2. Effect of temperature on the action of xylanase. a) 1.*Trichoderma viride* X1-6; 2.

Aspergillus niger A 7-5. b) *Penicillium canescens* B-41

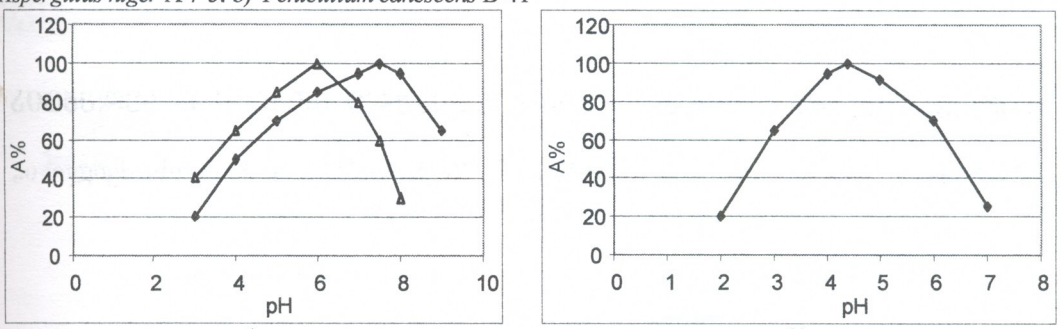


Fig. 3. Effect of pH on the action of xylanase. a) 1. *Trichoderma viride* X1-6; 2. *Aspergillus niger* A 7-5. b) *Penicillium canescens* B-41

Stable cellulases and xylanases from the extremophilic fungi have the potential to convert agricultural wastes and plant indestructible substances into non-toxic, rich in protein biomass. Xylanase of the extremophilic fungi are able to decompose and convert agricultural wastes containing xylan and cellulose. In pulp and paper industry the lack of cellulase synthesis of the selected strains, could be applied for high quality paper production.

Acknowledgments: This work was supported by a grant # **GNSF/ST06/-087** of the Georgian national Science Foundation.

References:

Bailey M.J., Biely P., Pountanen K. *Interlaboratory testing of methods for assay of xylanase activity.* J. Biotechnol., **23**, 257-270, 1992.

Beguin P., Aubert J.P. *The biological degradation of cellulose.* FEMS Microbiol. Rev., **13**, 25-58, 1994.

Berestetski O.A. *About changes of soil microflora by fruit plants in connection with fruit-garden soil toxicity.* In: Proceedings of first inter-university meeting on agrophytocenology, Kazan, 1969.

Diekman M.A., Green M.l. *Mycotoxins and reproduction in domestic livestock.* J. Anim. Sci. **70**, 1615-1627, 1992.

Duarte J.C., Costa-Ferreira M. *Aspergillus and lignocellulosics: enzymology and biotechnological application.* FEMS Microbiol. Rev., **13**, 377-386, 1994.

Ghose T.K. *Measurement of cellulase activities.* Pure Appl. Chem., p.257-268, 1987.

Kvesitadze G.I., Kvesitadze E.G. *Biotechnology.* p.419, Tbilisi, 1999.

Kvesitadze G.I. Enzymes of microscopic fungi cultures developing under extreme conditions. Works of Bach Ins. of Biochemistry of Acad. Sci. of USSR, Moscow, 1986.

Nelson N.A. *Photometric adaptation of the Somogyi method for the determination of glucose.* J. Biol. chem., 153-157, 1944.

Ohga M., Shimizu K., Morita Y. Agric.Biol.Chem. **30**, p.967, 1966.

Somogyi M. *Determination of reducing sugars.* J. Biol. Chem., 195-199, 1952.

Wipapat K., Lange L., Jones N. H., Bhumiratana A. *Alkaline tolerant fungi as a source for Alkaline Enzymes. Mycology Laboratory,* The National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand, 2002.

ქსილანაზის პროდუცენტი - მიკროსკოპული სოკოების სელექცია

კვესიტაძე ე., ქუთათელაძე ლ., ჯობავა მ., ზაქარიაშვილი ნ., ხოხაშვილი ი., ძალამიძე ი., ლასხიშვილი მ.

ს. დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 24.03.2008)

რეზიუმე

შექმნილი მიკროსკოპული სოკოების კოლექცია მოიცავს 2000-ზე მეტ კულტურას, რომლებიც გამოყოფილია სამხრეთ კავკასიის სხვადასხვა ეკოლოგიური ნიშიდან. სკრინინგის გზით, სიღრმული კულტივირების პირობებში შერჩეულია სოკოების შტამები – ქსილანაზის აქტიური პროდუცენტები. მათ შორის გამოვლენილია ექსტრემალურ პირობებში მზარდი კულტურები. ქსილანაზას პროდუცენტებს შორის განსაკუთრებით მაღალი აქტივობებით ხასიათდებოდნენ *Aspergillus*-ის, *Penicillium*-ისა და *Trichoderma*-ს გვარის წარმომადგენლები. ქსილანაზის შერჩეული აქტიური პროდუცენტებიდან დიდი ინტერესი გამოიწვია სამმა შტამმა: *Penicillium canescens* 41 (მეზოფილი), *Aspergillus niger* A 7-5 (ექსტრემალური ჰალოფილი, თერმოტოლერანტი), *Trichoderma viride* X 1-6 (ალკალიფილი), რომლებიც ქსილანაზის მაღალი აქტივობის ფონზე ხასიათდებოდნენ ცელულაზის ნულოვანი აქტივობით. ასეთი შტამები კი ბუნებაში იშვიათად გვხვდება. მიკო-ტოქსიკოლოგიური კვლევებით დადგენილია, რომ შერჩეული შტამები არაა პათოგენური და ტოქსიკური. აღნიშნული შტამების ფიზიოლოგიური და ბიოქიმიური თვისებების შესწავლით სიღრმული კულტივირების პირობებში მოხდა საკვები არეების ოპტიმიზაცია და ოპტიმალური პირობების (ტემპერატურა, pH) დადგენა. აღმოჩნდა რომ, შტამი *Penicillium canescens* 41 მაღალ ქსილანაზურ აქტივობას ამჟღავნებს 27°C-სა და pH 4.0-ზე, *Aspergillus niger* A 7-5 - 40-45°C და pH 6,0-ზე, *Trichoderma viride* X1-6 - 30°C-სა და pH 7,5-ზე. ოპტიმალური პირობების დადგენით, საკვები არეების შემადგენელი კომპონენტების ოპტიმიზაციით შტამების მიერ წარმოქმნილი ქსილანაზას აქტივობები გაზრდილია შესაბამისად 100, 75 და 60 %-ით. მიღებულია ფერმენტული პრეპარატები და ჩატარებული კვლევების შედეგად დადგენილია, რომ *Penicillium canescens* 41 მიერ წარმოქმნილი ქსილანაზა მოქმედების ტემპერატურული და pH ოპტიმუმებია 45-50°C და pH 4.4. *Aspergillus niger* A 7-5-ის ქსილანაზა - 65°C და pH 6.5, ხოლო *Trichoderma viride* X 1-6-ის ქსილანაზა 50-55°C და pH 7.8-8.5.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 1-2, 2008.

Ecology

ORIBATID MITES (ACARI: ORIBATIDA) OF *CASTANEA* FORESTS OF MTIRALA NATIONAL PARK AND KINTRISHI RESERVE

MURVANIDZE M.[1], KVAVADZE ER.[2], JGENTI L.[3]

[1] *I. Javakhishvili Tbilisi State University*
[2] *LEPL Institute of Zoology*
[3] *Batumi Sh. Rustaveli State University*
E-mail: maka.murvanize@gmail.com

(Received February 20, 2008)

Abstract

Oribatid mites of *Castanea* forests of Mtirala National Park and Kintrishi Reserve were studied. 103 species were identified. Two species of them – *Steganacarus flagellatissimus* and *Damaeobelba minutissima* are new for Caucasian fauna. 34 species are common for both protected territories. Cluster analyses showed that differences in faunal communities are caused by different structure of understorey, which is presented as evergreen vegetation and deciduous plants.

Key words: Oribatid mites, Mtirala National Park, Kintrishi Reserve, Ajara.

Introduction

Ajara represents one of the best natural reserves of ancient vegetation. It belongs to those regions of Caucasus, where owing to the favorable location, climatic conditions of tertiary epoch still remain, and the vegetable cover is hardly changed.

Flora of Ajara is rich. According to the last data [Reserves of Caucasus, 1990], 1640 species of vascular vegetation are presented there. Richness and antiquity of vegetation cause richness of fauna, including oribatid mites.

Mtirala National Park is created in June of 2006. The new established park covers 15 806 ha and protects a unique ecosystem of forests. Kintrishi Reserve is one of the oldest reserves in Georgia. It was established in 1959 to protect the middle Kolkhic mountains relict flora and fauna. The main protective object is chestnut and beech forests with evergreen elements. The oribatid mite fauna of Kintrishi Reserve is comparably well studied [Murvanidze, Jgenti, 2002; Murvanidze, Kvavadze, Jgenti, 2004; Arabuli et all. 2007]. However there are no data regarding oribatid fauna of Mtirala National Park. We collected material in chestnut forests in both protected territories to compare the faunal composition and structure of oribatid communities.

Material and Methods

Material was collected during scientific expeditions held in June 2007 (Mtirala National Park) and in 2002-2005 (Kintrishi Reserve). The description of sites is as follows:

Mt1. Mtirala National Park. Korolistavi. *Castanea sativa* forest with several *Criptomeria.* Understorey is made by *Rhododendron ponticum* and *Laurocerrassus officinalis.*

Mt2. Mtirala National Park. Tskiniori. *Castanea sativa* forest. Understorey is made by *Rhododendron ponticum, Sambucus nigra, Laurocerrassus officinalis, Dryopteris, Rubus, Phyllitus scolopendrium.*

Mt3. Mtirala National Park. Over Tskiniori. Colchic forest with predomination of *Castanea sativa*, several *Fagus.*

K1. Kintrishi Reserve. *Castanea* forest with *Buxus* understorey. H = 200m

K2. Kintrishi Reserve. Zera Boseli. Left bank of Kintrishi River. *Castanea* forest with *Buxus* understorey. H = 400m

K3. Kintrishi Reserve. Didvake. *Castanea* forest with *Rhododendron ungnerii* understorey. H = 835m

K4. Kintrishi Reserve. Khino. *Castanea* forest with *Carpinus* and *Alnus*. Understorey is made by *Vaccinium arctostaphylos*

K5. Kintrishi Reserve. Gogoturi. *Castanea* forest with *Carpinus, Alnus, Tilia* and *Picea.* Understorey is made by *Rhododendron ponticum.*

For each sample 10 cm^3 soil was taken and mites were extracted by Berlese-Tulgren apparatus. The specimens were preserved in ethanol and after clearing were studied in lactic acid in temporary microscope slide. The terminology of morphological structures follows Weigmann (2006). In this investigation only adult mites were identified and counted.

Results and Discussion

In the chestnut forests of Mtirala National Park and Kintrishi Reserve 103 species of oribatid mites are registered. One species of them *Ctenobelba heterosetosa* is new for science [Murvanidze, Weigmann, 2007] and 2 (*Steganacarus flagellatissimus* and *Damaeobelba minutissima*) are new for Caucasus region (Table 1.). 56 species were found in Mtirala National Park and 82 species – in Kintrishi Reserve. 34 species are common for both protected territories. 20 species were registered in Mtirala National Park only and 43 species were found only in Kintrishi Reserve.

With high percent of dominance were distinguished the following wide distributed species: *Steganacarus spinosus* – 24%, *Hypochthonius rufulus* – 23% and *Minunthozetes pseudofusiger* – 33%. Indexes of dominance of rare species are comparably low (Tab.1).

Table 1. List of species of oribatid mites of Mtirala National Park and Kintrishi Reserve *Castanea* forests (% of dominance; dominance < 1 is indicated as +)

#	species	Mt1	Mt2	Mt3	K1	K2	K3	K4	K5
1	*Mesoplophora pectinata*	+	7		+				1
2	*Hypochthonius luteus*							1	
3	*H. rufulus*					23			
4	*Enichthonius minutissimus*	+							
5	*Hoplophthiracarus vanderhammenii*				3	3			
6	*H. vicinus*	+		5				1	1
7	*Phthiracarus anonymus*	+			1				
8	*Ph. boresetosus*	+							
9	*Ph. crassus*								+
10	*Ph. ferrugineus*	2	4	4			21		+
11	*Ph. globosus*	+							

12	*Ph. italicus*				+				
13	*Ph. lanatus*		7	7					+
14	*Ph. laevigatus*							1	
15	*Ph. lentulus*					9			
16	*Steganacarus carinatus*	+		2					
17	*S. conjuctus*								+
18	*S. flagelatissimus*	+							
19	*S. patruelis*			2					
20	*S. spinosus*	24	+	5	3		8	3	11
21	*S. striculus*								+
22	*Mesotritia grandjeani*								+
23	*Rhyzotritia ardua*			2	1			2	1
24	*Euphthiracarus monodactylus*								1
25	*Hermannia gibba*								+
26	*Hermanniella punctulata*	3	11		8		5	1	11
27	*Nanhermannia nana*			4					+
28	*Nothrus silvestris*	2						7	+
29	*Platynothrus peltifer*								1
30	*Arthrodamaeus starki*								+
31	*Aleurodamaeus setosus*				1				
32	*Damaeobelba minutissima*								+
33	*Metabelba monilipedia*			4					
34	*M. rara*							1	
35	*M. papillipes*							+	+
36	*Porobelba spinosa*	+	+						
37	*Damaeolus ornatissimus*	+	+		4		3		
38	*Euphterotegeus ornatissimus*								1
39	*Amerobelba decedens*	+			+				
40	*Eremobelba geographica*								+
41	*Ctenobelba diversisetosa*	+							
42	*Gustavia microcephala*			2	1				
43	*Liacarus coracinus*								+
44	*L. lencoranicus*					2			
45	*L. tubifer*								+
46	*L. sp.*	4							
47	*Xenillus tegeocranus*	+	3		+				+
48	*Tectocepheus punctulatus*		3	5					
49	*T. velatus sarecensis*								+
50	*T. velatus velatus*	12	3		1		3		+
51	*Carabodes femoralis*	4					3		+
52	*C. labirinthicus*				+				
53	*C. procerus*	+			+		3		1
54	*C. rugosior*	+			3			1	+
55	*C. sp.*							1	
56	*Lamellocepheus personatus*					2			
57	*Banksinoma lanceolata*				+				
58	*Conchogneta delacarlica*				4	5	3		
59	*C. tragardhi*	7	18	17				9	4
60	*Quadroppia michaeli*	+			+				
61	*Dissorhina ornata*	4		4				15	4
62	*Oppiella acuminata*	+	2					4	
63	*O. fallax*	+	12	4		11		9	3

64	*O. nova*	1			+	2		1	+
65	*O. subpectinata*	1			3	2	13	9	+
66	*Ramusella insculpta*	+		2	+		3		
67	*Suctobelba atomaria*		1						+
68	*S. trigona*		+			2			
69	*S. aliena*							1	
70	*Suctobelbella acutidens*								+
71	*S. duplex*							1	
72	*S. falcata*	+							
73	*S. forsslundi*		+						
74	*S. subtrigona*		+						
75	*Cymbaeremaeus cymba*		+						
76	*Eupelops curtipilus*					2			
77	*E. torulosus*			4				11	3
78	*Achipteria longisetosa*						3		
79	*A. nitens*								2
80	*Parachipteria georgica*			4	+	11	18	2	11
81	*P. nicoleti*		4		1				
82	*P. punctata*				4				
83	*Acrogalumna longipluma ajarica*				+	2			2
84	*Galumna lanceata*		3						
85	*Oribatella colchica*				1	2		1	
86	*O. nigra*				1				
87	*Chamobates caucasicus*				5				
88	*Chamobates kieviensis*	+	3						
89	*Ch. voigtsi*	11		5				6	2
90	*Euzetes globosus*								+
91	*Ceratozetes gracilis*								2
92	*Melanozetes mallicomus*								+
93	*Trichoribates caucasicus*					+			
94	*Minunthozetes pseudofusiger*	+	4	2	33	2	5		
95	*M. semirufus*		+						
96	*Punctoribates punctum*			4	+				
97	*Liebstadia longior*	+							
98	*Scheloribates latipes*	6		5	1	6	8		+
99	*Sch. laevigatus*			2					
100	*Protoribates capucinus*		4	4			3		
101	*P. lophotrichus*	+							17
102	*Or. tibialis*							1	+
103	*Zygoribatula exilis*		7	2	2				
Total		**37**	**25**	**24**	**33**	**17**	**15**	**25**	**48**

Clustser of faunal likeness shows that oribatid mites were grouped in two categories. One group is made by mites of Castanea forests with evergreen understorey *(Buxus* and *Rhododendron ungnerii,* sites K1, K2 and K3*).* The second group create oribatid mites of chestnut forests with mixed broad-lived vegetation (sites Mt 1, Mt2, Mt3, K4 and K5) (Fig. 1).

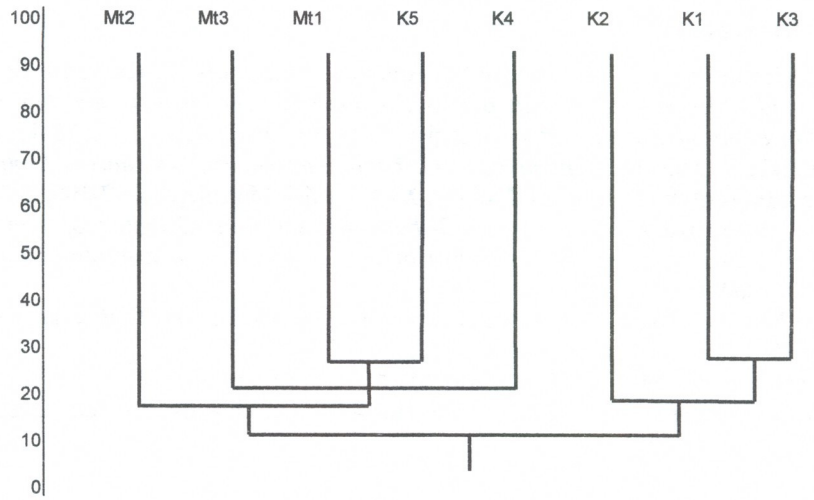


Fig. 1. Cluster of faunal likeness of oribatid mites of Mtirala National Park and Kintrishi Reserve

Cluster of dominance identities shows the similar results: oribatid mites of chestnut forests with evergreen understorey are separated from those of chestnut forests with mixed broad-lived vegetation (Fig. 2).

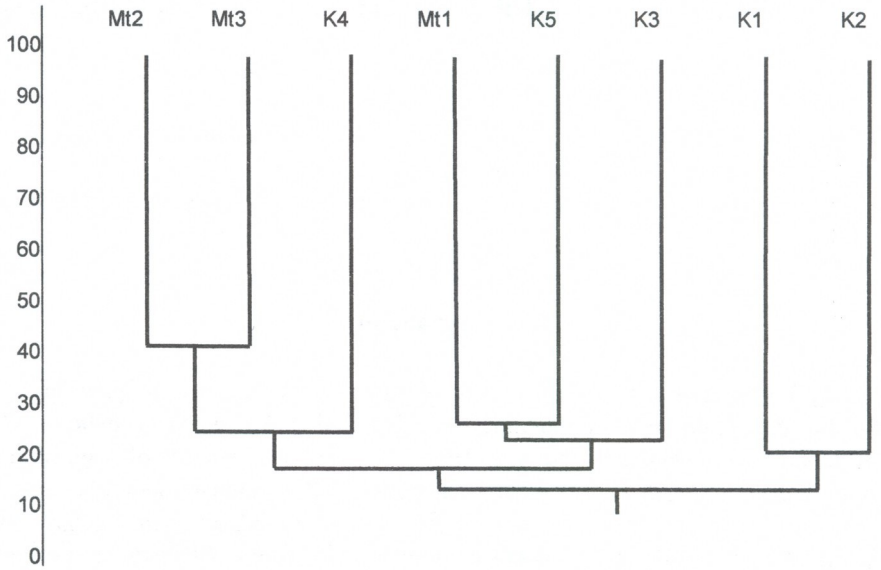


Fig. 2. Cluster of dominance identities of oribatid mites of Mtirala National Park and Kintrishi Reserve

The analysis of the data shows that oribatid fauna of chestnut forests of Mtirala National Park and Kintrishi Reserve is mainly similar. Differences in faunal communities are caused by different structure of understorey, which is presented as evergreen vegetation and deciduous as broad-lived plants.

References:

Arabuli G., Mosulishvili M., Murvanidze M., Arabuli T., Bagaturia N., Kvavadze Er. *The Colchic Lowland Alder Woodland with Boxwood Understory (Alneta barbatae buxosa) and their Soil Invertebrate Animals*. Proc. Georgian Acad. Sci., Biol. Ser. B, **5**, 2, 35-42, 2007.

Murvanidze M.O., Jgenti L.T. *The fauna of Oribatid mites (Acari, Oribatei) of Kintrishi Reserve.* Proceedings of the Institute of Zoology. **XXI**. Tbilisi. Metsniereba. 97-100, 2002.

Murvanidze M., Kvavadze E., Jgenti L. *Oribatid mites (Acari, Oribatei) of Ajara (Caucasus, Georgia) and their vertical-zonal distribution.* Proceedings of Institute of Zoology, **XXII**, 89-102, 2005.

Murvanidze M., Weigmann G. *A New Species of Ctenobelba (Acari: Oribatida: Ctenobelbidae) from the Caucasus Region.* Acarina, 15, 2, 283-285, 2007.

Reserves of Caucasus. M., "Misl", pp.146-155, 1990 (in Russian).

Weigmann G. *Hornmilben (Oribatida).* Die Tierwelt Deutschlands. 76 Teil. Goecke & Evers, Keltern. 520p, 2006.

მტირალას ეროვნული პარკისა და კინტრიშის ნაკრძალის წაბლნარების ჯავშნიანი ტკიპები (Acari: Oribatida)

მურვანიძე მ.[1], ყვავაძე ერ.[2], ჟღენტი ლ.[3]

[1] ი. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი
[2] სსიპ ზოოლოგიის ინსტიტუტი
[3] ბათუმის შ. რუსთაველის სახელობის სახელმწიფო უნივერსიტეტი

(მიღებულია 20.02.2008)

რეზიუმე

შესწავლილია მტირალას ეროვნული პარკისა და კინტრიშის ნაკრძალის ჯავშნიანი ტკიპები. რეგისტრირებულია 103 სახეობა. მათგან ორი – *Steganacarus flagellatissimus* და *Damaeobelba minutissima* ახალია კავკასიის ფაუნისათვის. 34 სახეობა საერთო აღმოჩნდა ორივე დაცული ტერიტორიისათვის. კლასტერულმა ანალიზმა აჩვენა, რომ განსხვავებები ფაუნისტურ კომპლექსებს შორის გამოწვეულია ქვეტყის განსხვავებული სტრუქტურით, რომელიც წარმოდგენილია მარადმწვანე და ფოთლმცვივანი მცენარეებით.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 1-2, 2008.

Entomology

NEW DATA ON *RAMULUS BITUBERCULATUS* REDT. (PHASMOPTERA, LANCHODIDAE) DISTRIBUTION IN THE CAUCASUS

DIDMANIDZE E., SKHIRTLADZE I., PETROV V.

National Museum of Georgia, Zoological collections of Nature history

(Received February 11, 2008)

Abstract

Representative of Phasmoptera family, mature female of species *Ramulus bituberculatus* was found in Vashlovani Reserve (Eastern Georgia). In the Caucasus region distribution of this species is not noted in the literature. Only 5 species of Phasmoptera in larvae phase are presented in the collections of the National Museum of Georgia, which were found in Armenia (Bos-Dag) and Azerbaijan (Geok-Tapa) and were defined as *Gratidia bituberculata* Redt. While comparing the adult species found in Vashlovani reserve and larval species kept in museum collections it was revealed that they are identical. Finding of adult forms of *Ramulus bituberculatus* Redt. in Georgia enables us to say that they are distributed throughout the Caucasus region.

Key words: *Ramulus bituberculatus* Redt., adult form, larvae.

Introduction

In the Caucasus fauna of Phasmoptera practically is not studied [Key of insects of the European part of the Soviet Union, 1948; 1964]. Within this region only one species - *Ramulus nana* Mitsh. was found in Nakhichevan along the gorge of the river Arax [Mishchenko, 1941], and as a rare species it was included in The Red Book of the USSR [Red Book, USSR 1984].

While comparing the species found in the Vashlovani reserve with the specimens kept in the collections of National Museum of Georgia, it was found that on the territory of the Transcaucasus and the Caucasus in general, another species of Phasmoptera - *Ramulus bituberculatus* Redt. is also occurred.

Ramulus bituberculatus Redt. from semi-deserts and deserts of Turkmenistan, Tajikistan, Kazakhstan and Northern China (Jungaria) is known.

As a result of our study *Ramulus bituberculatus* Redt. has been noted for the first time in the entomological list of Georgia, Azerbaijan and Armenia.

Materials and Methods

Studied species *Ramulus bituberculatus* Redt. was found in Vashlovani reserve by route method carried out by zoological department of the Georgian National Museum in May, 1976.

The paper is based on the data of abovementioned species and species kept in the collections of The National Museum of Georgia.

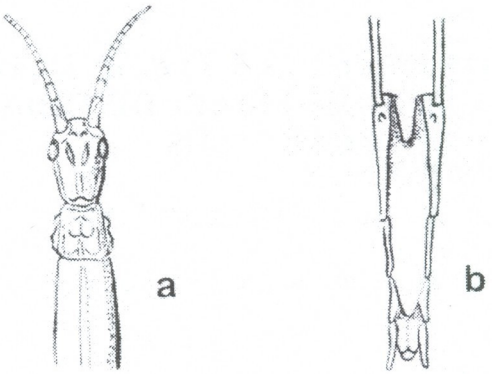


Fig. 1. Ramulus bituberculatus Redt. a – Front part of the Body; b – Lower part of Stomach.

Material: *Gratidia bituberculata Redt.*, Det. L.Mitshenko, 1940.

Caucasus Elisavetopol Leg. Babajanides V.1912 (Larva, 2 ex) - Azerbaijan; Bos-Dag Caucasus Leg. A. Shelkocnikov 21.IV.1915 (Larva, 1 ex) - Armenia; Geok-Tapa, Caucasus Leg. A. Shelkovnikov 25.VI.1915 (Larva, 1 ex) - Azerbaijan; Feruisa C. Annger 24.VI.1960 Leg. (greatly damaged 1 ex.) - Turkmenistan.

Ramulus (=Gratidia) bituberculatus Redt., Det. V. Petrov, 2007.

Vashlovani reserve, convallis, Kumuro, female (mature stage), Leg. E. Didmanidze 12.V.1976.

Results and Discussion

Interest towards the fauna of Phasmoptera was caused by the fact that in 1976 in Vashlovani reserve the adult form of *Ramulus bitubetculatus* Redt. was discovered (Fig.1), which up today has not been known for the Caucasus fauna.

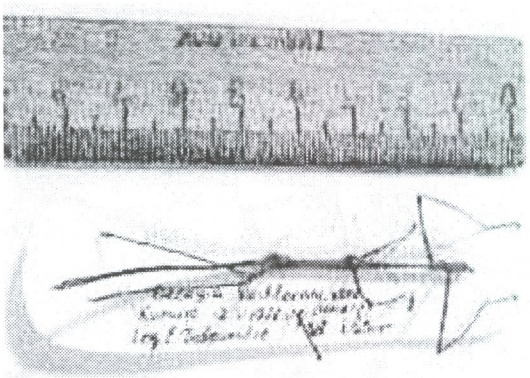


Fig. 2. Ramulus bitubercultus Redt.

After processing the literature data it was revealed that in 1912 in the environs of Elizavetopol (Ganja) 9 specimens of Phasmoptera in larvae stage were found by I. Babadzhanidi, which were defined as *Gratidia bituberculata* Redt. [Babadzhanidi, 1915].

Comparing the adult specimen from Vashlovani reserve with larval specimens kept in the collections of the National Museum of Georgia it was found that they are identical and all of them belong to *R. bituberculatus* Redt., which is in accordance with the determinations of museum specimens done by L.Mishchenko (Fig.1).

Thus, after our studies it can be concluded that the museum specimens belong to species *R. bituberculatus* Redt., and which identification was doubtful up today due to undeveloped, larvae phase. Along with this fact, distribution of this species is noted for the first time not only for the region of Georgia, but for the whole Transcaucasus and Caucasus region. We consider that *R. bituberculatus* Redt., as a rare species for the Caucasus should be listed in The Red Book.

References:

Babadzhanidi I.V. *Several observations over South Caucasus Phasmoptera (Gratidia sp.).*Bull. of Caucasus Museum, **IX**, 58-59, 1915 (in Russian).

Mishchenko L. *Species of the genus Gratidia Stal. (Plasmodea, Clitumnidae) in the fauna of the USSR.* Bulletin of the Academy of Sciences of the Georgian SSR, **II**, 1-2, 145-148, 1941 (in Russian).

Key of insects of the European part of the Soviet Union. M-L, 127-128, 1948 (in Russian).

Key of insects of the European part of the Soviet Union. M-L, 203-204, 1964 (in Russian).

The Red Book of USSR. M-L, 223, 1984 (in Russian).

ახალი მონაცემები ჩხირასებრთა (Phasmoptera, Lonchodidae) გავრცელების შესახებ კავკასიაში

დიდმანიძე ე., სხირტლაძე ი., პეტროვი ვ.

საქართველოს ეროვნული მუზეუმი, ზოოლოგიური ფონდების დეპარტამენტი

(მიღებულია 11.02.2008)

რეზიუმე

ჩხირასებრთა ოჯახიდან (Phasmoptera, Lonchodidae) Ramulus bituberculatus Redt.-ის ზრდასრული მდედრი საქართველოს ტერიტორიაზე, ვაშლოვანის ნაკრძალში მოპოვებულია ე. დიდმანიძის მიერ 12.05.1976 წელს. ამ სახეობის გავრცელება კავკასიაში დღემდე არ იყო ცნობილი. ლიტერატურულ წყაროების და საქართველოს ეროვნული მუზეუმის ზოოლოგიურ ფონდებში დაცულ ჩხირასებრთა კოლექციის ნიმუშების შესწავლის შედეგად აღმოჩნდა, რომ ჩხირას 5 ეგზემპლარის მატლური ფაზა ჯერ კიდევ გასული საუკუნის 40-იან წლებში იყო მოპოვებული აზერბაიჯანისა და სომხეთის ტერიტორიებზე და გარკვეული სავარაუდოდ როგორც *Gratidia bitubercalatus* Redt. საქართველოს ტერიტორიაზე მოპოვებული R. bitubercalatus Redt.-ის ზრდასრული ფორმის შედარებამ მუზეუმში დაცულ მატლურ ფაზების ნიმუშებთან გამოავლინა მათი იდენტურობა, რაც საეჭვოს ხდის ამ სახეობის არსებობას არა მარტო საქართველოში, არამედ სომხეთისა და აზერბაიჯანის ტერიტორიებზე.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 1-2, 2008.

Entomology

THERMAL ADAPTATION OF ENTOMOPATHOGENIC NEMATODES FOR INFECTION AND REPRODUCTION

LORTKIPANIDZE M., KOKHIA M., MELASHVILI N., GORGADZE O., KUCHAVA M.

Institute of Zoology

(Received February 11, 2008)

Abstract

To establish the optimal temperature of infection of insect-host with nematodes, their death-rate and maximal reproduction, thermal adaptation of entomopathogenic nematodes distributed in various regions of Georgia of species *Steinernema carpocapsae, Steinernema feltiae, Heterorhabditis bacteriophora* and *Steinernema* sp. in the range of 10-40°C was studied. As a result of experiment it was concluded that various species of entomopathogenic nematodes have well defined thermal breadth, on which their development and reproduction is greatly depended.

Key words: infective juveniles, thermal breadth, Steinernematidae, Heterorhabditidae, Tenebrio molitor, symbiotic association

Introduction

Temperature is a significant factor for development and reproduction of living organisms. Thermal adaptation has an important role for animals, and especially for ectothermal organisms [Cossins & Bowler, 1987]. Entomopathogenic nematodes (EPN) (Rhabditida: Steinernematidae and Heterorhabditidae) are soil inhabitant insect parasites, which have potential of biocontrol agents [Gaugler & Kaya, 1990]. Symbiotic association with bacteria xenorhabdus and phoxorhabdus favors development and reproduction of nematode parasite forms [Poinar, 1990].

Parasitic cycle of EPN begins from infective juveniles, when organism of insect-host as a nutrition matrix is entirely assimilated by nematodes. At this stage infective juveniles pass into environment - in soil, and begin free vital activity, where their main function is to find a new insect-host, to infest them and to develop new population.

In unfavorable conditions infective juveniles are provided with survival mechanism. Their stability in soil, maintaining the infesting ability, development and reproduction in new insect organism is entirely determined by temperature effect [Grewal, 1994; Kaya & Gaugler, 1993].

The goal of our research was to study thermal adaptation of EPNs *Steinernema carpocapsae, Steinernema feltiae, Heterorhabditis bacteriophora* and *Steinernema* sp. collected in various regions of Georgia.

Materials and Methods

The aim of our study was to establish thermal breadth of EPN for insect-host infection and for maximal reproduction.

We used the following species: 1. Steinernema carpocapsae, 2. Steinernema feltiae, 3. Heterorhabditis bacteriophora and 4. Steinernema sp.

Cultivation of those species was carried out on pupa and larvae of bread beetle *Tenebrio molitor* at 25°C by Dutky method [Dutky et al., 1964]. Age of nematodes received after cultivation was 1-2 months.

Experiment 1. From every species of EPN noted above we prepared suspension: 200 ml distilled water / 50 nematodes, sprayed into Petri dishes, where several larva of *Tenebrio molitor* (10-12 ones) were prepared for preinfection, closed with polyethylene film and placed in thermostat at different temperature regimes, in range +10 - +40°C. Infection of insects by nematodes and their death rate were detected 4 hours after beginning of experiment, at every 10 hours with increasing the temperature one-by-one degree.

Dead pupa of *Tenebrio molitor* were taken from sand, washed in distilled water and cut under microscope. Then nematode number in every dead pupa at definite temperature was determined. Duration of experiment was about 1 month (Fig. 1).

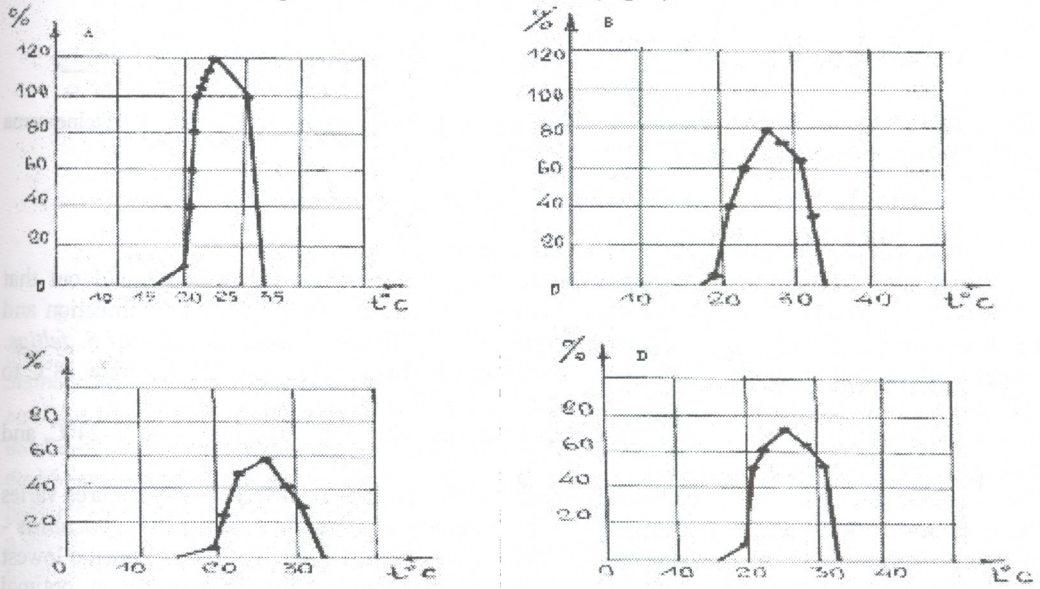


Fig. 1. Optimal temperature of EPN reproduction. A – Steinernema caprocapsae; B –Steinernema Sp.; C – Heterorhabditue bacteriophora; D – Steinernema feltiae.

Experiment 2. Potential of maximal reproduction of nematodes was studied at various temperature regimes, within the range 10-40°C. For this aim along with 5 larva of *Tenebrio molitor* 500 infective juveniles of every species noted above were placed on Petri dishes. 8-12 hours after larva died they were put into Petri dishes covered with filter paper and placed into water traps to obtain new infective juveniles. Traps were put in thermostat at various temperatures. Cultivation of nematodes lasted 12-14 days. Nematode suspension was pumped out every day from water traps and nematode number was determined under microscope according to Abbott method [Abbott, 1925]. To receive new infective juveniles distilled water was added in water traps. By this method total number of infected juveniles cultivated in insect-host at each temperature increased by one degree was determined. As a result of experiment optimal temperature for maximal reproduction of nematodes was established (Fig. 2).

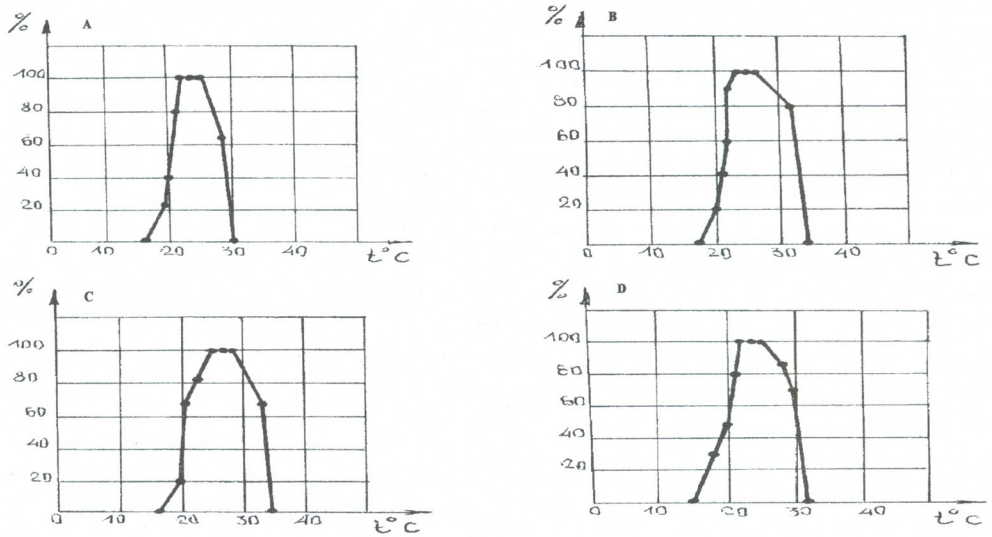


Fig. 2. Thermal breadth of nematode infection (%). A – Steinernema caprocapsae; B –Steinernema Sp.; C – Heterorhabditue bacteriophora; D – Steinernema feltiae.

Results and Discussion

Various species have different infection temperatures (Fig. 1). It was found out that *S.carpocapsae* is characterized by the widest temperature range - 16-36°C for host infection and mortality, while the most narrow temperature range 17-32°C was detected in case of *S. feltiae.* Temperature breadth for *Heterorhabditis bacteriophora* (HRb) was relatively high - from 18°C to 34°C, and for *Steinernema* sp. - 16-33°C, which is close to that of *S. feltiae.*

Optimal temperature at which *S.carpocapsae* and HRb infest the host was 23-24°C, and for *S. feltae* and *Steinernema sp.* - 21-22°C.

As is seen from Fig. 2 cultivation potential of nematodes at different temperatures varies among studied species. The highest index of reproduction was noted in case of *S. carpocapsae* - 120 000 nematodes at 22-25°C, for HRb - 80 000 nematodes at 24-26°C. *S. feltiae* has the lowest index - 60 000 nematodes at 23-25°C and *Steinernema* sp. 70 000 nematodes at optimal temperature 22-26°C. In whole duration of cultivation lasted two weeks.

As a result of the experiment it was shown that development cycle of nematodes is depended greatly on temperature. Different species of EPN differ by potential of insect-host infection, death-rate and degree of reproduction. On the basis of those facts we conclude that EPNs have well-defined thermal breadths for their development and reproduction.

References:

Abbott W.S. *A nematode of computing the effectiveness of an insecticide.* J. Econ. Entomol., **18,** 265-279, 1925.

Cossins A.R. and Bowler K. *Temperature Biology of animals.* Chapman and Hall, NY, 1987.

Dutky S.R. Tompson J.V. and Cantwell G.E. *A technique for mass propagation of the DD-136 nematode.* J. Insect Pathol., **6,** 417-422, 1964.

Gaugler R. and Kaya H.K. *Entomopathogonic Nematodes in Biological control.* CRC Press, Boca Raton FL, 1990.

Grewal P.S. *Thermal adaptation of entomopathogenic nematodes: niche breadth for infection, establishment, and reproduction.* J.therm. Biol., **19**, 4, 245-253, 1994.

Kaya H.K.and Gaugler R. *Entomopathogenic nematodes.* Ann. Rev.Entomol., **38**, p.181-206. 1993.

Poinar G.O. Jr. *Texonomy and biology of Steinernematidae and Heterorhabditidae.* In: Entomopathogenic Nemetodes in Biological Control. Ed. R. Gaugler and H.K. Kaya. CRC Press, Boca Raton FL., p. 23-26, 1990.

ენტომოპათოგენური ნემატოდების თერმული ადაპტაცია ინვაზირებისა და რეპროდუქციისათვის

ლორთქიფანიძე მ., კოხია მ., მელაშვილი ნ., გორგაძე ო., კუჭავა მ.

ზოოლოგიის ინსტიტუტი

(მიღებულია 11.02.2008)

რეზიუმე

შესწავლილია საქართველოს სხვადასხვა რეგიონში მოპოვებული ენტომოპათოგენური ნემატოდების (ეპნ) სახეობების: *Steinernema carpocapsae, Steinernema feltiae, Heterorhabditis bacteriophora* და *Steinernema sp.*-ის თერმული ადაპტაცია 10°C-40°C-ის ფარგლებში, რათა დაგვედგინა ნემატოდების მიერ მწერი-მასპინძლის ინვაზირების, სიკვდილიანობის გამოწვევისა და მათი მაქსიმალური რეპროდუქციის მიღების ოპტიმალური ტემპერეტურა. ნაჩვენებია, რომ ენტომოპათოგენური ნემატოდების სხვადასხვა სახეობებს გააჩნია მკვეთრად განსაზღვრული ტემპერატურული ინტერვალი, რომელზედაც მნიშვნელოვნად დამოკიდებულია მათი განვითარება და გამრავლება.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 1-2, 2008.

Genetics

FEATURES OF EXPRESSION OF ABO SYSTEM ANTIGEN-ANTIBODIES IN NEWBORNS

NAGERVADZE M., TSKVITINIDZE S., *KHUKHUNAISHVILI R., DIASAMIDZE A.

Batumi Sh. Rustaveli State University
E-mail: rusudan_777@mail.ru

(Received February 25, 2008)

Abstract

Features of expression ABO system antigen-antibody in newborns were studied. In immunoserology widely approved cross-agglutination method was used, which means ability of simultaneous detection of A and B antigens fixed on erythrocyte membranes and anti-A and anti-B antibodies in plasma. 20 newborns' blood samples were studied on group antigens. In 10 cases A antigen was detected, whereas B antigen was not revealed at all. It should be noted that in all those 10 cases weak expression of A-antigen was registered. Accordingly, among newborns and adults distinct agglutinabilities of A antibodies were revealed.

Key words: immunoserological method, erythrocytic group antigen, monoclonal antibodies, ABO system.

Introduction

Blood erythrocytic group antigen is genetically deterministic characteristic [Instee, 1990; Schenken-Brunner, 2000]. Their specificity is used widely in biology and medicine.

At present up to 25 erythrocitic group systems are distinguished, in which about 300 antigens are included [Mineeva et al., 1995]. In clinical viewpoint ABO system is the most significant [Ogasawara et al., 1996; Yamamato, 1995].

The aim of our research was to study the features of expression of antigen-antibodies of ABO system in newborns. In literature about this issue different opinions occur. Some scientific groups think that ABO system antigens absent in newborns, and some groups report that those antigens due to inadequate synthesis are characterized with weak expression and their serological detection is difficult [Bronnikova, Garkavi, 1964]. As for group-specific antibodies, according to one hypothesis induction of those antibodies take place in an embryo of 2-3 months age under influence of enteric microflora. Hence, this phenomenon is considered as a result of bacterial immunization [Drannik, 2003].

It is very likely that distinct from adults in some newborns A and B antigens are weakly expressed on erythrocyte surface, and in serum corresponding antibodies may not occur, which leads to incorrect determination of blood group.

Materials and Methods

20 newborns' blood samples were researched on antigen-antibodies. The material was provided from Batumi Maternity Hospital. To reveal erythrocitic group-specific antigen-antibodies both, plasma and erythrocitic mass were used.

In immunoserology widely approved cross-agglutination method was used, which means ability of simultaneous detection of A and B antigens fixed on erythrocyte membranes and anti-A and anti-B antibodies in plasma. In the studies anti-A and anti-B monoclonal antibodies and standard erythrocytes of I, II, III and IV groups were applied.

To avoid mistakes all experimental conditions provided by method were kept. Among them: titre of reagents, temperature, charge ratio, detection of pseudo agglutination and panagglutination, period of observation, etc.

Degree of agglutination was carried out by electron microscope (Axsioplan imagine 2, Karl Zeiss).

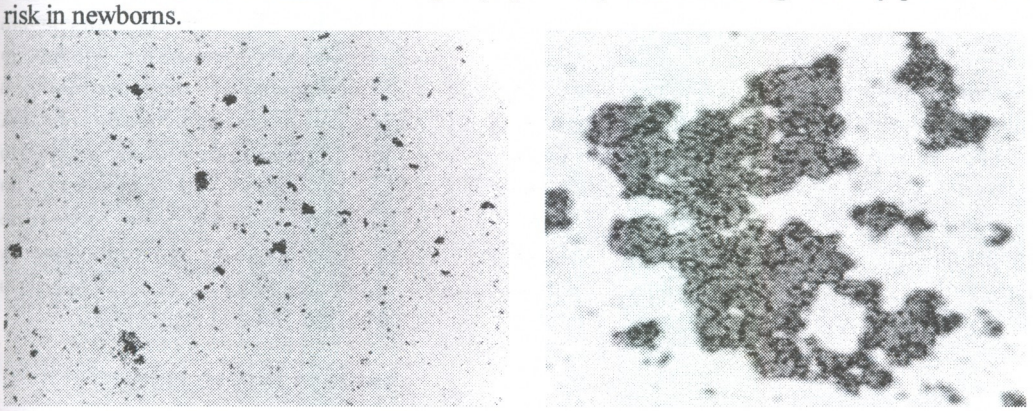
Results and Discussion

Among studied 20 newborns blood samples in 10 cases A-antigen was detected, but B antigen was not revealed at all. It should be noted that in all those 10 cases A-antigen was weakly expressed. Accordingly, among newborns and adults distinct agglutinabilities of A antibodies were revealed (Fig.1).

Group-specific antibodies were registered only in one case. Within 9 newborns being carriers of O(I) group only in 2 ones both, anti-A and anti-B antibodies were observed, in the rest 7 ones those antibodies were not revealed at all.

During investigation interesting fact was noted, namely, in A group carriers anti-A antibody was detected, which as a rule is not found in carriers on this group. We can suppose that this case is a result of immunization caused by ABO-system discrepancy between mother and fetus.

According to obtained results we can conclude that usage of one definite immunoserological method is not sufficient for exact determination of blood group-specificity of newborn. Only combination of approved methods enables to establish newborns' group belonging without error. Exact determination of group-specificity will reduce significantly posttransfusion risk in newborns.



a b

Fig. 1. Agglutination caused by A antigen in a) newborns and b) adults

References:

Bronnikova M.A., Garkavi A.S. *Development of isoserological systems in humans during embryogenesis.* Proceedings of VII "MKAEN", imprint, pp.10, 1964 (in Russian).

Drannik G.N. *Clinical immunology and allergology.* M., "Medical News Agency", 2003 (in Russian).

Mineeva N. V., Kirina O.N., Ivanova N.E. *Modern requests for providing safe hemetransfusion.* News in Transfusiology, **10**, 3-8, 1995 (in Russian).

Prokop O., Geller V. *Human blood groups.* M., "Medicina", pp. 510, 1991 (in Russian).

Instee O. J. *Blood group active surface molecules of the human red blood cells.* Vox Sang., **58**, 1-20, 1990.

Ogasawara K. et al. *Molecular genetic analysis of variant phenotypes of the ABO blood group system. Blood.* **88**, 7, 2732-2737, 1996.

Schenken-Brunner H. *Human blood groups.* In: Chemical and biochemical basis of antigen specificity, pp. 636, 2000.

Yamamato F. *Molecular Genetics of the ABO histo-blood group system.* Vox Sang., **69**, 1-7, 1995.

ABO სისტემის ანტიგენ-ანტისხეულების გამოვლენის თავისებურებანი ახალშობილებში

ნაგერვაძე მ., ცქვიტინიძე ს., ხუხუნაიშვილი რ., დიასამიძე ა.

შ. რუსთაველის სახ. ბათუმის სახელმწიფო უნივერსიტეტი

(მიღებულია 25.02.2008)

რეზიუმე

შესწავლილია ახალშობილებში ABO სისტემის ანტიგენ-ანტისხეულთა ექსპრესიის თავისებურებები. გამოყენებულ იქნა იმუნოსეროლოგიაში ფართოდ აპრობირებული ჯვარედინიზაციის მეთოდი, რომელიც ითვალისწინებს ერითროციტის მემბრანაზე ფიქსირებული A და B ანტიგენისა და პლაზმაში ანტი-A და ანტი- B ანტისხეულების ერთდროულად გამოვლენის შესაძლებლობას. ჯგუფურ ანტიგენებზე შესწავლილ იქნა 20 ახალშობილის სისხლი. 10 შემთხვევაში დაფიქსირდა A ანტიგენი, ხოლო B ანტიგენი არც ერთ შემთხვევაში არ იქნა გამოვლენილი. აღსანიშნავია, რომ აღნიშნულ ათივე შემთხვევაში დაფიქსირდა A ანტიგენის სუსტი ექსპრესია. შესაბამისად გამოვლინდა A ანტიგენის აგლუტინაციის განსხვავებული უნარი ახალშობილებსა და ზრდასრულებში.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 1-2, 2008.

Genetics

INFLUENCE OF γ-IRRADIATION AND PESTICIDE RIDOMIL ON GROWTH OF SOYBEAN (GLYCINE MAX (L) MERR) PLANTLETS

SHATIRISHVILI A.[1], CHITANAVA ZH.[2], ZARNADZE T.[3]

[1]*Chavchavadze Tbilisi State University*
[2]*Batumi Rustaveli State Universiti*
[3]*Kutaisi Tsereteli State University*

(Received March 5, 2008)

Abstract

The single and combined treatment with different concentrations of pesticide Ridomill solution and 2,4,6,8 and 10 Grey doses of γ-irradiation of annual seeds received from heterozygous ($Y_{11}y_{11}$) plants of soybean genetic line (L65-1237) have been studied. Ridomil and γ-irradiation revealed different effects on the growth of plantlets developed from seeds of various genotypes.

Key words: heterozygous plants, homozygous plants, pesticide, irradiation

Introduction

Pollution of environment with various toxicants is transformed into accompanied phenomenon of technogenic progress. Polluting factors of environment stipulate for formation of biotechnosphere [Dubinin, Pashin, 1978; Lekiavichus, 1983]. Among polluting agents according to stress-index pesticides are on the first place. A lot of them are stress-stable and during many years they circulate in biosphere. Many species are characterized with potential of accumulation of toxicants in their organism, and due to this fact concentration of this compound is much more higher in subsequent trophic chain than it is actually in environment [Dubinin, Pashin, 1978; Lekiavichus, 1983; Durmishidze, 1988]

Majority of pesticides besides toxicity reveal gene-toxicity also. They change genetic system and as a result they become dangerous for whole living system including current and following generations of humans. Toxicants concentrated in environment with natural background of enhanced radiation fully affect living organisms. Chronic effect of toxicants and radiation on living system increases genetic load [Dubinin, 2000; Dubinin, Pashin, 1978; Lekiavichus, 1983].

Materials and Methods

Tests were conducted on genetic line L65-1237 of soybean (Glycine max (L) Merr). Origin of this genetic line of soybean and conditions of test-system usage are described in earlier articles [Baratashvili et al., 2003a; 2003b]. Experiments were carried on seeds obtained from heterozygous plants of light-green color.

Annual seeds of soybean were treated with Ridomil solution of the following concentrations: 0.02, 0.04, 0.06, 0.08 and 0.1%.

100 dry-air seeds were exposed by preparation of every concentration for 24 hours. After treatment for elimination of preparation, seeds were washed by flowing water for 4 hours. Seeds were sown into wooden boxes with soil (mixture of black sand and soil, 1:1).

Dry-air seeds of soybean were exposed by γ-irradiation of 2, 4, 6, 8 and 10 Grey doses (100 seeds in each variant). Irradiation was carried out by ^{60}Co rays, 135 rad/sec using PXM-γ (20) in department of biophysics of Kanchaveli Plant Protection Institute. Irradiated seeds were placed in distilled water for 24 hours. Then swelled seeds were sown into wooden boxes in corresponding order.

To study combined effect of γ-irradiation and Ridomil dry-air seeds were exposed by irradiation of 4 Grey dose and further they were treated with Ridomil solution of noted above concentrations. Time of exposition was 24 hours. 100 seeds were used in every variant. Each test variant had its independent control variant. To exclude various effects of abiotic factors on plantlets all seeds were sown at one and the same day.

In the test-system of soybean Y_{11} allele of gene is polydominant, it controls chlorophyll synthesis. Recessive allele y_{11} don't lead this process, so homozygous recessive plants do not contain chlorophyll. From seeds received from heterozygous plants, plantlets of three phenotypic classes are developed: green ($Y_{11}Y_{11}$), light-green ($Y_{11}y_{11}$) and yellow ($y_{11}y_{11}$), in ratio 1:2:1. As chlorophyll synthesis is disabled in the plants of yellow phenotype, the plantlets die at bifoliate stage. In plants of green and light-green coloration measurements were carried out in 10 plantlets of the same age at 5-day intervals during the month; in plants of yellow phenotype – at 2-day intervals during 15 days (after this period reserve of organic matter is exhausted in seed lobes and they die).

Obtained results were treated statistically [Plokhinski, 1970].

Results and Discussion

Results of studies of growth intensity of plants germinated from seeds of control variant and irradiated with various doses of γ-rays are presented in Table 1. It is seen from the data that the doses of 2 and 4 Gr have stimulating effect on homozygous ($Y_{11}Y_{11}$) plants of green phenotype at initial stages. 6, 8 and 10 Gr have inhibiting effect on the growth of plantlets. In the consequent period of plantlet development (15-20 days) only those plantlets revealed stimulating growth on following stage, which were irradiated with 2 Grey. Namely, plantlets of age of 25 days do not differ by growth potential from control variant. Plants irradiated with higher doses retarded from control variant in growth and phenomenon of dose-effect was sharply revealed.

In heterozygous ($Y_{11}y_{11}$) plantlets of light-green color at initial stages of development (at 5^{th} day of germination) the same effect was seen as in soybean of green phenotype. In particular, stimulating effect was revealed in plantlets developed from seeds irradiated with 2 and 4 Gr, and in plantlets of age of 15-20 days – only 2 Gr showed stimulating effect.

Plantlets of age of 25-days retarded in growth from control plants and phenomenon of dose-effect was drastic. Plantlets of light-green coloration germinated from seeds irradiated with 6, 8 and 10 Gr within the period of observation retarded from control plants by growth potential.

All doses of irradiation have inhibiting effect on yellow homozygous plants ($y_{11}y_{11}$), and 9 days after stimulating effect of irradiation with 2 Grey was registered.

Test plants of different genotypes and control plants of soybean by potential of growth differ from each other. Rate of growth in plantlets of green coloration was significantly higher than in plants of light-green color, which is a result of processing of photosynthesis with different intensities. As for plantlets with yellow phenotypes, they retarded significantly from the both

abovementioned by growth rate. Yellow plantlets use those reserves of organic compounds for assimilation, which are in lobes.

Table 1. Effect of γ-irradiation on plant growth

Dose, Grey	Phenotype	Time, days				
		5	9	13	17	21
2	Green	69±1.43	115±1.14	137±0.71	137±1.35	153±1.40
	Light-green	69±0.63	98±0.40	121±1.00	121±0.92	126±0.72
	Yellow	39±0.67	53±1.25	61±1.35	70±1.25	80±1.00
4	Green	67±0.90	112±1.30	128±1.30	128±1.20	135±1.15
	Light-green	68±0.90	92±1.20	108±1.10	115±0.65	122±0.61
	Yellow	38±0.67	50±1.20	58±0.75	64±1.30	73±1.20
6	Green	64±0.70	102±1.45	120±0.75	120±1.00	128±1.55
	Light-green	58±0.65	79±1.15	96±1.15	96±0.75	108±1.10
	Yellow	35±0.70	37±1.40	50±0.85	58±1.55	69±1.30
8	Green	56±0.80	95±1.20	110±1.25	115±1.20	118±1.40
	Light-green	54±0.60	79±0.95	94±0.70	94±0.80	105±1.30
	Yellow	28±0.95	35±1.35	45±0.90	50±1.36	65±1.40
10	Green	54±1.00	73±0.93	86±1.00	98±1.30	110±1.30
	Light-green	45±0.95	65±1.30	90±0.70	90±1.00	100±0.85
	Yellow	28±1.00	30±1.25	40±1.45	45±1.50	60±1.35
control	Green	65±1.10	120±1.50	132±1.25	132±1.60	154±0.95
	Light-green	61±1.00	101±1.10	115±0.90	115±0.60	138±1.10
	Yellow	43±0.80	52±1.00	60±1.50	64±1.25	74±1.65

Many researchers have noted the stimulating effect of low doses of γ-irradiation, but even these low doses are characterized with mutagenic effect. It is established that there is not the threshold of radiation effect on genetic system. Every dose of radiation is harmful for living organisms and induces mutations [Dubinin, 2000]. Species associated in different taxa reveal distinct radioresistances. Artificial populations (cultivars) of different genotypes belonging to one and the same species showed various relations towards γ-irradiation [Valeva, 1969].

In plantlets of green phenotype germinated from the seeds treated with pesticide Ridomil of concentrations 0.02% and 0.04% stimulating effect was observed at initial stages (Table 2). In the following period of plantlet development only in plants treated with preparation of concentration 0.02% in comparison with control high growth rate was noted. All other concentrations of preparation cause inhibiting effect.

Nearly the same situation was noted in heterozygous plantlets of light-green coloration. At the same time plants of this phenotype retarded significantly by growth rate from plantlets of green phenotype. All used concentrations of Ridomil have inhibited effect on plantlets of yellow phenotype of soybean and the phenomenon of dose-effect was revealed. Thus, soybeans of various genotypes respond by ambiguous physiological reaction on toxicant effect.

Results of combined effect of pesticide Ridomil of various concentrations and γ-irradiation (4 Grey) on the growth of plantlets are presented in Table 3. In plantlets of green phenotype as compared with control inhibition of growth was noted during the whole period of study. At all stages of growth phenomenon of dose-effect was sharply observed.

Entirely distinct situation was registered while studying the growth of heterozygous plantlets of light-green color. At initial stages of plantlet development (5 days) test plants retarded by growth from control ones. In plantlets of 10-20-days age at combined effect of Ridomil of concentration 0.02% and irradiation growth rate was enhanced as compared with control at initial stage, but at following stages it was reduced. In other test cases decrease of growth rate as compared with control was observed. Similar effect was revealed at single effects of Ridomil

(0.02%) and low doses of γ-irradiation. 10 days after reserve of organic compounds is exhausted in plantlets and they use those compounds, which are received during photosynthesis. Therefore, for transition of plant into such important stage in genetic system of plant expression of definite genes is occurred. Thus, low doses of radiation and toxicant have stimulating effect only on development and only at this important stage.

Table 2. Effect of Ridomil on plant growth

Concentration, %	Phenotype	Time, days				
		5	9	13	17	21
0.02	Green	70±1.00	95±1.15	129±1.00	147±1.00	153±1.00
	Light-green	70±0.90	84±0.80	115±1.00	126±1.65	140±1.00
	Yellow	39±1.00	45±1.25	50±1.60	55±1.25	65±1.00
0.04	Green	67±1.45	75±1.50	100±1.60	130±1.40	140±1.10
	Light-green	68±1.00	75±1.20	100±1.35	110±1.45	130±0.55
	Yellow	35±1.50	40±1.40	45±1.15	50±0.90	62±1.35
0.06	Green	60±1.20	70±0.85	90±1.35	115±1.45	125±1.45
	Light-green	60±1.20	70±1.25	90±1.00	100±1.50	115±1.60
	Yellow	30±1.45	35±1.00	40±1.00	43±1.00	53±1.0
0.08	Green	50±1.00	60±0.80	75±1.30	95±1.00	110±1.45
	Light-green	60±1.65	70±0.65	80±1.45	90±0.90	105±0.65
	Yellow	30±1.10	35±1.65	37±1.25	40±0.90	47±1.50
0.1	Green	35±1.25	45±1.00	60±1.30	85±1.40	95±1.35
	Light-green	40±0.90	65±0.55	75±1.30	90±1.00	100±0.90
	Yellow	25±1.00	30±1.55	35±1.00	40±0.65	42±1.55
control	Green	65±1.10	120±1.50	132±1.25	132±1.60	154±0.95
	Light-green	61±1.00	101±1.10	115±0.90	115±0.60	138±1.10
	Yellow	43±0.80	52±1.00	60±1.50	64±1.25	74±1.65

Table3. Combined effect of γ-irradiation (4 Grey) and Ridomil on plant growth

Concentration, %	Phenotype	Time, days				
		5	9	13	17	21
0.02	Green	76±1.40	110±1.55	121±1.60	127±1.20	130±1.40
	Light-green	74±1.30	105±1.40	119±1.30	121±1.20	122±1.85
	Yellow	44±1.35	54±1.45	72±1.20	81±0.80	94±1.35
0.04	Green	73±1.15	103±1.45	117±0.90	121±1.45	126±1.35
	Light-green	72±0.30	95±0.40	112±0.90	118±1.10	120±1.45
	Yellow	41±0.80	49±0.95	61±1.20	70±0.95	80±1.00
0.06	Green	71±0.90	96±1.10	117±0.85	122±1.20	122±0.80
	Light-green	71±0.90	89±0.75	103±1.25	106±1.25	115±0.85
	Yellow	39±1.20	49±1.25	57±1.25	66±0.85	80±1.20
0.08	Green	70±1.45	88±1.35	103±0.80	110±1.350	115±0.95
	Light-green	64±0.85	82±1.20	95±0.60	100±1.45	110±0.90
	Yellow	34±0.80	45±1.35	55±0.95	60±0.95	75±1.35
0.1	Green	66±0.75	86±1.40	103±0.90	1088±1.45	112±1.40
	Light-green	62±0.60	76±0.45	84±0.80	96±1.35	106±0.65
	Yellow	30±0.95	45±1.35	55±0.95	60±0.95	75±1.35
control	Green	65±1.10	120±1.50	132±1.25	132±1.60	154±0.95
	Light-green	61±1.00	101±1.10	115±0.90	115±0.60	138±1.10
	Yellow	43±0.80	52±1.00	60±1.50	64±1.25	74±1.65

Combined effect of radiation and toxicant on plantlets of yellow phenotype showed somehow different effect. 0.02% solution of Ridomil and radiation have stimulating effect during

all stages of development. Ridomil preparations of higher concentrations and radiation inhibited plantlet development at initial stages, and stimulated - at late stages. Only at combined effect of pesticide of 0.1% concentration and radiation at all stages of development inhibition of plantlet growth rate as compared with control was recorded.

Effect of Ridomil on matured seeds has been studied. Different effects of preparation on physiological processes occurring in all plantlets of various genotypes were revealed [Kadagishvili et al., 2006; Vig, Paddok, 1970]. It is established that intrusion of xenobiotics, and among them pesticides, and their effect on plant cell is complicated process run in two phases. In the second phase plant defense systems affect the xenobiotics and decompose them into intermediate products. Those intermediate products have distinct effects on cellular components. Some of them are much more toxicant than initial agent and cause significant inhibition of growth and development processes. Some of them are less toxic and have stimulating effect. As a result enhancement of other physiological processes is noted in some cases [Durmishidze, 1988]. In spite of stimulating effect genetically active xenobiotic causes significant changes of genetic system and mutational load [Dubinin, 2000; Dubinin, Pashin, 1978; Lekiavichus, 1983].

Literature:

Baratashvili N., Chitanava Zh., Shatirishvili A. Effect of fungicides Ridomil and Khomecyn on germination of soyabean (Glycine max) seeds. In: "Actual problems of biology and medicine" 298-303, Tbilisi, 2003

Baratashvili N., Chitanava Zh., Shatirishvili A. Genetic changes in the soybean Glycine max L. induced by pesticides (Khomecyn, Ridomil). Proc.Georgian Acad.Sci., Biol. Ser. B, **1**, 1-2, 29-31, 2003.

Dubinin N.P. Radiation and chemical mutagenesis. vol.**2**, M., "Nauka", 2000.

Dubinin N.P., Pashin Iu. V. Mutagenesis and environment. M., "Nauka", 1978.

Durmishidze S. Plant biochemistry and preservation of environment. In: "Biotransformation of xenobiotics in plants", 4-7, Tbilisi, "Metsniereba", 1988.

Kadagishvili E., Doborjginidze T., Shatirishvili A., Baratashvili N., Chitanava Zh. The effect of pesticide cyfluthrin (Buldok) on growth of soybean (Glycine max) seedlings of different genotypes. Bull.Georgian Acad.Sci., **174**, 4, 134-136, 2006.

Lekiavichus R.K. Chemical mutagenesis and pollution of environment. Vilnius, "Mokslas", 1983.

Plokhinski I.A. Biometry. M., Publ. of Moscow State University, 1970.

Valeva S.A. Modern problems of radiation genetics. M., "Atomuzdat", 280-301, 1969.

Vig. B.K., Paddok E.F. Studies on the expression of somatic crossing-over in Glycine max L. Theoret. Applied Genetics, 40, 316-321, 1970.

γ-დასხივების და პესტიციდ რიდომილის მოქმედება სოიას (Glycine max(L) Merr) აღმონაცენთა ზრდაზე

შათირიშვილი ა.[1], ჭითანავა ჟ..[2], ზარნაძე თ.[3]

[1]ი.ჭავჭავაძის თბილისის სახელმწიფო უნივერსიტეტი
[2]შ.რუსთაველის ბათუმის სახელმწიფო უნივერსიტეტი
[3]ა.წერეთელის ქუთაისის სახელმწიფო უნივერსიტეტი

(მიღებულია 05.03.2008)

რეზიუმე

სოიას გენეტიკურ ხაზის L65-1237 ჰეტეროზიგოტი მცენარეებიდან მიღებული ერთწლიანი თესლები დამუშავებულია ჰერბიციდ რიდომილის განსხვავებული კინცენტრაციის ხსნარით, ასევე γ-გამოსხივების 2, 4, 6, 8 და 10 გრეი დოზით. პარალელურად შესწავლილია რადიაციისა და რიდომილის ერთობლივი მოქმედება. რიდომილმა და γ-გამოსხივებამ განსხვავებული გავლენა მოახდინა თესლებიდან განვითარებული განსხვავებული გენოტიპის აღმონაცენების ზრდაზე.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 1-2, 2008.

Microbiology

USAGE OF POLYMERASE CHAIN REACTION FOR LABORATORY DIAGNOSTICS OF BRUCELLOSIS OF BOVINE ANIMALS

LOMTATIDZE Z.[1], *GHVINJILIA M.[2], GHVINJILIA G.[3]

[1]*Tbilisi Botanic Garden and Institute of Botany*
[2]*Laboratory of Ministry of Agriculture of Georgia*
[3]*National Service of Food Safety, Veterinary and Plant Protection*
E-mail: m.ghvinjilia@mail.ru

(Received March 10, 2008)

Abstract

For effective control over epizootic and epidemic factors in endemic regions of brucellosis laboratory methods of investigation are used. Isolation of pathogenic agent is one of the most effective characteristics. For detection and identification of brucellosis and laboratory confirmation of diagnosis method based on polymerase chain reaction (PCR) was used. For laboratory diagnostics of brucellosis of bovine animals in cases of acute and chronic processing of the disease high specificity and sensitivity of this method was shown.

Key words: Rose-Bengal Reaction, serological methods, polymerase chain reaction (PCR)

Introduction

Brucellosis is widespread zoonotic infectious disease, especially within the regions of developed livestock farming. At present brucellosis is considered as extremely dangerous infection causing economic damage to animal farming [Kosilov, 1992].

Brucellosis control is significant for public health care because of direct or indirect transmission of infection from infected animals to humans causing illness, disability and cripple [Fiodorov & Gorshenko, 1982].

Incubation period lasts from 6 to 30 days. Brucellosis is characterized with polymorphism of clinical features. In some cases infection is processed as primary-latent form without clinical symptoms, which is revealed only at laboratory research. In other cases the disease is proceeded as acute (acute-septic) or chronic forms [Butkin, 1981].

Brucella is gram-negative, facultative intracellular pathogen. Genus Brucella consists of 7 species: *Br. abort us*, *Br. suit, Br. melitensis, Br. neotomae, Br. ovis, Br. canis* and new species - *Br. maris*, which differ by biochemical, metabolic, antigen and virulent characteristics [Devrishov, 2006].

Taxonomic characterization of Brucella along with morphological, cultural and serological characteristics also includes biochemical and genetic tests. Many of them are expensive, laborious and time-consuming [Paior, 2006].

The goal of our research was to demonstrate potential of usage of molecular biological method, and in particular of PCR-method for determination of presence of specific sequences of Brucella DNA in tested samples as high specific, sensitive and fast method of laboratory diagnostics of brucellosis.

Material and Methods

For conducting of PCR in the test-system primers synthesized on the basis of BCSP31 gene sequence coding surface protein of outer membrane of *B. abortus* of size 31kD were used. Sequences of this gene are identical for all species of brucella and therefore those primers are genus specific.

Primer pair Br1/Br2 (direct primer Br1 5'-AGTCAGACGTTGCCTATTG-3' and reverse primer Br2 5'GTGTTCAGCCTTGATATCG-3') were used for flanking DNA fragment of 260 bp size providing high specificity of reaction.

Reaction of brucella agglutination (Rose-Bengal reaction) reveals immunoglobulines IgG1 and IgG2. 30 μl of serum is placed on special chart of drop-like form and 30 μl of special antigen composition is added. Serum is mixed with antigen all along the drop-like area by stirrer, the chart is put on shaker and stirred for 4 min.

Results: Ned - particles are dispersed, no lumpiness; Pos. - agglutination is seen with naked eye.

Indirect variant ELISA reveals immunoglobulins IgG (depends on used conjugate). Material for research - blood serum or milk. Used apparatus - spectrophotometer for reading of ELISA plates and plate washer.

Results: Ned - dyeing absent; Pos. - dyeing.

Results and Discussion

50 samples of bovine blood serum were researched. Primary serological diagnosis was made via Rose-Bengal reaction. Then samples were tested by PCR.

It is known that Rose-Bengal method is specific and fast method for brucellosis diagnostics. Though, it is worthy to pay attention to the data obtained (Table 1). N20 and N31 samples of blood serum showing negative and ambiguous results according to Rose-Bengal reaction appeared positive according to PCR, which indicate the beginning stage of disease. Samples N34, 35, 36 being ambiguous by Rose-Bengal reaction and needing repeated research, give stable negative result by PCR. It is likely that in this case we have to take into account the fact that bovine animals may be carriers of some nonpathogenic bacteria, which are able to provoke obtaining of incorrect or ambiguous results in Rose-Bengal reaction, however it is absolutely excluded in case of PCR. Though in this experiment such cases are single, they need special way of approach.

Table1. Detection of brucellosis in samples of bovine blood serum by Rose-Bengal and PCR methods

N of sample	Result by Rose-Bengal	Result by PCR
1-19	+	+
20	-	+
21-30	+	+
31	+-	+
32	+	+
33	+	+
34-36	+-	-
37-50	+	+

Assessment of effectiveness of PCR for laboratory diagnostics of brucellosis was realized by parallel study of 150 samples of blood serum of bovines diseased with brucellosis. All animals were investigated during acute infectious process.

Blood serum samples were tested by PCR and serological reactions: Rose-Bengal and ELISA. Serological reactions conform intensity of infectious process and coincide completely with positive results obtained by PCR.

Table 2. Detection of brucellosis in samples of bovine blood serum by Rose-Bengal, ELISA and PCR methods

N of sample	Result by Rose-Bengal	Result by ELISA	Result by PCR
1-50	+	+	+
51-100	+	+	+
101-150	+	+	+

At the next step of research 100 bovine animals being in contact with diseased livestock or suspected on disease were tested.

At PCR setting DNA of brucella was found in all studied samples. It should be noted that in the given animal group clinical symptoms of brucellosis infection were not revealed. Complete investigation and in proper time of just those risk groups is significant for epizootic control of brucellosis.

After confirmation of reaction specificity Br1/Br2 primers were used for determination of sensitivity of the method. For this aim PCR setting with lysates, which are contained in brucella cells of all species in various concentrations was realized. Quantity of cells brought in was determined by titration of suspension on solid nutrient medium. Usage of those primers enables to reveal specific fragment of 260 bp in all lysate samples received from 10 brucella cells.

Studies carried out showed high effectiveness of PCR for epizootic control of brucellosis. PCR appeared to be high specific and sensitive. However such sensitivity of PCR needs special approach in the viewpoint of clinical interpretation of results. It is necessary to take into account the fact that contact of animals with small doses of weak pathogenic species of brucella may not cause infectious process. Moreover, for animal vaccination farmers may use live vaccines, which give unsterile immunity and vaccine strain is isolated into environment long time.

Persons looking after those animals are also contacted with vaccine strains; they may be carriers and have positive response in PCR test.

All those facts should be taken into account at obtaining PCR positive results without clinical symptoms and positive results of serological tests. In such cases accurate anamnesis and complex laboratory research is important.

Due to high specificity of PCR it is helpful at differential diagnostics of brucellosis and other infectious diseases, which pathogens have common with brucella antigen determinants.

References:

Butkin E.I. *Epizootology with Microbiology.* M.,"Mir", 1981 (in Russian).

Kosilov I.A. *Brucellosis of farming animals.* M., "Veterinaria", 1992 (in Russian).

Payeur J.B. *Methods of laboratory diagnostics of brucellosis.* In: "Collection of works in microbiology and Virology", N24915, 2006.

Devrishov D.A. *Results of sporadic analysis of animal brucellosis.* M., "Veterinaria", 2006 (in Russian).

Fiodorov Iu.N., Gorshenko V.V. *Brucellosis.* M., "Veterinaria", 1982 (in Russian).

პოლიმერაზული ჯაჭვური რეაქციის გამოყენება მსხვილფეხა საქონლის ბრუცელოზის ლაბორატორიული დიაგნოსტიკისათვის

ლომთათიძე ზ.[1], ღვინჯილია მ.[2], ღვინჯილია გ.[3]

[1]თბილისის ბოტანიკური ბაღი და ბოტანიკის ინსტიტუტი
[2]საქართველოს სოფლის მეურნეობის სამინისტროს ლაბორატორია
[3]სურსათის უვნებლობის, ვეტერინარიისა და მცენარეთა დაცვის ეროვნული სამსახური

(მიღებულია 10.03.2008)

რეზიუმე

ბრუცელოზის ენდემურ რაიონებში ეპიზოოტური და ეპიდემიური ფაქტორების აქტივობაზე კონტროლის მიზნით გამოყენებულია კვლევის ლაბორატორიული მეთოდები. ერთერთ ყველაზე ეფექტურ მაჩვენებელს წარმოადგენს დაავადების გამომწვევის გამოყოფა. ბრუცელოზის დეტექციის, იდენტიფიკაციისა და დიაგნოზის ლაბორატორიულად დადასტურების მიზნით გამოყენებულია პოლიმერაზული ჯაჭვური რეაქციის მეთოდი. ნაჩვენებია ამ მეთოდის მაღალი სპეციფიკურობა და მგრძნობელობა მსხვილფეხა საქონლის ბრუცელოზის მწვავე და ქრონიკული ფორმების დიაგნოსტიკისათვის.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 1-2, 2008.

Microbiology

HALOPHYLIC ACTINOMYCETES FROM SALINE SOILS OF EASTERN GEORGIA AND THEIR BIOLOGICAL PROPERTIES

PATARAYA D., GURIELIDZE M., BERISHVILI T., CHOLOKAVA N., ZAALISHVILI G., NUTSUBIDZE N.

Durmishidze Institute of Biochemystry and Biotechnology
**E-mail: pataraia38@yahoo.com*

(Received March 17, 2008)

Abstrsct

On the bases of morphological, cultural, physiological, biochemical and antagonistic properties halophilic actinomycetes isolated from saline soils of Eastern Georgia has been classified. Specific and generic composition has been established. Protease and amylase activities of halophilic actinomycetes from saline soils have been studied. Protease activity was defined in 48 strains among 197 strains isolated from solonchak and solonetz soils. The activity varied from 0,03- 1,23 unit/ml. The capability of halophilic actinomycetes to grow on hydrocarbons – hexane, benzene, benzpyrene, crude oil containing nutrient medium has been investigated. It has been established that halophilic actinomycetes are distinguished with their ability to grow on naphthaline containing medium. Benzene and hexane are absorbed by the studied cultures with moderate intensity. Halophiles weakly develop on crude oil containing medium. The collection of halophilic actinomycetes which consists of producers of biologically active compounds and cultures with hydrocarbon detoxification ability has been created. The studied cultures can be used for novel, highly effective, modern biotechnologies.

Key words: actinomycetes, halophiles, identification.

Introduction

Biodiversity of living organisms, their distribution and classification into definite groups, species and categories is performed on the basis of profound study of their morphological and biological properties. Special interest is focused on adaptation mechanisms of living organisms to extreme environment. From this point of view saline environment with a special group of extreme microorganisms living there, halophilic actinomycetes, is very interesting. Most of halophilic bacteria are divided into two groups on the basis of growth ability at different NaCl concentration. Moderate halophiles grow at 2-20% (0,3-0,4M). Extreme halophiles require at least 2,6M sodium chloride for growth and grow even in saturated brines (about 30%[wt/vol] or 5M) [Ventosa, 1998]. Halophilic actinomycetes synthesize biopolymers, performing complicated functions they serve as unique "natural technologies".

Actinomycetes – actinobacteria are widely distributed in nature. Due to high enzymatic activity and adaptation ability they occupy a wide area. There are no natural substrates without actinomycetes. They are found in soil, water reservoirs, vegetative and animal wastes, in various geographic zones: extreme north, arctic, tropics, highest summits of mountains, deserts.

Actinomycetes and their representatives play an important role for soil formation and fertility restoration. Solonetz and solonchak soils are widely distributed in Eastern Georgia, in desert valleys and valley zones. They occupy over 205 000 ha in Dedoflistskaro, Signagi, Lagodekhi, Gurjaani, Sagarejo, Gardabani, Marneuli, Bolnisi and Kvemo Kartli regions. In general, saline soils are not cultivated and used as a pasture. The cultivated part is characterized by low crop capacity but as a result of definite measures crop capacity of saline soils increases [Gogoberidze, 1984].

There are a lot of producers of antibiotics, enzemes, hormons, vitamins and other compounds among actinomycetes.

We have studied distribution of actinomycetes in typical for Georgia soils, their group and specific compositions, new species of actinomycetes have been described [Pataraya, 1968]. Chemical composition of solonchak and solonetz soils and that of their microflora has been investigated [Gurielidze, 2007].

The carried out investigation had following objectives:

1. Isolation of actinomycetes pure cultures from solonetz and solonchak soils of Eastern Georgia.
2. Study and identification of their biological properties.
3. Creation of halophilic actinomycetes collection, which can be used for restoration of saline soils fertility.

Materials and Methods

The method of dilution was used for isolation of actinomycetes from soil. Incubation was performed in thermostat at 26-28°C, 55-60°C, 14 days. Separate colonies developed on Petri dish were placed into flasks on specific nutrient medium [Krassilnikov, 1966]. Morphology of the isolated actinomycetes, their phases of development was studied by light microscope at ×200-300. Form of spore surface was investigated by electron microscope, at ×19000-23000. Growth ability of the culture was studied on different synthetic and organic nutrient media. Pridham's method was used to study carbon source uptake ability. 1% source of carbon, in particular, monosaccharides – glucose, fructose, galactose, arabinose, xylose, ramnose, alcohols – mannitol, sorbitol, inositol, dulcite, glycerol, disaccharides – saccharose, lactose, maltose, polysaccharides - starch, organic acids – sodium citrate, sodium lactate, and sodium succinate, was added to nutrient medium [Pridham, 1948]. To establish uptake of different sources of nitrogen Fedorov's nutrient medium was used. Nitrogen containing organic and inorganic compounds was used as nitrogen source.

Actinomycetes ability to grow on hydrocarbons – crude oil, hexan, benzpyrene containing nutrient medium was investigated.

Hydrocarbon absorption ability of actinomycetes was defined according to growth intensity.

Antagonistic properties were studied by agar block method [Egorov, 1965]. As test-cultures the following strains were selected: *Staphilococcus aureus, Echerichia coli, pseudomonas aeroginosa, Azospirillum brassilens* G-3, *Mycobacterium phlei, Rodoccocus* spp., *Saccharomyces cerevisiae, Candida utilis, Rhizoctonia* spp., *Fusariom solani.*

Protease activity was established by Anson's method, modified by Petrova [Petrova, 1976]. Actinomycetes identification was performed according to Bergey's manual [Bergey, 1997].

Results and Discussion

197 actinomycetes cultures have been isolated from solonchak and solonetz soils of Eastern Georgia (environs of the Kumisi lake, Sagarejo Region – village Krasnogorka, territory of

the former lake, Alazani Valley – Tsnori, Millary valley – Dedoplistskaro). According to Krassilnikov's method, by coloring of aerial mycelium, and pigment forming ability the isolated cultures have been attributed to the following groups: *Griseus, Cromogenes, Violaceus, Fradiae, Rube, Glaucus, Albus, Viridis, Fluorecens, Globisporus.*

On the basis of morphological properties the isolated cultures mainly develop long or short straight sporiferous aerial mycelium. Cultures with spiral or wavy sporiferous aerial mycelium are very seldom. Cultures which develop sporangium on aerial mycelium are found. Most cultures with straight sporiferous aerial hyphae have smooth spore surface but cultures with spiral spore aerial hyphae have spiny surface. Arial mycelium of some cultures undergo fragmentation into rod- or coccus-like elements (Fig. 1-7). On the basis of morphological, cultural properties actinomycetes have been attributed mainly to genera *Streptomyces, Streptosporangium, Nocardia, Saccharopolyspora, Micromonospora, Promicromonospora.* Growth ability of halophilic actinomycetes on nutrient medium containing 2-15% NaCl has been investigated. Most of cultures grow well on medium with 2-5% NaCl. There are actinomycetes strains capable to grow on 7-10% NaCl. Actinomycetes isolated from saline soils of Eastern Georgia are attributed to the group of moderate halophiles. Optimum pH for most of the studied strains is neutral or alkaline. The cultures are mesophilic – 15-45°C.

Physiological and biochemical properties of the isolated actinomycetes have been studied. The strains differ by their ability to uptake different sources of carbon, which is considered to be a hereditary trait and important for actinomycetes identification [Krassilnikov, 1970]. The studied halophilic actinomycete strains uptake intensively glucose, fructose from monosaccharides, among disaccharides – maltose with different intensity, among polysaccharides – starch (Table 1). From inorganic nitrogen sources the strains uptake intensely KNO_3, among aminoacids – arginin, β-alanine, glycine and leucine. In nutrient medium, where peptone is used as a source of nitrogen all strains develop very well (Table 2). H_2S formation, milk peptonization and coagulation, gelatin dilution, starch hydrolysis ability have been investigated. As seen from Table 3 most actinomycetes obtain catalytic activity and starch hydrolysing ability. The cultures differ in physiological properties.

Protease activity of halophylic actinomycetes isolated from saline soils of Eastern Georgia have been studied. Among 197 strains isolated from solonetz and solonchak soils 48 strains obtain protease activity. Protease activity varies within 0.03-1.23 unit/ml. Among the studied cultures protease activity was stated in 48% of actinomycetes isolated from environs of the Kumusi Lake, 11% - Krasnogorka, 26% - Millary Valley and 15% - Alazani Valley. Strain 173H, isolated from environs of the Kumisi Lake is distinguished by protease activity – accounting 1.23 units/ml. 42 Strains obtain amylase activity. Halophilic actinomycetes ability to grow on nutrient medium containing hydrocarbons (hexane, benzene, benzpyrene, crude oil) has been investigated. Actinomycete abilities to develop on different hydrocarbons containing mineral nutrient medium differ. Nutrient media with naphthalene in concentrations 0.02, 0.1, 0.5, 1% have been used for selection of active strains. Among the studied 197 strains most ones develop on nutrient medium with 0.02 and 0.1% naphthalene used as a single source of carbon, 37 strains develop well at 0.5%, average – 34, weakly – 56 and no development has been observed in 73 strains. Nutrient medium contained 0.5% hexane, benzene, naphthalene, crude oil and 0.005% benzpyrene. Among the studied strains good growth of 26 halophilic actinomycetes cultures has been observed on hexane containing nutrient medium, average – 18, good growth of 28 – on benzene containing nutrient media and average – 19, on crude oil containing nutrient media good growth of 3 strains and weak – 38 has been observed; at given concentration of benzpypene good growth of 19 strains, average – 3 has been found. Halophilic actinomycetes are most distinguished by their growth intensity on naphthalene containing medium. Moderate uptake of benzene, hexane and weak development of the investigated cultures on crude oil containing medium have been established.

Table 1. Uptake of carbohydrate sources by different halophilic actinomycetes [a]

#	Strains	Monosaccharides			Disaccharides		Alcohols			Polysaccharides		Sodium lactate
		Glucose	Fructose	Galactose	Saccharose	Maltose	Mannitol	Sorbitol	Glycerol	Starch	Cellulose	
1	11H	3	0	0	0	0	0	0	0	3	0	0
2	12H	5	5	5	0	5	2	0	3	5	0	0
3	33H	5	5	5	2	5	5	1	3	5	0	3
4	77H	2	3	1	0	2	0	0	2	5	0	0
5	115H	4	1	1	1	2	0	1	0	4	0	0
6	142H	4	0	0	0	1	0	0	0	4	0	0
7	165H	3	0	0	0	0	0	0	0	4	0	0
8	169H	2	0	1	0	2	0	1	2	4	0	0
9	171H	3	5	5	1	5	3	0	3	4	1	1
10	276H	5	5	5	3	6	6	3	3	5	3	4
11	278H	4	5	5	0	5	3	1	3	5	0	1
12	283H	3	5	3	0	5	1	0	0	5	0	0
13	286H	5	3	5	1	5	4	0	0	5	0	1
14	287H	3	3	3	0	3	1	0	1	5	0	0
15	289H	3	4	4	0	5	1	1	0	4	0	2
16	294H	2	3	3	0	1	0	0	0	5	0	0
17	295H	2	1	2	0	4	1	0	0	5	0	0
18	297H	2	5	4	0	3	0	0	0	5	0	0
19	300H	4	5	5	4	5	4	0	3	5	1	0
20	301H	2	4	3	0	5	0	0	0	5	0	1
21	303H	3	5	5	0	5	4	0	3	5	0	0
22	306H	3	5	2	0	4	0	1	0	5	0	1
23	307H	2	5	0	0	2	2	0	2	5	0	0
24	310H	3	4	4	1	3	1	0	1	5	0	0
25	311H	2	3	0	0	0	0	0	0	5	0	0
26	312H	2	5	3	0	5	2	1	1	4	0	0
27	326H	3	5	5	0	4	0	0	3	5	0	0
28	327H	5	5	5	0	5	0	0	0	5	0	0
29	328H	4	3	3	0	5	0	0	0	5	0	0
30	329H	5	4	5	5	5	4	0	3	4	0	1
31	331H	1	1	0	0	4	0	0	0	5	0	0

[a] 0 - no uptake; 1 and 2 - weak uptake; 3 – moderate uptake; 4 and 5 – strong uptake.

As for antagoistic properties, among the studied strains 10 reveal antagonistic ability to *Staphilococcus aureus,* 13 strains to *Esherichia coli,* 12 strains to *Pseudomonas aeroginosa,* 2 strains to *Candida utilis,* 1 strain to *Saccharomyces cerevisiae,* 1 strain to *Fusarium solani,* 8 strains to *Rhizoctonia* spp. (Fig.8-11).

Table 2. Uptake of nitrogen sources by different halophilic actinomycetes [a]

#	Strains	Inorganic sources of N			Amino acids					Pepto-ne
		KNO_3	NH_4Cl	$(NH_4)_2SO_4$	β-alanine	Arginine	Glycine	Leucine	Trypto-phane	
1	11H	2	0	0	3	2	3	2	1	3
2	12H	5	4	5	5	5	5	5	2	5
3	33H	5	4	4	5	5	5	5	4	5
4	77H	4	1	1	4	5	5	4	1	5
5	115H	3	1	1	3	5	5	5	1	5
6	142H	2	1	2	3	3	5	3	0	4
7	165H	4	0	0	3	2	3	1	0	3
8	169H	4	2	2	4	3	4	5	2	4
9	171H	5	2	4	5	5	5	5	0	5
10	276H	5	2	3	5	5	5	5	3	5
11	278H	4	2	3	5	5	5	5	3	5
12	283H	5	2	3	5	5	5	5	1	5
13	286H	5	3	3	5	5	5	5	2	5
14	287H	4	3	3	5	5	5	5	1	5
15	289H	2	4	4	5	5	5	5	2	5
16	294H	4	2	2	5	5	5	5	0	5
17	295H	5	4	5	5	5	5	5	5	5
18	297H	5	3	4	5	5	5	5	4	5
19	300H	4	1	1	5	5	5	5	4	5
20	301H	4	1	2	5	5	5	5	1	5
21	303H	5	1	1	5	5	5	5	2	5
22	306H	5	1	3	5	5	5	5	4	5
23	307H	5	2	3	5	5	5	5	4	5
24	310H	4	2	3	5	5	5	5	0	5
25	311H	4	1	1	3	4	4	3	0	5
26	312H	5	2	3	5	5	5	5	4	5
27	326H	5	5	3	5	5	5	5	5	5
28	327H	5	5	3	5	5	5	5	5	5
29	328H	3	5	3	5	5	5	5	1	5
30	329H	5	3	4	5	5	5	5	3	5
31	331H	2	3	3	5	5	5	3	0	4

[a] 0 - no uptake; 1 and 2 - weak uptake; 3 – moderate uptake; 4 and 5 – strong uptake.

On the bases of morphological, cultural, physiological, biochemical and antagonistic properties actinomycetes isolated from solonetz and solonchak soils of Eastern Georgia could be attributed to the following species: *Streptomyces noboritoensis,* Jsono et al., 1957, *Streptomyces albocrustosus,* Krassilnikov 1970, *Streptomyces raffinosus,* Nikitina 1957, *Spertomyces globivulgaris,* Krassilnikov, 1970, *Streptosporangium album,* Nonomura, Ohara, 1960, *Streptomyces ravus,* Krassilnikov, Pataraya, 1970, *Streptomyces alborobeus,* Krassilnikov, 1970. *Streptomyces ravulus,* Krassilnikov, 1970, *Streptomyces* sp. 124H, *Nocardia septisporus,* Krassilnikov, 1970, *Nocardia* spp., 154H. *Nocardia albatus*, Krassilnikov, 1970, *Nocardia aborectus,* Krassilnikov, 1970, *Streptomyces sindenensis*, Nakazava et Fujii, 1957, *Streptomyces bacillaris,* Nikitina, 1957, *Streptomyces rectiviolaceus,* Artamanova, 1965, *Saccharopolispora* spp., 121H, *Streptomyces canosus,* Krassilnikov, Pataraya, 1970, *Streptomyces globisporus,* Krassilnikov, 1941, *Sreptomyces streptomycini,* Krassilnikov, 1949, *Streptomyces sp. 317H, Streptomyces sporostellatus Krassilnikov, Pataraya* 1970, *Streptomyces griseofavillus,*

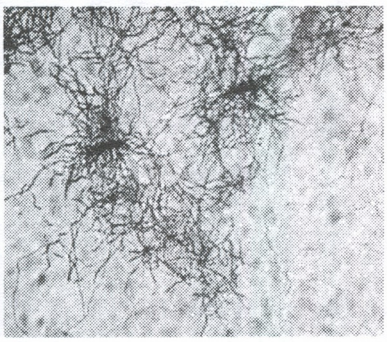
Krassilnikov, Pataraya, 1970, *Streptomyces rameus,* Okami et al., 1959, *Streptomyces argentocanus,* Krassilnikov, 1970, *Streptomyces cin erofulvus,* Krassilnikov, Pataraya, 1970, *Streptomyces* spp., 11H, *Sreptomycs* spp., 12H, *Sreptomyces* spp., 21H, *Sreptomycs* spp., 318H.

We have classified halophilic actinomycetes isolated from saline soils of Eastern Georgia.

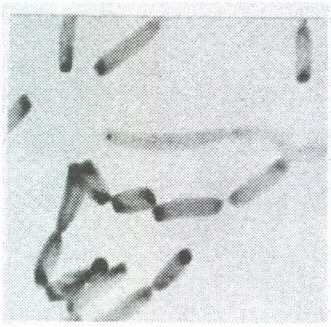
Table 3. Some physiological and biochemical properties of halophilic actinomycetes[a]

#	Strains	pH	T°C	H_2S production	Mick		Dilution of gelatine	Hydrolyze of starch	Catalase activity	Nitrat-reductae activity
					patronization	coagulation				
1	11H	7-9	15-35	-	+	-	-	-	+	+
2	12H	7-11	15-35	+	+	-	+	+	+	-
3	33H	7-11	15-35	+	+	-	-	-	-	+
4	77H	7-11	15-35	-	-	-	-	+	-	-
5	115H	7-11	15-35	-	+	-	-	+	-	+
6	142H	7-8	15-35	-	-	-	-	+	+	+
7	165H	7-11	15-35	-	+	-	-	+	+	-
8	169H	7-11	15-35	-	-	-	-	-	+	-
9	171H	7-11	15-35	+	+	-	+	-	+	+
10	276H	7-9	15-45	-	+	-	-	+	+	+
11	278H	7-9	15-45	+	+	-	-	+	-	-
12	283H	7-11	15-45	+	+	-	+	+	+	+
13	286H	4-9	15-35	-	+	-	+	+	+	-
14	287H	7-9	15-35	-	+	-	-	+	+	+
15	289H	7-8	15-35	+	+	-	+	+	+	+
16	294H	7-11	15-35	+	+	+	+	+	+	-
17	295H	4-9	15-35	+	+	-	+	+	+	-
18	297H	7-11	15-45	+	+	-	-	-	+	-
19	300H	7-11	15-45	-	-	-	-	+	+	+
20	301H	7-11	15-45	-	-	-	-	+	+	+
21	303H	7-11	15-35	+	+	+	+	-	+	-
22	306H	7-11	15-45	+	+	-	+	-	-	+
23	307H	7-9	15-45	+	+	-	-	+	+	-
24	310H	7-11	15-45	+	-	-	-	+	+	-
25	311H	7-9	15-45	-	-	-	+	+	+	+
26	312H	7-11	15-45	+	-	-	-	+	+	-
27	326H	4-9	15-45	+	-	-	+	+	+	+
28	327H	4-9	15-45	+	-	-	+	+	+	-
29	328H	7-11	15-45	+	-	-	+	+	+	-
30	329H	4-9	15-35	-	-	-	+	+	+	+
31	331H	7-11	15-35	-	+	-	+	-	+	+

[a] + positive result, - negative result.

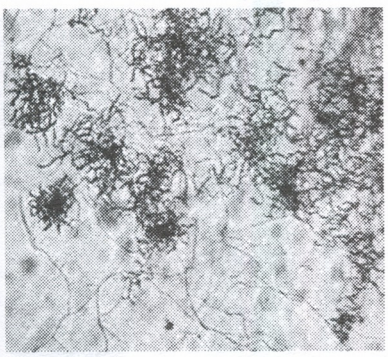


a

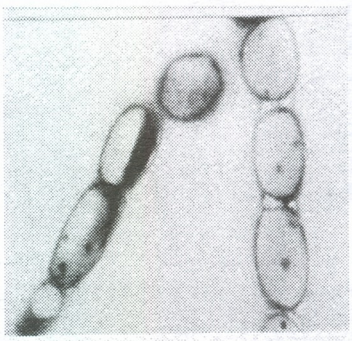


b

Fig. 1. *Streptomyces bacillaris* 171H. a) Aerial mycelium b) spores.

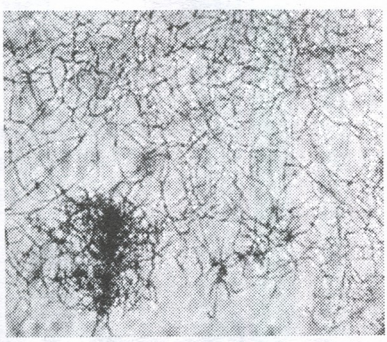


a

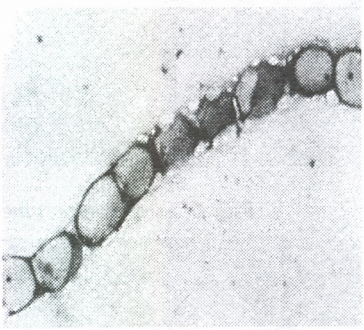


b

Fig. 2. *Streptomyces globisporus* 289H. a) Aerial mycelium, b) spores.

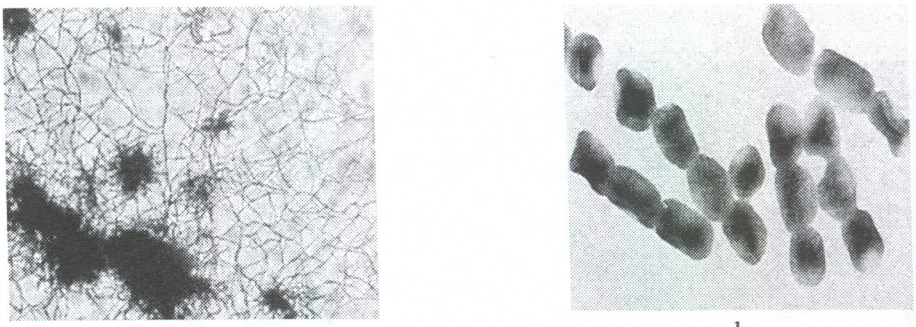


a



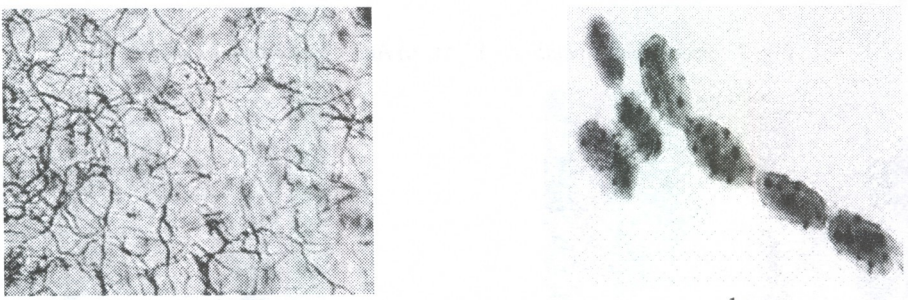
b

Fig. 3. *Streptomyces streptomycini* 295H. a) Aerial mycelium, b) spores.



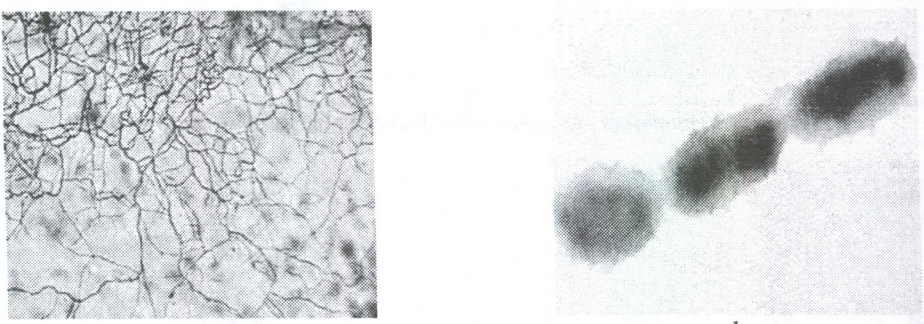
a b

Fig. 4. *Streptomyces* spp.318H. a) Aerial mycelium, b) spores.



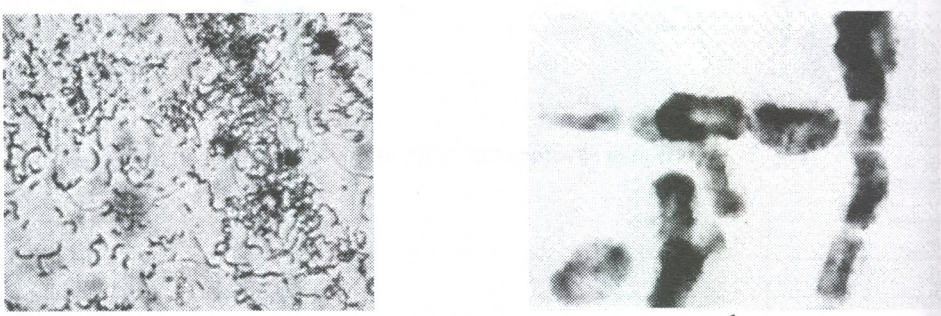
a b

Fig. 5. *Streptomyces argentocanus* 329H. a) Aerial mycelium, b) spores.



a b

Fig. 6. *Streptomyces cinerofulvus* 331H. a) Aerial mycelium, b) spores.



a b

Fig. 7. *Nocardia septisporus* 138H. a) Aerial mycelium b) spores.

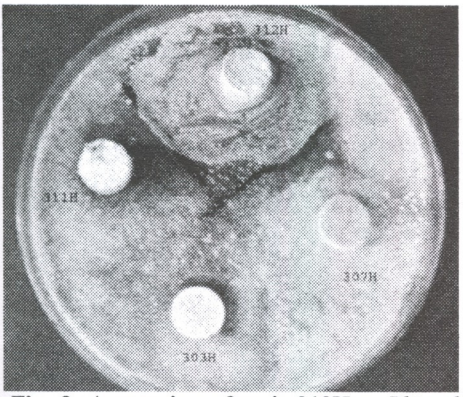


Fig. 8. Antagonism of strain 312H to *Rhizochtonia* spp.

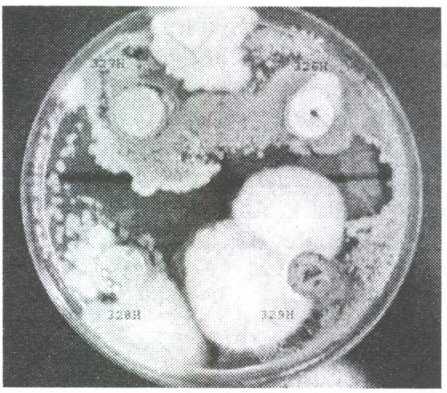


Fig. 9. Antagonism of strain 327H to *Fusarium solani*

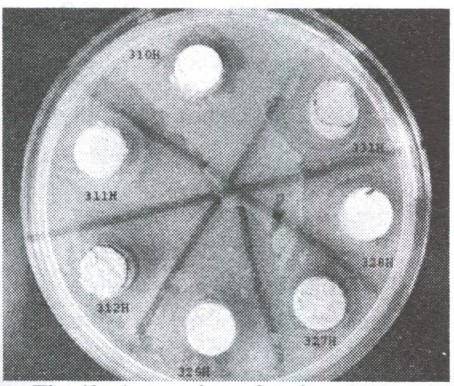


Fig. 10. Antagonism of strains - 310H, 327H, 328H and 331H to *Pseudomonas aeroginosa.*

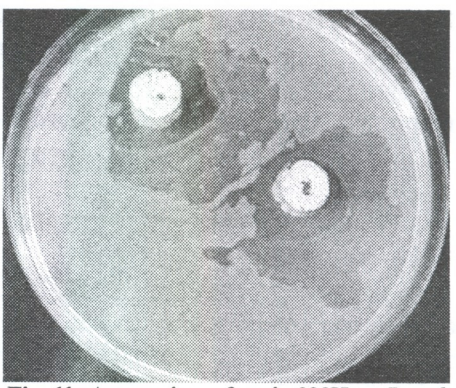


Fig. 11. Antagonism of strain 329H to *Pseudomonas aeroginosa.*

Acknowledgement: The designated project has been fulfilled by financial support of Georgian National Science Foundation (Grant #GNSF/ST06/5-077). Any idea in this publication is possessed by the author and may not represent the opinion of Georgian National Science Foundation itself.

References:

Bergey's Mannual of Determinative Bacteriology (eds J.G.Holt, N.R.Krieg, P.H.A.Sneath, J.T.Staley & S.T.Williams), V.**2**, Moscow,"Mir" pp. 613-706, 1997.

Egorov N.S. *Microbes-antagonists and the biological methods of determination of antibiotic activity.* Moscow, "Visshaja Shkola", p. 152, 1965 (in Russian).

Gogoberidze I. *Saline soils of Eastern Georgia.* Tbilisi, "Tsodna", p.24, 1984.

Gurielidze M., Berishvili T., Cholokava N., Pataraya D., Nutsubidze N. *Chemical composition and microflora of solonchaks and solonetsz soils of Eastern Georgia.* Proc. Georgian Acad. Sci., Biol.Ser.B, **5**, 2, 57-65, 2007.

Krassilnikov N.A. *Method of soil microorganisms and their metabolites study.* M., Publishing House of Moscow University, pp. 7-46, 1966 (in Russian).

Krassilnikov N.A. *Actinomycetes*. Moscow, Nauka, 1970.
Pataraya D. *Actinomycetes of different soils of Georgia.* Author's abstract of dissertation. Tbilisi, 28 p., 1968 (in Russian).
Petrova I.S. *Proteolytical enzemes of actinomycetes.* Moscow, "Nauka", 1976.
Pridhem T.C., Gottlieb D. *The utilization of carbon compounds by some Actinomycetales as an aid for species determination.* J. Bacteriol., **56**, p. 107-114, 1948.
Ventosa A., Nieto J.J., Aharon O. *Biology of Moderatety Halophilic Aerobic Bacteria*, Microbiol. Mol. Biol. Rev 62, 2, 504-544, 1998.

აღმოსავლეთ საქართველოს დამლაშებული ნიადაგებიდან გამოყოფილი ჰალოფილური აქტინომიცეტები და მათი ბიოლოგიური თვისებები

პატარაია დ., გურიელიძე მ., ბერიშვილი თ., ჭოლოკავა ნ., ზაალიშვილი გ., ნუცუბიძე ნ.

ს. დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 17.03.2008)

რეზიუმე

შესწავლილია აღმოსავლეთ საქართველოს დამლაშებული ნიადაგებიდან გამოყოფილი ჰალოფილური აქტინომიცეტების მორფოლოგიური, კულტურალური, ფიზიოლოგიურ-ბიოქიმიური და ანტაგონისტური თვისებები. დადგენილია მათი ჯგუფობრივი და სახეობრივი შემადგენლობა. გამოკვლეულია დამლაშებული ნიადაგებიდან გამოყოფილი ჰალოფილური აქტინომიცეტების პროტეაზული და ამილაზური აქტივობები. ბიცობიანი და მლაშე ნიადაგებიდან გამოყოფილი 197 შტამიდან პროტეაზულ აქტივობას ავლენდა 48 შტამი. აქტივობა მერყეობდა 0.03-1.23 ერთ/მლ ფარგლებში. შესწავლილი იქნა ჰალოფილური აქტინომიცეტების ზრდის უნარი ნახშირწყალბადების – ჰექსანი, ბენზოლი, ბენზ-პირენი, ნედლი ნავთობის შემცველ საკვებ არეებზე. გამოვლინდა, რომ ნაფტალინის შემცველ არეზე ზრდის მიხედვით გამოირჩევა ჰალოფილური აქტინომიცეტები. ბენზოლსა და ჰექსანს შესწავლილი კულტურები ითვისებენ საშუალო ინტესივობით. ნავთობის შემცველ არეზე ჰალოფილები სუსტად ვითარდება. შექმნილია ჰალოფილური აქტინომიცეტების კოლექცია, რომელიც შედგება ბიოლოგიურად აქტიური ნივთიერების პროდუცენტი და ნახშირწყალბადების დეტოქსიკაციის უნარის მქონე კულტურებისაგან. შესწავლილი კულტურები შესაძლებელია გამოყენებული იქნეს ახალი, მაღალეფექტური, თანამედროვე ბიოტექნოლოგიების შესაქმნელად.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 1-2, 2008.

Mycology

CONTRIBOTION TO THE MYCOBIOTIC DIVERSITY OF GEORGIA: FUNGI ASSOCIATED WITH RED LIST SPECIES (R L) OF WOODY PLANTS OF GEORGIA

GVRITISHVILI M., KACHEISHVILI-TAVARTKILADZE K., MURVANISHVILI I.

Tbilisi Botanic Garden and Institute of Botany

(Received March 6, 2008)

Abstract

The papers deals with new records of fungi associated with RL species of woody plants (*Celtis glabrata, Nitraria schoberi, Populus euphratica, Quercus pontica, Sambucus tigranii)* and new fungus-plant combinations to Georgia.

Key words: Red List woody plants, fungi, Georgia.

Introduction

There are no data or little is known about fungi associated with RL woody species under consideration. In particular, *Nitraria schoberi, Populus euphratica* and *Sambucus tigranii* are not listed among the host plants of fungi of Georgia. Only one parasitic fungus, *Cylindrosporium celtidis* Eearle is known on *Celtis glabrata* (Nakhutsrishvili, 1986). On *Quercus pontica* 4 species: *Ascochyta quercus* Sacc. & Speg., *Diplodia quercina* West., *Gnomonia setacea* (Pers.) Ces.& De Not. (Shainidze, 1999) and *Microsphaera alphitoides* Griffon & Maubl. (Mikaberidze, 1980) are reported.

Materials and Methods

The collection of fungi investigated have been gathered during field observations carried out in Tbilisi Botanic Garden (TBG), Tbilisi environs (TE), Mtskheta district (M), Washlovani State Reserve (WSR), East Georgia; Guria floristical region (G), West Georgia and partly in Meskheti region (MR), South-West Georgia. Routine light microscopic method has been used for identification of collected specimens on the base of macro and micromorphologocal features using classic and modern guide books for identification of fungi.

Results and Discussion

Celtis glabrata Stev. ex Planch.

On dead stems and twigs:
**Hendersonia celtidis* Ell.& Ev., vill. Karsani (M);

***Massaria foedans* Fr. ibid., in association with *Cytospora pruinosa* (Fr.) Sacc., TBG riv. Tsavkisistskali, near TBI;
***Microdiplodia melaena* Allesch, Mtskheta distr.;
***Microsphaeropsis olivacea* (Bonord.) Höhn., TBG;
**Phoma sp.* 1 (conidia 4-5x1.5μm), in old conidiomata of *Stigmina obtecta*. Karsani (M);
**Phoma sp.* 2 (conidia 5-11x3-5 μm), in old conidiomata of *S. obtecta*, ibid.;
***Stigmina obtecta* (Petrak & Esfandiari) M.B.Ellis. TBG; Karsani (M);
***Trimmatostroma salicis* Corda, TBG;
***Valsa cypri* (Tul.)Tul.& C.Tul. in association with its conidial stage, *Cytospora pruinosa* (Fr.) Sacc., TBG; Karsani (M);

Nitraria schoberi L.

On this small shrubbery not referred as host plants of any fungi in Georgia the following 6 species of microfungi have been found on dry stems and twigs:
***Alternaria alternata* (Fr.) Keissl;
***Camarosporium sp.;*
** *Hendersonia vagans* Fuckel;
***Microdiplodia microsporella* (Sacc.) Allesch.;
** *Pleospora herbarum* (Pers.) Rabenh. ex Ces. & De Not.;
***Stigmina obtecta* (Petrak & Esfandiari) M.B. Ellis.

Meskheti region, between vill. Rustavi and Aspindza, dry habitat. Coord.: 40° 35' 359 N; 43° 10' 155 E, 1026 m a.s.l., 28. 09. 2006. M. Gvritishvili.

It must be noted that all of these fungi including *Camarosporium sp.* are not specific to *N. schoberi* having more or less wide range of host plants.

Populus euphratica Olivier

On this endangered plant following 8 microfungi are recorded on dead twigs and leaves:
***Camarosporium propinquum* (Sacc.) Sacc., WSR;
***Cladosporium macrocarpum* Preuss, WSR;
***Cytospora leucosperma* (Pers.) Fr. together with its teleomorph, *Valsa ambiens* (Pers.) Fr., TBG.
***Microdiplodia salicis* Died., TBG;
***Microdiplodia rosarum* Died., TBG;
***Mycosphaerella populi* (Auersw.) J. Schröt., on leaves, WSR;
**Platystomum populinum*, WSR;
**Pleospora sclerotioides* Speg., WSR.

Quercus pontica C.Koch

On dead stems and twigs:
**Coryneum megaspermum* H. & P. Syd. This species is known from Canada on Quercus sp. (Sutton, 1975);
***Cytospora leucosperma* (Pers.) Fr.;

---

*New record to Georgia
**New fungus-plant combination

***C. leucostoma** Fr.;
***C. sacculus**(Schwein.) Gvrit., together with *Valsa ceratosperma* (Tode) Maire;
****Diaporthe leiphaemia** (Fr.) Sacc.;
**** *Naemospora microspora* Desm. (conidia 7-8 x 1.2 μm);
***Phomopsis quercina** (Sacc.) Höhn.

P. quercina differs from other species of *Phomopsis* associated with *Quercus* spp. in having larger conidia (8-13(-21)x2-4 μm). As mentioned by Wehmeyer (1933) two types of conidia were formed on agar. The alfa conidia were long, fusoid-cylindric, one-celled, hyaline, and 11-20x2.5-5. According to him these conidia agree fairly well with "these conidia of the European species mentioned above", i.e. *P. quercina.*
***Septomyxa aesculi** Sacc.;
****Stereum hirsutum** Fr.

West Georgia, Chokhatauri distr., along the road to Bakhmaro, Tskhratskaro, beech-spruce forest, *Quercus pontica* stand. 19.08.1998. M. Gvritishvili, K.Kacheishvili-Tavartkiladze.
Sambucus tigranii Troitzk.

8 species of microfungi listed bellow have been recorded on this plant represented by the only very small population with 19 shrubs including 7 fruit-bearing ones.
****Coniothyrium fuckelii** Sacc.;
****Cytospora leucosperma** (Pers.) Fr.;
****C. pruinosa** (Fr.) Sacc.;
****C. rubescens** Fr. (syn. *C. cincta* Sacc.).;
****Microdiplodia microsporella** (Sacc.) Allesch.;
****Sclerophoma** sp. (conidia 3-4x1.5 μm);
****Trichothecium roseum** (Pers.) Link, on fruits.;
****Tuberculariа vulgaris** Tode.

Meskheti region, between vill. Rustavi and Aspindza, right bank of the river Kura (Mtkvari), opposite of fortress Tmogvi. Coord.: 41°23' 317 N; 43°18' 502 E, 1300 m a. s. l., 15. 10. 2007. M. Gvritishvili.

It must be noted absence in this list such common parasitic fungi associated with *Sambucus* spp. as *Cercospora depazeoides* (Desm.) Sacc., *Cercosporella prolificans* (Ellis & Holw.) Sacc. and *Ramularia sambucina* Sacc.

References:

Mikaberidze M. *Spreading of Microsphaera alphitoides Griff et Maubl., the causal agent of powdery mildew of oaks in Georgia.* Trans. Georg. Inst. Plant. Prot. **30**, 15-18, 1980.
Nakhutsrishvili I. G. (ed.) *Flora of spore-producing plants of Georgia. Catalogue.* Tbilisi "Metsniereba", 1986 (in Georg.).
Sutton B.C. *Coelomycetes.* 5. Coryneum. Mycol. Pap. **138**, 1-324, 1975.
Uecker F.A. *A world list of Phomopsis names with notes on nomenclature, morphology and biology.* Mycol. Memoir, N13, J. Cramer, Berl., Stuttg., 1988.
Wehmeyer L.E. *The genus Diaporthe Nitscke and its segregates.* Univ. Mich. Press, 1933.

მასალები საქართველოს სოკოების მრავალფეროვნების შესწავლისათვის: საქართველოს წითელი ნუსხის მერქნიან მცენარეებთან ასოცირებული სოკოები

გვრიტიშვილი მ., ყაჭეიშვილი-თავართქილაძე ქ., მურვანიშვილი ი.

თბილისის ბოტანიკური ბაღი და ბოტანიკის ინსტიტუტი

(მიღებულია 06.03.2008)

რეზიუმე

სტატიაში მოყვანილია ახალი მონაცემები საქართველოს წითელი ნუსხის მცენარეებთან (*Celtis glabrata, Nitraria schoberi, Populus euphratica, Quercus pontica, Sambucus tigranii*) ასოცირებული სოკოების შესახებ.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 1-2, 2008.

Paleobiology

THE MICROFAUNISTICAL AND PALYNOLOGICAL CHARACTERISTIC OF MIDDLE SARMATIAN DEPOSITS OF EASTERN GEORGIA (KARTLI)

MAISURADZE L.[1], *KOIAVA K.[2], SPEZZAFERRI S.[3], SHATILOVA I.[1], MCHEDLISHVILI N.[1], SHUBITIDZE L.[2], STRASSER A.[3]

[1]*Georgian National Muzeum, Institute of Paleobiology*
[2]*Djanelidze Institute of Geology, koiava_ka@yahoo.com*
[3]*University of Fribourg, Departament of Geosciences, Ch. du Musee 6, 1700 Fribourg, Switzerland, silvia.spezzaferri@unifr.ch, andreas.strasser@unifr.ch*

(Received February 11, 2008)

Abstract

The section of Sarmatian deposits near village Nadarbazevi (Kartli) from paleontological point of view is one of more interesting on the territory of Eastern Georgia. The complex investigation of the section is realized at first by microfaunistical and palynological methods. The description of associations of foraminifers is given and their change in time is traced. The list of flora is composed according to palynological data and data of study of large remains of plants publish in literature. Three diagrams are given: one microfaunistical and two palynological. The microfaunistical diagram reflects the quantitative distribution of genera of foraminifers in layers of section. One of palynological diagrams is built by landscape-phytocenological method and gives the possibility to trace the changes of area of main vegetational formations in dependence of climatic fluctuations.

Key mords: Eastern Georgia, Sarmatian, foraminifers, pollen and spores.

Introduction

On the territory of Eastern Georgia the Sarmatian deposits are widely distributed. They are represented by three parts: Volynian substage (Lower Sarmatian), Bessarabian substage (Middle Sarmatian) and Khersonian substage (Upper Sarmartian). The Lower Sarmatian is divided into two parts and Middle Sarmatian on three parts. On the territory of Eastern Georgia the Lower and Middle Sarmatian are built by marine deposits, but Upper Sarmatian, except some regions, is represented by continental sediments. The mollusks from Eastern Georgia Sarmatian deposits are learned in details, unlike microfauna, which was investigated sporadically. The works (Koiava 2006, 2006a) devoted to results of micropaleontological study of Sarmatian deposits of Eastern Georgia were published not long ago. The distribution of foraminifers in time was traced by author, who used the regularity of this process for stratigraphy.

Till today our knowledge about flora and vegetation of Eastern Georgia of Sarmatian were founded on the data about imprints of leaves [Uznadze, 1965; Chelidze, 1987]. From palynological point of view they are practically not studied. There is only work of Mtchedlishvili (1953) who learned the samples from Central part of Eastern Georgia. 28 forms were determined, out of them 3 belonged to cryptogamous plants, 7 – to conifers and 18 - to angiosperms. We also have seen all these forms in new materials.

Material and Methods

The work is devoted to the results of complex investigation of representatives of marine (foraminifers) and terrestrial (flora) Sarmatian bios. The samples from Middle Sarmatian deposits of gorge Nadarbazevi, near station Metekhi (Eastern Georgia, Kartli, region of Kaspi) were analyzed. The list of foraminifers from these deposits is given below (Tab.I).

Table 1. The List of Sarmatian Foraminifers

Family	Genera	Species, subspecies
Elphidiidae Galloway, 1933	Elphidium Montfort, 1808	Elphidium macellum (Fichtel et Moll.)
		Elphidium crispum (Linne)
		Elphidium aff. rugosum (d'Orbigny)
		Elphidium fichtelianum (d'Orbigny)
		Elphidium aculeatum (d'Orbigny)
		Elphidium aff. flexuosum (d'Orbigny)
		Elphidium aff. mirandum Krasheninnikov.
		Elphidium angulatum (Egger)
		Elphidium ukrainicum Krasheninnikov.
		Elphidium reginum (d'Orbigny)
		Elphidium hauerinum (d'Orbigny)
	Elphidiella,Cushman, 1936	Elphidiella artifex Serova
Nonionidae Schultze, 1854	Nonion Montfort, 1808	Nonion bogdanowiczi Voloshinova
		Nonion aff. tumidulus Pishvanova
		Nonion sp.(1)
		Nonion sp.(2)
	Porosononion Putrja in Voloshinova, 1958	Porosononion subgranosum (Egger)
		Porosononion subgranosum umboelatum (Bogdanowicz)
		Porosononion granosum (d'Orbigny)
		Porosononion martkobi (Bogdanowicz)
		Porosononion hyalinum (Bogdanowicz)
		Porosononion aragviensis (O.Djanelidze)
Miliolidae Ehrenberg, 1939	Cycloforina Luczkowska, 1972	Cycloforina complanata (Gerke et Issaeva)
		Cycloforina aff.hauerina (d'Orbigny)
		Cycloforina latelacunata (Venglinski)
	Sinuloculina Luczkowska, 1972	Sinuloculina consobrina (d'Orbigny)
		Sinuloculina consobrina sarmatica (Gerke)
		Sinuloculina aff.mayeriana (d'Orbigny)
	Varidentella Luczkowska, 1972	Varidentella reussi (Bogdanowicz)
	Miliolinella Wiesner, 1931 emend. Luczkowska, 1972	Miliolinella sp.
	Articulina d'Orbigny, 1826	Articulina sarmatica (Karrer)
		Articulina problema (Bogdanowicz)
	Articularia Luczkowska, 1974	Articularia articulinoides (Gerke et Issaeva)
	Meandroloculina Bogdanowicz, 1935	Meandroloculina conicocamerale Bogdanowicz
		Meandroloculina sp.
	Sarmatiella Bogdanowicz, 1952	Sarmatiella sp.
Ellipsolagenidae A.Silvestri, 1923	Fissurina Reuss, 1850	Fissurina sp.1 Fissurina sp.2

From palynological point of view the Nadarbazevi section turned out to be very rich and interesting. Bellow is given the common lists of flora (Tab. 2), composed by palynological data and the data of study of large remains of plants of Sarmatian deposits of Kartli [Chelidze, 1979; 1987]. The quantitative composition of separate taxa are given in Tab. 3.

Table 2. The list of taxa of Sarmatian flora determined by macro-remains of plants (m) and by palynological data (p)

Class	Family	Form	m	p
Briopsida	Sphagnaceae	Sphagnum sp.		p
Lycopodiopsida	Lycopodiaceae	Lycopodium serratum Tunb.		p
Isoetopsida	Selaginellaceae	Selaginella sp.		p
Ophyoglossopsida	Ophyoglossaceae	Bothrychium sp.		p
Polypodiopsida	Osmundaceae	Osmunda sp.	m	p
	Schizaeaceae	Schizaea sp.		p
	Anemiaceae	Anemia sp.		p
		Mohria sp.		p
	Lygodiaceae	Lygodium sp.		p
	Pteridaceae	Cryptogramma sp.		p
		Pteridacidites longifoliiformis Sh., St.		p
		Pteris sp.*		p
	Marsileaceae	Marsilea sp.		p
	Adiantaceae	Anogramma sp.		p
		Onychium sp.		p
		Pityrogramma sp.		p
	Gleicheniaceae	Clavifera sp.		p
		Gleichenia sp.		p
		Gleicheniaceae gen.indet.		p
	Polypodiaceae	Polypodium aureum L.		p
		Polypodium pliocenicum Ram.		p
		Polypodium verrucatum Ram.		p
		Polypodium sp.1-7		p
		Polypodium sp. (aff.Cyclophorus sp.)		p
		Verrucatosporites histiopteroides W.Kr.		p
		Pyrossia sp.		p
		Polypodiaceae gen.indet.*		p
	Hymenophyllaceae	Hymenophyllum sp.		p
	Thyrsopteridaceae	Cibotium sp.		p
	Dicksoniaceae	Dicksonia sp		p
	Cyatheaceae	Alsophyla sp.		p
		Cyathea sp.		p
		Hemitelia sp.		p
		Leiotriletes Naum.*		p
	Aspleniaceae	Asplenium sp.		p
	Aspidiaceae	Cystopteris sp.		p
	Davalliaceae	Microlepia sp		p
		Filicales fam.indet.		p
Ginkgoopsida	Ginkgoaceae	Ginkgo sp.		p
Pinopsida	Podocarpaceae	Dacrydium sp.		p
		Podocarpus sp.*		p
	Phyllocladaceae	Phyllocladus sp.		p
	Araucariaceae	Araucaria sp.		p
	Pinaceae	Abies ciliticaeformis N.Mtchedl.		p
		Abies nordmanniana (Stev.) Spach.		p
		Abies sp.*		p
		Cathaya sp.		p
		Cedrus sauerae N.Mtchedl.		p
		Cedrus sp.*		p

Pinopsida	Pinaceae	Keteleeria caucasica Ram.		p
		Picea minor N.Mtchedl.		p
		Picea sp.*		p
		Pinus sp.*	m	p
		Pseudolarix sp.		p
		Pseudotsuga sp.		p
		Tsuga aff. canadensis (L.) Carr.		p
		Tsuga aff. pattoniana Engelm.		p
		Tsuga sp.*		p
		Pinaceae gen.indet.*		p
	Sciadopityaceae	Sciadopitys sp.		p
	Taxodiaceae	Cryptomeria sp.		p
		Cunninghamia sp.		p
		Sequoia sp.		p
		Sequoiadendron sp.		p
		Taxodium sp.		p
		Taxodiaceae gen.indet.		p
	Cupressaceae	Libocedrus salicornioides (Ung.)Heer	m	
		Libocedrus sp.		p
		Juniperus sp.		p
		Cupressaceae gen.indet.		p
Ephedropsida	Ephedraceae	Ephedra sp.		p
Dicotyledoneae	Myricaceae	Comptonia sp.		p
		Myrica laevigata (Heer) Sap.	m	
		Myrica sp.1 (cf.M.acuminata Ung.)	m	
		Myrica sp.2 (cf.M.lignitum (Ung.)Sap.)	m	
		Myrica sp.		p
		Myricaceae gen.indet.*		p
	Juglandaceae	Carya sp.*		p
		Engelhardia sp.		p
		Platycarya sp.		p
		Pterocarya sp.*		p
		Juglans sp.*		p
	Salicaceae	Salix sp.	m	
	Betulaceae	Alnus subcordata C.A.M.	m	
		Alnus sp.*	m	p
		Betula sp.		p
		Carpinus sp.*		p
		Corylus sp.*		p
	Fagaceae	Castanea sp.		p
		Castanopsis sp. (cf. C. echidnocarpa A.DC)	m	
		Castanopsis sp.		p
		Lithocarpus sp.		p
		Fagus sp.		p
		Quercus neriifolia A.Br.	m	
		Quercus sp.*	m	p
	Ulmaceae	Celtis sp.		p
		Ulmus sp.*		p
	Ulmaceae	Zelkova sp.		p
		Ulmaceae gen.indet.*		p
	Eucommiaceae	Eucommia sp.		p
	Moraceae	Moraceae gen.indet		p

Dicotyledoneae	Polygonaceae	Polygonaceae gen.indet.		p
	Caryophyllaceae	Caryophyllaceae gen.indet.		p
	Chenopodiaceae	Artemisia sp.		p
		Chenopodiaceae gen.indet.*		p
	Magnoliaceae	Liriodendron sp.		p
		Magnolia megafigurata (Krutsch) comb. nov. Ram.		p
		Magnolia dianae Ung.	m	
		Magnolia dzundzeana (Pal.) Takht.	m	
		Magnolia sp.1,2*		p
	Annonaceae	Annona sp.		p
	Lauraceae	Cinnamomum lanceolatum (Ung.) Heer	m	
		Cinnamomum sp.	m	
		Laurus sp.	m	
		Ocotea sp.	m	
		Lauraceae gen.indet.		p
	Ranunculaceae	Ranunculus sp.		p
	Menispermaceae	Menispermum sp.		p
	Nymphaeaceae	Nuphar sp.		p
		Nymphaea sp.		p
		Nymphaeaceae gen.indet		p
	Cruciferae	Cruciferae gen.indet.		p
	Papaveraceae	Papaver sp.		p
	Platanaceae	Platanus sp.		p
	Hamamelidaceae	Corylopsis sp.		p
		Disanthus sp.		p
		Hamamelis sp.		p
		Fothergilla sp.		p
		Parrotia sp.		p
		Sycopsis sp.		p
		Liquidambar sp.		p
	Cercidiphyllaceae	Cercidiphyllum sp.		p
	Rosaceae	Rosaceae gen.indet.	m	p
	Caesalpiniaceae	Cassia ambigua Ung.	m	
		Cassia sp.	m	
		Podogonium knorrii Heer	m	
	Fabaceae	Sophora europaea Ung.	m	
		Fabaceae gen.indet.	m	p
	Geraniaceae	Geranium sp.		p
	Anacardiaceae	Rhus sp.		p
	Hippocastanaceae	Aesculus sp.		p
	Aquifoliaceae	Ilex falsani Sap.et Mar.	m	
		Ilex sp.*		p
	Celastraceae	Euonymus sp.		p
	Staphyleaceae	Staphylea sp.		p
	Rhamnaceae	Rhamnus sp.(cf.R.winogradowii)	m	
		Zizyphus sp.	m	
	Vitaceae	Parthenocissus sp.		p
	Tiliaceae	Tilia sp.*		p
	Sterculiaceae	Sterculia sp.		p
	Violaceae	Viola sp.		p
	Myrtaceae	Myrtus sp.1,2	m	
		Myrtaceae gen.indet.	m	p

Dicotyledoneae	Alangiaceae	Alangium sp.		p
	Nyssaceae	Nyssa sp.		p
	Cornaceae	Thelycrania (Cornus) sanguinea (L.)Fourr.	m	
		Cornaceae gen.indet.		p
	Araliaceae	Aralia sp.		p
		Dendropanax sp.		p
		Araliaceae gen.indet.		p
	Apiaceae	Apiaceae gen.indet.*		p
	Sapotaceae	Sapotaceae gen.indet.		p
	Ebenaceae	Diospyros sp. (cf.D.brachysepala A.Br.)	m	
	Symplocaceae	Symplocos sp.	m	p
	Apocynaceae	Apocynophyllum linearifolium Kol.	m	
		Apocynophyllum sp.	m	
	Oleaceae	Fraxinus sp.		p
		Oleaceae gen.indet.		p
	Caprifoliaceae	Lonicera sp.		p
	Labiatae	Labiatae gen.indet.		p
	Plantaginaceae	Plantago sp.		p
	Asteraceae	Achillea sp.*		p
		Asteraceae gen.indet.		p
Monocotyledoneae	Liliaceae	Liliaceae gen.indet.		p
	Poaceae	Poaceae gen.indet.*		p
	Arecaceae	Nipa sp.		p
		Arecaceae gen.indet.		p
	Sparganiaceae	Sparganium sp.		p
	Typhaceae	Typha sp.		p
Artificial taxon		Tricolporopollenites wackersdorfensis Thiele-Pfeiffer.		p

*** These forms were determined also by Mtchedlishvili (1953).**

Table 3. Number of taxa in Sarmatian flora of Eastern Georgia determined by palynology (p) and by macro-remains of plants (m)

Systematic units	The common composition of flora		Cryptogamous		Gymnosperms		Angiosperms	
	p	m	p	m	p	m	p	m
Form	164	35	40	1	32	2	88	32
Genus	116	23	29	1	23	2	64	20
Family	81	19	20	1	9	2	52	16
Class	10	3	5	1	3	1	2	1

As it can be seen from the Tables 2 and 3 between palynological data and data about the large remains of plants are big differences. The macrofloras are nearly devoid of ferns and conifers. At whole the large remains of plants don't give the correct idea about the composition of these groups of plants and their part in forest communities. By number of forms the angiosperms determined by pollen grains also prevail over the number of plants determined by imprints of leaves. The differences are traced as in composition of deciduous plants and grasses, which pollen grains are the constant components of palynocomplex, so in composition of some subtropical forms (*Hamamelidaceae, Alangiaceae* and others). In the same time the large remains of plants give more full information about systematic composition of some taxa. Especially about the family *Lauraceae,* which pollen grains are bad preserved in fossil materials. At whole the data of both

methods added to each other, give possible full idea about character of flora, vegetation and climate.

Results and Discussion

In section Nadarbazevi the Sarmatian deposits are represented by clays, clayey sandstones and marls, with interleyers of oolitic sandstones and lumachell full by mollusk fauna. The sratigraphy and macrofauna were learned by Buleishvili [Buleishvili, 1960] and Siradze [Siradze, 1958]. Nevertheless the analysis of all layers of section by help of micropaleontological methods illustrated newly the chronology and paleobiological history of development of marine and terrestrial biocenosis.

The description of section and complexes of foraminifers (from bottom to up) is given below.

1. The strata of sandstones and sandy clays. In complex of foraminifers are dominated *Elphidium crispum* (L.) 27% and *E.macellum* (F.et M.) 24%, with thick walls of test. Other species of foraminifers *E.reginum* 5%, *E.aff.flexuosum* 8%, *E.hauerinum* 2%, *E.aff.rugosum* 2%, *Nonion bogdanowiczi* 4% are also big and are rare components of complex. In composition of complex in equal correlation are *Porosononion subgranosum* 15% and *Ostracodae* 13%. They have thick rough, large, but smooth (very rounded) wall of tests (sample 10).

2. Oolitic sandstones with numerous fragments of shells of several species of mollusks. With big number of specimens is represented the family *Miliolidae* 45% (genera: *Cycloforina* 12%, *Varidentella* 14%, *Sinuloculina* 10%, *Miliolinella* 9%) and nearly by equal quantity *Elphidium* 20% (*E.macellum* 8%, *E.aculeatum* 5%, *E.aff.flexuosum* 2%, *E.hauerinum* 3%, *E.aff.rugosum* 2%) and *Porosononion* 26% (*P.subgranosum* 20% and *P.subgranosum umboelata* 6%). In complex *Nonion bogdanowiczi* and *Ostracodae* are represented by 4% and 5% (sample 9).

3. The strata of dark-gray sandstones with shell detritus is characterized by scanty fauna of foraminifers, in which composition are single *Elphidium macellum, Porosononion subgranosum, Nonion sp.(1), Nonion sp.(2)* and *Ostracodae* (sample 8).

4. The blue weakly-sandy clays. In composition of complex are *Porosononion* 40% (*P.subgranosum* 22%, *P.subgranosum umboelata* 10%, *P.martkobi* 7%, *Porosononion* sp. 1%), *Elphidium* 28% (*E.macellum* 8%, *E.fichtelianum* 5%, *E.aculeatum* 5%. *E.aff.rugosum* 6%, *E.reginum* 4%), *Nonion* 10% (*N.bogdanowiczi* 6%, *Nonion* sp.(1) 4%) and *Ostracodae* (4-5 species). In complex nearly all species are represented by big number of specimens and in spite of clayey character of deposits the big tests are dominated, although it is known, that in clays the fauna is smaller and more delicate (sample 7).

5. The strata of clay-marl and clayey sandstones with interlayers of lumachell. In complex of foraminifers are dominated *Porosononion* 32% (*P.subgranosum* 16%, *P.granosum* 7%, *P.martkobi* 6%, *Porosononion sp. Nonion* 22% (*N.bogdanowiczi* 7%, *N.aff.tumidulus* 6%, *Nonion sp.(1)* 4%, *Nonion sp.(2)* 5%). The representatives of genus *Elphidium* composed 18%, that is somewhat less in comparison with mentioned above genera. The species *E.fichtelianum* 5%, *E.aff.rugosum* 5%, *E.crispum* 4% and *E.hauerinum* 4% are equally distributed. The big place in complex is occupied by *Elphidiella artifex* 5% and several species of *Ostracodae* 22%. The complex is characterized by: the abundance of specimens of dominate genera *Porosononion* and *Nonion*, their high intraspecific changeability, the diversity of *Ostracodae*, the big sizes of *Elphidium* and *Nonion*, especially of *E.fichtelianum, E.crispum, N.bogdanowiczi, N.aff.tumidulus* (sample 5).

6. The clays with interleyers of sandstones. In complex of foraminifers, in comparison with previous one, is observed the following peculiarity: the correlation between genera *Elphidium* 38% (*E.crispum* 10%, *E.macellum* 10%, *E.aff.flexuosum* 4%, *E.ukrainicum* 2%, *E.aff.mirandum*

2%, *E.fichtelianum* 1%, *E.angulatum* 1%) and *Porosononion* 24% (*P.subgranosum* 10%, *P.subgranosum umboelata* 8%, *P.granosum* 6%) is changed. The genus *Nonion* composed 8%, which is 1.5 times smaller than in strata 5, besides it is somewhat poorer by species. *Elphidium artifex* preserved its position also as the big form of *Ostracodae* (sample 4).

7. Gray-blue sandstone clays. The complex of foraminifers composed from big number of specimens of *Elphidium* 33% (*E.crispum* 11%, *E.macellum* 10%, *E.aff.rugosum* 4%, *E.reginum* 2%, *E.aff.flexuosum* 2%, *E.fichtelianum* 1%, *E.aculeatum* 1%), *Porosononion* 29% (*P.subgranosum* 14%, *P.subgranosum umboelata* 8%, *P.hyalinum* 3%, *P.granosum* 2%, *P.martkobi* 2%), *Nonion* 12% (*N.bogdanowiczi* 4%, *N.aff.tumidulus* 3%, *Nonion sp.(1)* 2%, *Nonion sp.(2)* 1%) and *Elphidiella* 6% (*E.artifex*). *Ostracodae*, which composed 20% of complex, are characterized by abundance of specimens, by diversity of their specific composition and also big sizes of tests. At whole foraminifers preserved all peculiarities characteristic for Middle Sarmatian representatives of *Elphidium*, *Porosononion* and *Nonion* (the sizes, changeability, the abundance of specimens). The complex is also enriched by new species (sample 3).

8. The sandy clays with interlayers of oolitic sandstones. In complex of foraminifers, in comparison with preceding one, is enriched by typical Middle Sarmatiam species, as are *Porosononion hyalinum*, *P.aragviensis*, *Articularia articulinoides* and *Meandroloculina conicocamerale*. In this complex foraminifers are distributed by following way: *Porosononion* 36% (*P.subgranosum umboelata* 13%, *P.subgranosum* 11%, *P.hyalinum* 10%, *P.aragviensis* 2%), *Elphidium* 25% (*E.crispum* 12%, *E.macellum* 5%, *E.fichtelianum* 3%, *E.aff.flexuosum* 3%, *E.aff.mirandum* 2%), *Nonion* 14% (*N.bogdanowiczi* 4%, *N.aff.tumidulus* 4%, *Nonion sp.(1)* 3%, *Nonion sp.(2)* 3%), *Miliolidae* 8% (*Cycloforina latelacunata* 3%, *Articularia articulinoides* 3%, *Meandroloculina conicocamerale* 2%), *Elphidiella artifex* 5% and *Ostracodae* 12%. The big number of forms are contained in kernel of oolits and can't be determinated (sample 2).

9. Blue-gray sandy clays with enclosing of oolits, without fauna. The complex of foraminifers contains: *Elphidium* 38% (*E.crispum* 18%, *E.macellum* 9%, *E.aff.rugosum* 4%, *E.fichtelianum* 6%, *E.reginum* 1%), *Porosononion* 25% (*P.subgranosum umboelata* 10%, *P.subgranosum* 8%, *P.hyalinum* 5%, *P.aragviensis* 2%), *Miliolidae* 14% (*Cycloforina latelacunata* 5%, *Articularia articulinoides* 6%, *Meandroloculina conicocamerale* 2%, *Meandroloculina sp.* 1%, *Sarmatiella sp.*1%), *Nonion* 6% (*N.bogdanowiczi* 2%, *N.aff.tumidulus* 2%, *Nonion sp.(1)* 2%, *Nonion sp.(2)* 2%), *Elphidiella artifex* 1% and *Ostracodae* 15% (sample 1).

The composition of foraminifers and lithologic peculiarity of deposits of section indicate the coastal-shallow character of this plot of Sarmatian basin, in which more eurofaciale and eurohaline foraminifers (*Elphidium, Porosononion, Nonion, Miliolidae)* and *Ostracodae* were lived. The analysis of complexes shows, that the small changes of lithologic composition of deposits are reflected mainly on the percentage correlation of genera and groups of species and in small degree on the specific composition of complex (Fig.1). Such nuance is very significant as for ecology, so for detail subdivision of Sarmatian deposits, which were formed in shallow conditions.

Taking into account the character and ecology of species of foraminifers distributed in this section it is possible to distinguish the following complexes:

Complex A probably was formed near the coast, in the plot of deposition of coast-grained sediments. In complex *E.macellum* and *E.crispum* are dominated, with participation also of *E.fichtelianum* and *E.reginum*. The study of recent [Myers, 1943; Phleger, 1960; Murray, 1973; Boltovskoy, Wright, 1976; Hansen and Lykke-Andersen, 1976, etc.) and fossil [Bogdanovich, 1947; Krasheninnikov, 1960; Voloshinova, Kuznetsova, 1964, etc.) *Elphidiidae* shows, that the test of *Elphidium* has delicate, but strong and light construction, which is adapted to shallow conditions. The *Elphidium* are able to accustom the marine bottom with mobile, not fasten ground, which underwent the hydrodynamic activity of water. Such conditions were not favorable for other foraminifers, which formed the zone nearly devoid of competitors.

Complex B had occupied also the coast zone, but somewhat far from mentioned above zone, where the hydrodynamics activity of water is lower and terrigenous material is better assorting. Here was arisen the environment favorable for formation the other complex, in which *Porosononion* are dominated, although *Elphidium* are also numerous. The test of *Porosononion* is less strong, as of *Elphidium,* but the numerous pseudopodiums help to hold on sand bottom and not bury in ground.

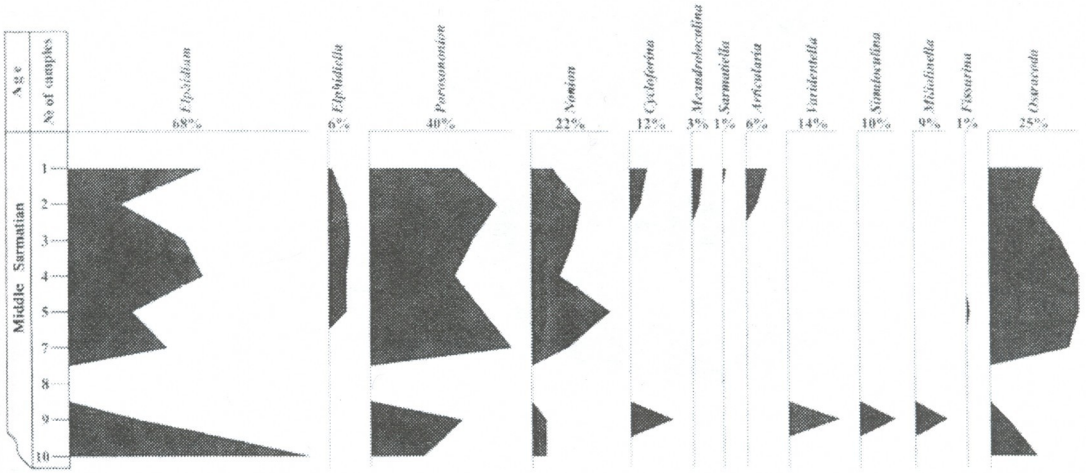


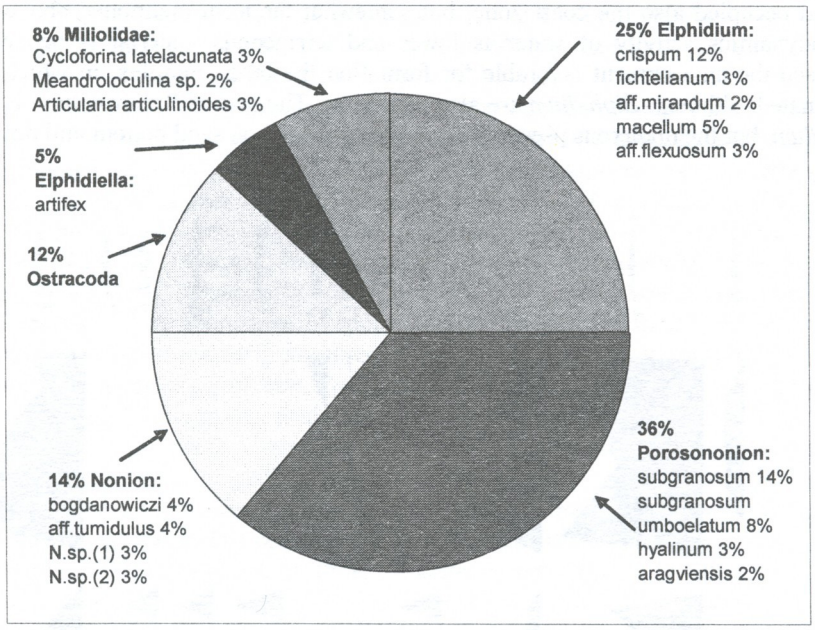
Fig. 1. Genera composition of foraminifers in Nadarbasevi section.

The durability of *Porosononion* test is reached by formation of additional skeleton either in umbilical area (*P.subgranosum umboelata* and *P.hyalinum*), or on whole surface of test (*P.aragviensis*).

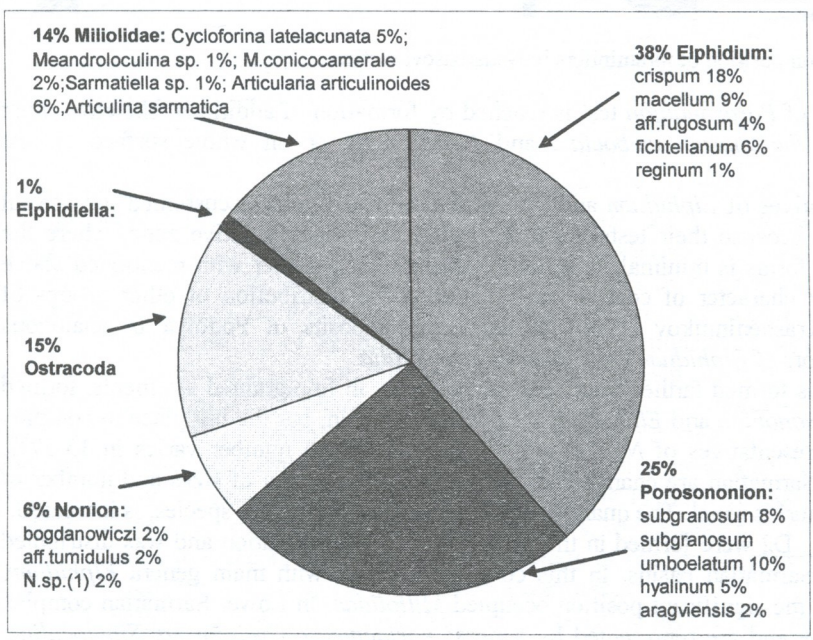
The representatives of *Elphidium* and *Porosononion* nearly fully accustomed the coastal line of Sarmatian Sea, because their tests are more stable for living in active zone, where the competition with other forms is minimal. It is not excepting, that together with mentioned above factors, the fresh-water character of coastal zone disturbed the distribution of other groups of microorganisms. By Krasheninnikov (1960) in Sarmatian deposits of Podolya in analogous conditions the competitors of *Elphidium* are *Cibicides* and *Rotalia.*

Complex C was formed farther from coast as previous, in fine-grained sediments, formed in quiet situation. *Porosononion* and *Elphidium* are dominated again, but the big place in complex is occupied by the representatives of *Nonion* and *Elphidiella,* which number varies in 13-27%. *Nonionidae* in Middle Sarmatian are changeable. For them the increasing of sizes and number of chambers on the last whorl is usual. The quantity of specimens, as in all other species, is increased.

Complexes D1, D2 were formed in the condition of oolite-formation and was distributed in Lower and Middle Sarmatian basins. In this complex together with main genera *Elphidium, Porosononion, Nonion,* the significant position occupied *Miliolidae*. In Lower Sarmatian complex D1 they composed 45% and are represented by genera: *Varidentella, Cycloforina, Sinuloculina, Miliolinella.* The complex D2 was distributed in Middle Sarmatian, where 8% was composed of *Miliolidae,* which are represented by other association (Fig.2). In its composition are genera: *Articularia, Meandroloculina, Sarmatiella, Cycloforina,* with very bad preserved big rough thick-walled tests. Many specimens of foraminifers are covered with white carbonate pellicle, which hide the details of morphological structure of test. In sediments of sample the remains of *Bryozoa* and *Algae* are seen. The representatives of genera *Articularia, Meandroloculina, Sarmatiella* were attached on them.



Complex D1



Complex D2

Fig. 2. The cyclograms for complexes D1, D2.

The big attention attracted the complex of *Ostracodae.* They are presented in all samples, but in some - in very great number (20-25%). The generic and specific variability of *Ostracodae,* the big number of specimens, between which very large forms with thick walls were dominated, allows to suppose that the conditions in Sarmatian basin in studied region were enough favorable, may be optimal in Middle Sarmatian, that promoted the violent flourish of *Ostracodae.* The

favorable conditions were connected with absence of competitors, among which by our opinion the main representatives were higher *Crustacea* - *Mysidae*. As it is known in Sarmatian basin of Paratethys the fossil statoliths of *Mysidae* are presented in great quality [Voicu, 1974; Maissuradze and Popescu, 1987]. By Bagdasarjan (1983) in Sarmatian basin *Mysidae* composed the main part of zooplankton and had big role in trophic chain of many inhabitant of Sarmatian Sea.

In complexes described by us the statoliths of *Mysidae* were not seen, because the conditions probably were not favorable for this group of *Crustacea*, in contrast to ostracods. Although the abundance of foraminifers created the good trophic resource for ostracods and mollusks, which in big numbers are presented in the complex.

The character of morphological peculiarity of tests of foraminifers and ostracods (thickness of walls, intensive of sculpture, big sizes, abundance of specimens) allows us to suppose that they were living in shallow warm basin, saturated by $CaCO_3$ in which it was not deficiency of phytoplankton – the single source of food for foraminifers. Such warm basin can be existed only in conditions of warm climate that is confirmed by data of paleobotanical investigations.

Before the description of vegetation and climate we want to touch briefly the method, which was use for reconstruction of the paleoclimatic conditions. This is so-called landscape-phytocenological or zonal method. It doesn't give the exact climatic parameters, but allows enough good to restore the displacement of boundaries of landscape-climatic zones. By data of Borzenkova (1992) such zonal communities are tundra, forest and steppe, which in the plain territories occupied the large spaces and their change in time by themselves indicates the fluctuation of climate. Much complicated situation was in southern mountain regions, where on comparable small area existed simultaneously some altitudinal belts, with different climatic conditions. So, for reconstruction of the paleoclimate of Georgia by use of landscape-phytocenological method we offer to restore the conditions of every altitudinal belt separately, in dependence with character of vegetation [Shatilova et al., 2004].

Via landscape-phytocenological method the diagram was built, which curves correspond to main vegetation formations distributed on different levels of mountain relief (Fig.3). In Sarmatian such formations were subtropical and warm-temperate polydominant forests of plains, lower and middle mountain belts, the temperate forests of upper belt. Separately on diagram the curve of pine is given – of intrazonal plant and indicator of humidity. The part of separate taxa in composition of these communities is given on Fig.4, which was built on base of more number of samples that diagram on Fig.3.

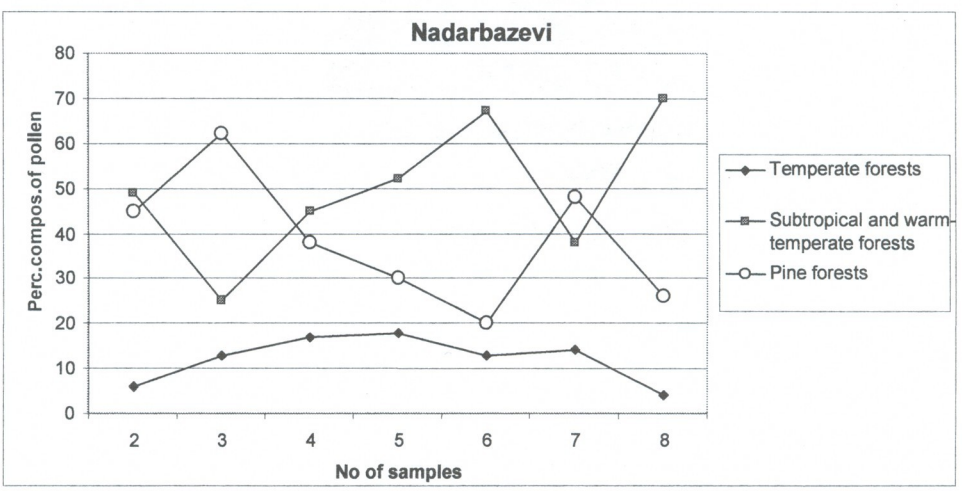


Fig. 3. The diagram reflected the changes of area of separate vegetational formations.

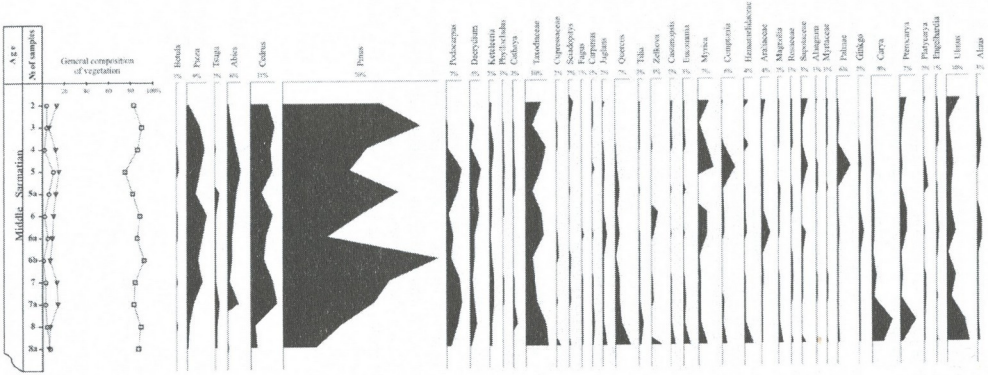


Fig. 4. The pollen diagram of Sarmatian deposits of Eastern Georgia (Nadarbazevi section)

□ - pollen of trees; ▽ - spores; ○ - pollen of grasses.

The polydominant forests consisted of evergreen and deciduous plants (representatives of families *Myricaceae, Juglandaceae, Betulaceae, Fagaceae, Ulmaceae, Magnoliaceae, Lauraceae, Hamamelidaceae, Altingiaceae, Arecaceae)* and warm-require conifers *(Podocarpus, Dacrydium, Keteleeria, Phyllocladus, Cathaya,* some *Pinus, Cedrus, Cryptomeria, Sequoia, representatives* of family *Cupressaceae*). In mixed forests the ferns were represented by great number of forms, among which the genera of families *Polypodiaceae, Gleicheniaceae, Schizaeaceae, Cyatheaceae* predominated. Along rivers and on flooding places the riparian and swamp forests existed, main components of which were *Taxodium, Carya, Pterocarya, Ulmus, Liquidambar* and *Nyssa.*

On plain and lower mountain belt the climate was subtropical. Hypsometrical higher subtropical climate was changed by warm-temperate and by temperate in upper mountain belt. Here dark-coniferous formation dominated, which were growing far from the accumulation basin. Main components of forests were *Abies, Picea, Tsuga* and some *Pinus.*

The above described picture of altitudinal distribution of vegetational formations was characteristic for early stretches of Middle Sarmatian (palynocomplexes of strata 3 in description of section; on Fig.3, 4 – sample 8, 8a), when the polydominant forests had the biggest area and the pine, probably, didn't form yet the separate cenosis. Then the situation was changed and judging by diagrams (Fig.3, 4) during the following stretches of Middle Sarmatian the dynamics of vegetation was expressed mainly in periodical domination of either polydomonant forests or pine forests and sometimes in possession by both formations of the equal areas. These changes were connected with fluctuations of temperature and humidity.

At whole the development of vegetation on territory of Kartli was gone under influence of common paleogeographical changes, which had place at the end of Middle Sarmatian, one of turning-points in Neogene history of Caucasus. In this time as a result of orogenic movements the water area of sea decreased, which bay (so-called Rionian) was preserved only in western part of Georgia.

Conclusion

The Sarmatian deposits of Eastern Georgia were studied by two methods of micropaleontology (foraminifers, pollen and spores) for the first time.

The detail analysis of microfaunistical material allows to trace the changes of association of foraminifers during the Middle Sarmatian and to conclude about bionomical conditions and ecology of Middle Sarmatian basin on the territory of Eastern Georgia.

The results of our investigations can be used while studying the other sections of Sarmatian deposits formed in same shallow conditions.

The comparison of palynological data with data of study of large remains of plants revealed definite differences in list of flora. The quantitative differences can be seen in all groups of plants, but especially in composition of ferns and conifers.

The use of landscape-phytocenological method allows to reconstruct the common direction in development of vegetation and climate.

In the early stretches of Middle Sarmatian, when the polydominant forests were dominated on the largest part of territory of Kartli, the humid subtropical climate, transitive into warm-temperate and temperate with increasing level of relief prevailed. Later the role of pine increased and the territory of polydominant forests reduced, which area no longer reached the former sizes. It can be traced the succession of two main formation – polydomonant forests and pine forests, among which one of them occupied periodically the dominant position.

So, in early stretches of Middle Sarmatian the climate was subtropical, which further was changed by conditions with lower indexes of temperature and humidity. This process was not even

and expressed in often fluctuation, which had place on territory of Kartli during the whole Middle Sarmatian.

Acknowledgments: The authors thank the Swiss National Science Foundation SCOPES Project Ref.IB7320-11069/1 and the European Union INTAS Project N.1000017-8930 that made the collaboration between Georgia and Switzerland possible. Thanks also to all members of the two projects for fruitful discussions.

References:

Bagdasarjan K.G. *The ecologic systems of Sarmatian sea.* Journal of Paleontology of Acad. Sci. USSR, **4**,.3-11, 1983 (in Russian).

Bogdanovich A.K. *On the results of investigation of foraminifers from Miocene deposits of Crimea-Caucasus region.* In: The microfauna of oilfields of Caucasus. Emba and Middle Asia. Proceedings of VNIGRI, p.5-55, 1947 (in Russian).

Boltovskoy E., Wright R. *Recent Foraminifera.* The Hague, p.515, 1976.

Borzenkova I.I. *The change of climate in Cenozoic.* St. Petersburg, "Gidrometeoizdat", p.246, 1992 (in Russian).

Buleishvili D.A. *Geology and oil and gas content intermountain trough of Eastern Georgia.* Leningrad, "Gostoptekhizdat", p.238, 1960 (in Russian).

Chelidze L.T. *The Sarmatian flora of Karthli.* Bullet.Georgian Acad.Scien, **95**, 2, 473-475, 1979 (in Russian).

Chelidze L.T. *The Late Miocene flora and vegetation of Transcaucasus.* Tbilisi, "Metsniereba", p.112, 1987 (in Russian).

Hansen H.J., Lykke-Andersen A.L. *Wall structure and classification of fossil and recent elphidiid and nonionid Foraminifers.* In: Fossils and Strata, Oslo, **10**, 1-37, 1976.

Koiava K.P. *The biostratigraphy of Sarmatian deposits of Eastern Georgia by Foraminifers.* Thesis of PhD, Geol. Inst. of Georgia, Tbilisi, 20 p. 2006 (in Georgian).

Krasheninnikov V.A. *The Elphidiids of Miocene deposits of Podolye. Transsec.* Geological Inst.Acad.Sci.USSR, **21**, 3-133, 1960 (in Russian).

Maissuradze L., Koiava K.P. *The new data on distribution of Sarmatian Foraminifers in deposits of Kacheti.* "Oil and Gas in Georgia", Tbilisi, **19**, p.49-61, 2006 (in Georgian).

Maissuradze L.S., Popescu G. *Carpatho-Caucasian comparative study of Sarmatian Mysids.* "Dari de Seama". Inst.Geol.Geophysics, **72-73**, 3, 75-80, 1987.

Mchedlishvili P.A., Mchedlishvili N.D. *The study of development of flora of Eastern Georgia in Sarmatian Age by data of palynological analysis.* Proceedings of Acad. Sci.USSR, new ser., **XCI**, 3, 621-623, 1953 (in Russian).

Murray J.W. *Distribution and ecology of living benthic foraminiferids.* London, p.274, 1973.

Myers E.H. *Life activities of Foramimnifera in relation to marine ecology.* Proc. Amer. Phil. Soc., **86**, 3, 439-580, 1943.

Phleger F.B. *Ecology and distribution of recent Foraminifers.* The Johns Hopkins Press, Baltimore, p. 297, 1960.

Shatilova I., Rukhadze L., Mchedlishvili N., Makharadze N. *On the use of the Landscape-phytocenological method for the restoration of vegetation and climate of mountain regions (on the example of Colchis).* Bull.Georg.Acad.Scien., **169**, 3, 623-625, 2004.

Siradze K. *The Atlas of leading fossils of Sarmatian deposits of oil-bearing regions of Georgia.* VNIGNI, the Report of theme No 273, Tbilisi, 1958.

Uznadze M.D. *The Neogene flora of Georgia.* Tbilisi, "Metsniereba", p.180, 1965 (in Russian).

Voicu G. *Identification des mysides fossils dans les deports du Miocene Superieur geographique.* Geol.zborn. "Geol.Carpathica", Bratislava, **25**, 2, p.231-239, 1974.

Voloshinova N.A., Kuznetsova V.N. *New data on morphology and evolutional development of some representatives of family Elphidiidae.* Problems of Micropaleontology, Geol. Inst.Acad.Sci.USSR, **8**, 138-149, 1964 (in Russian).

აღმოსავლეთ საქართველოს (ქართლი) შუასარმატული ნალექების მიკროფაუნისტური და პალინოლოგიური დახასიათება

მაისურაძე ლ.[1], ქოიავა კ.[2], სპიზაფერი ს.[3], შატილოვა ი.[1], მჭედლიშვილი ნ.[1],შუბითიძე ლ.[2], სტრასერი ა.[3]

[1]საქართველოს ეროვნული მუზეუმი, პალეობიოლოგიის ინსტიტუტი
[2]ჯანელიძის სახ. გეოლოგიის ინსტიტუტი
[3]ფრაიბურგის უნივერსიტეტი, გეოლოგიურ მეცნიერებათა დეპარტამენტი

(მიღებულია 11.02.2008)

რეზიუმე

სოფელ ნადარბაზევთან (ქართლი) შუასარმატული ნალექების ჭრილი პირველადაა შესწავლილი მიკროფაუნისტური და პალინოლოგიური მეთოდების გამოყენებით. მოცემულია ფორამინიფერების ასოციაციათა აღწერა და მათი ცვალებადობა დროში. ფლორის სია შედგენილია პალინოლოგიური მონაცემებისა და ლიტერატურაში გამოქვეყნებული მცენარეთა მსხვილი ნაშთების შესწავლის შედეგების მიხედვით. ლანდშაფტურ-ფიტოცენოლოგიური მეთოდის გამოყენებით დადგენილია ძირითად მცენარეულ ფორმაციათა არეალების ცვლილებები და მათი დამოკიდებულება კლიმატურ ფლუქტუაციებზე.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 1-2, 2008.

Parasitology

PARASITOLOGICAL INVESTIGATION OF THE ANIMALS OF TBILISI ZOOLOGICAL PARK

*MURVANIDZE L., GOGEBASHVILI I., NIKOLAISHVILI K., LOMIDZE TS., KAKALOVI E.

Georgian Institute of Zoology
**E-mail: lali.murvanidze@hotmail.com*

(Received April 7, 2008)

Abstract

Several coprological studies were carried on the animals preserved in cages and open-air cages of Tbilisi Zoological Park. In 2003-2005 6 species of birds (44 specimens) and 22 species of mammals (77 specimens), in 2007 – 9 species of birds (14 specimens) and 12 species of mammals (15 specimens) were studied. Cists, eggs and larvae of parasites causing different protozoan and helminth diseases were found. In both birds and mammals prevail invasions provoked by helminthes, namely by nematodes. High percent of invasion was observed in predator birds and animals. Together with the preserved animals, researches were carried on lake frogs and invertebrate animals (terrestrial mollusks and earthworms) inhabiting surroundings of zoological garden as far as they represent the intermediate hosts of helminthes. Massive invasion of *Helix lucorum* (92%) by trematode larvae – cercaria is registered. Their biochemical studies were conducted. To prevent helminth distribution usage of preparation of moluskocidic piretroid group is suggested.

Key words: Tbilisi Zoological Park, Animals, Parasites

Introduction

Invasive diseases and especially helminthoses are widely distributed among the animals living in Zoological Park. Long-term parasitological studies have a great theoretical and practical meaning, with regard to conduct the sanitary-prophylactic measures and improve parasitological situation. The first data on parasitological investigations of animals of Tbilisi Zoological Park were published by Prof. N. Kamalov in 1948 [Kurashvili et al., 1983]. During 1973-1975 parasitological researches of animals of zoological park were carried by scientists of the Laboratory of Parasitology of Institute of Zoology. The results of the researches were published in 1983 [Kurashvili et al., 1983]. During the next 30 years parasitological studies in Tbilisi Zoological Park were not conducted.

Last years in zoological park living conditions of captive animals improved significantly. The systematic sanitary-prophylactic measures reduce the possibility of species invasion; but in spite of that, their invasion through different ways (by water, food, intermediate hosts) by helminthes or protozoan parasites is not excluded.

By the initiative of Prof. Kurashvili, in 2003-2005 and 2007 years birds and mammals preserved in Tbilisi Zoological Park were investigated. The goal of the investigation was establishment of protozoan and helminth diseases in captive animals.

Material and Methods

To establish the ways of circulation of parasite invaders, the parasitological situation of some vertebrate and invertebrate animals living on the territory of zoological park was studied.

In 2003-2005 years were studied: 6 species of birds (44 specimens) and 22 species of mammals (77 specimens); among them 6 species of predators, 2 species of primates and 14 species of hoofed (ungulate) animals. In 2007 were studied: 9 species of birds (14 specimens) and 12 species of mammals (15 specimens); among them 5 species of predators, 1 species of primates and 6 species of hoofed (ungulate) animals. From other animals found on the territory – *Rana ridibunda* (14 specimens), *Helix lucorum* (65 specimens) and 5 species of earthworms – *Allolobophora jassyensis* (18 specimens), *A. chlorotica* (10 specimens), *A. calliginosa trapezoides* (9 specimens), *Dendrobaena veneta* (4 specimens), *Eisenia foetida* (23 specimens) were investigated.

Within parasitological researches coprological-ovoscope, and coprological-larvoscope (Bergmann) methods were used. Within investigation of other animals found on the territory of the garden methods of total helminthological cut, individual cut and compressive methods were used. Coprological investigations were lead on microscope MBI-3 with augmentation 7 x 20. For biochemical studies of parasite-host system, total quantity of protein in *Helix lucorum* was calculated by spectrophotometric method according to Lowry [Lowry, 1951].

Results and Discussion

The parasitofauna of invaded animals investigated in Tbilisi Zoological Park is represented by following protozoan and helminthes:

Birds:

Pheasant – *Phasianus colchicus*. From investigated 8 specimens only 2 appeared invaded by eggs of nematode (suborder Oxyurata Skryabin, 1929, Family Heterakidae Railliet, 1914). In Georgia representatives of this family are found in domestic as well as in wild birds [Kurashvili, 1957, Kurashvili et al., 1976].

Peacock – *Pavo cristatus*. From investigated 2 specimens in one individual eggs of nematode *Capillaria* sp. (I) (order *Trichocephalidae* Skrjabin et Schulz, 1928, family Capillariidae Neveu-Lemaire, 1936) are found. The morphological characters of these eggs are identical to the eggs of *C. caudinflata* (Nolin, 1858), which in Georgia are registered in domestic animals [Kurashvili et al., 1976]. In the same peacock transit oocists of *Isospora* sp. were found.

Parrot – *Psittacus erithacus*. In feces of the one investigated specimen larvae and eggs of nematode *Askaridia* sp. (order Ascaridiida Skrjabin et Schulz, 1938, family Ascaridiidae Skrjabin et Mosgovoy, 1952) are found. The typical representative of this family *A. hermaphrodita* (Froelich, 1789) Railiet et Henry, 1914, is wide distributed species in parrots; it is registered in parrots preserved in Zoological Gardens of Germany, USA, Mexico and former USSR [Mosgovoi, 1953].

Field Eagle – *Aquila nipalensis* (Hodgs.). In feces of the one investigated specimen intensive invasion by eggs of Nematodae (I) has been registered. One or two big, round eggs of diameter of 0.055-0.062 mm with two envelopes were found.

Mountain Eagle – *Aquila chrysaetus.* In one investigated specimen eggs of *Capillaria* sp. (II) in blastomer stage was found with protruding cork-shaped projections on their poles. Length of the eggs is 0.050- 0,057 mm, width – 0.025-0.027 mm.

Eagle – *Gyps fulvus.* In one investigated specimen larva of Nematodae (II) were found. Intensity of invasion was not high – 1 larvae in 6-8 sight areas of microscope.

Ostrich – *Struthio camelus*. In one investigated specimen larva of Nematodae (III) were found.

Goose – *Anser anser.* Among 12 investigated specimens in two individuals pear-shaped oocists of coccid *Eimeria anseris* Cotlain, 1932 with one layer envelope were found.

In four species of birds (swan, crane, duck, griffon) no parasitic invasions were registered.

Mammals

Predators:

Lion – *Pantera leo* in two samples (1 – male, 1 – female) investigated in 2003-2005 eggs of *Toxaskaris leonina* (Linstow, 1912) Leiper, 1907 (suborder Ascaridata Skryabin, 1915, family Ascaridae Baird, 1853) were found. Intensity of invasion was quite high. In every sight area of microscope (7 x 20) 3-5 eggs were found. In May, 2007 feces of only one lion were investigated (the second individual died by noninvasive disease), where eggs of *Toxaskaris leonina* were registered again, but with low intensity (1 egg in 7-10 area of sight).

In tiger – *Pantera tigris,* snow leopard – *Panthera uncia,* leopard – *Pantera pardus,* brown bear – *Ursus arctos* and hyena-like dog – *Lycaon pictus,* only *Toxaskaris leonine* was registered. Among the predators no invasion was found only in wolf (*Canis lupus*) and jackal (*Canis aureus*).

Primates:

In 3 from investigated 4 specimens of Silver gibbon *Hylobates moloch* and in two from 6 investigated specimens of *Cercopithecus aethiops* – identical eggs and larvae of nematodes are found. In 4 from 15 investigated specimens of hamadryas (sacred) baboon -*Papio hamadryas* eggs of *Trichocephalus* sp. were found. Eggs of *Trichocephalus* in *Papio hamadryas* were observed in studies carried in 1973-75 years as well [Kurashvili et al., 1983].

Hoofed (ungulate) animals

In coprological material of antelope Niala *Tragelaphus anges* and antelope Cudu – *Tragelaphus strepsiceros* identical eggs of *Ascaris* sp. (I) were found. Percentage of invasion was much higher in antelope Niala.

Yak - *Bos mutus* – in one investigated animal eggs of Trichostrongilidae were found. Prof. N. Kamalov has found Trichostrongilidae in pathanatomic cut of aurochs and chamois and announced them as the reason of death [Rodonaia, 1971].

In Fallow deer- *Cervus dama* elliptical, transparent eggs of *Dictyocaulidae* with fine envelope were found. Length is – 0.079-0.095 mm, width – 0.42-0.48mm. Representatives of this family in Georgia are registered by T. Rodonaia [1971] in European roebuck and Caucasian Deer.

In one investigated specimen of wild horse - *Equus przewalskii* eggs of *Parascaris equorum* (Goeze, 1782) Yorke et Maplestone, 1926 were found.

Pony – *Equus caballus* - out of 7 investigated animals in 2 tests *Ascaridae* sp. was found. In coprological material of one specimen together with eggs of Ascaridae great number of infusorians were also registered, which are very harmful for host animal. They feed on food of host species and withdrew organic compounds, which are essential for the animal.

Camel – *Camelus bactrianus* – from two investigated specimens only in one oocists of coccid *Eimeria bactriani* Levine, Ivens,1970 were found.

The parasitological-coprological researches carried on the animals preserved in Tbilisi Zoological Park showed that in studied mammals and birds prevailed nematodes and mainly Ascaridoses. Eggs and larvae of 13 species of nematodes and 3 species of coccids were found.

Highest percentage of helminthes invasion was registered in predator birds and predator mammals, lesser – in primates and ungulates. In birds 2 species of coccids and 7 species of helminthes were found (table 1). In predator birds – mountain eagle, valley eagle and eagle – eggs and larvae of 3 different species of nematodes were registered.

Table 1. Invasion of animals preserved in Tbilisi Zoological Park by parasites

№	Investigated invaded animals	Parasites	
		2003-2005	2007
	Birds		
1	*Phasianus colchicus* – pheasant	Free	Eggs of *Heterakidae* sp.
2	*Pavo cristatus* – peacock		Eggs of *Capillaria sp.* (I)
3	*Psittacus erithacus.* – parrot		Eggs of *Ascaridia* sp.
4	*Aquila nipalensis* – valley eagle	Free	Eggs and larvae of Nematodes
5	*Aquila chrysaetus* – mountain eagle	Free	*Capillaria* sp. (II)
6	*Gyps fulvus* – eagle	Eggs of Nematodes	Eggs and larvae of Nematodes
7	*Struthio camelus*-ostrich	Free	Larvae of Nematodes
8	*Anser anser* – goose	*Eimeria anseris*	
	Mammals		
	Predators		
9	*Panthera leo* – lion	Eggs of *Toxascaris leonina*	Eggs of *Toxascaris leonina*
10	*Panthera tigris* – tiger	*Toxascaris leonina*	*Toxascaris leonina*
11	*Panthera uncia* – snow leopard	*Toxascaris leonina*	
12	*Pantera pardus* – leopard		*Toxascaris leonina*
13	*Ursus arctos* – brown bear		*Toxascaris leonina*
14	*Lycaon pictus* – hyena-like dog	*Toxascaris leonina*	*Toxascaris leonina*
	Hoofed (ungulate) animals		
15	*Tragelaphus angasi* – antelope Niala	Eggs of *Ascaris* sp.	free
16	*Tragelaphus strepsiceros* – antelope Cudu	Eggs of *Ascaris* sp.	Eggs of *Ascaris* sp.
17	*Bos mutus* – yak		Eggs of Trichostrongilidae
18	*Cervus dama* – fallow - deer		Eggs of Dictyocaulidae
19	*Equus caballus* –(Mongolian, Przewalskis) wild horse	Eggs of *Parascaris equorum*	
20	*Equus caballus* - pony	Eggs of Acaridae; infusorians	
21	*Camelus bactrianus* – camel		*Eimeria bactriani*
	Primates		
22	*Hylobates moloch* – silver gibbon	Eggs and larvae of nematodes	
23	*Cercopithecus aethiops* – green monkey	Eggs and larvae of nematodes	
24	*Papio hamadryas* – hamadryas (sacred) baboon	Eggs of *Trichocephalus* sp.	Free

In predator mammals – lion, tiger, snow leopard, leopard, brown bear and hyena-like dog – 1 species of Ascaridae *Toxascaris leonina* (Linstow, 1902) Leiper, 1907 was found, which is widely distributed in predators, leads to the pathological changes and aborts development of next

generations. Here should be mentioned, that in 2007, investigation of animals was carried out one month after dehelminthisation. Toxascaridose was registered in every animal, but less intensively. So, it may be concluded, that even in conditions of maximal care, the source of invasion remains in animal cages leading to the reinvasion of animals. Eggs of Ascaridae may be found not only on the floor of the cages, but on the animal fur as well. To prevent Toxascaridoses of animals, complex measures (periodical dehelminthisation, high sanitary-hygienic conditions) should be provided. Most part of intestine and lung nematodes, eggs and larva of which were found in birds, ungulates and primates belong to the geohelminthes and the host animals catch parasitological disease from feces, food and water polluted by eggs.

Except the preserved animals, lake frogs, mollusks and earthworms inhabited on the territory of Zooological Park, were also investigated. They are important in circulation and reservation of helminthes. From 14 investigated lake frogs, in peripheral blood slides of two specimens protozoa *Lankesterella minima* was found and in urine bladder of one specimen – trematode *Gorgodera cignoides.*

Parasitological investigations carried on earthworms gave negative results. This fact indicates the absence of those helminthes in animals, intermediate hosts of which are earthworms.

Mollusks serve as intermediate hosts for great number of helminthes. On the territory of Zoological Park 65 species of *Helix lucorum* were investigated, 60 of them (92%) were invaded by larvae of Trematoda – cercarias. According to the data of 1983 [Kurashvili et al., 1983], no helminthes were found in *Helix lucorum.* Currently parasitological situation is changed in regard to new hearth what led to the mass invasion of mollusks with trematodes. Life cycles of Trematodae families Dicrocoelidae, Brachylaemidae and Lecithodendridae as well as representatives of genera Protostrongylus and Muellerius in lungs of ungulate animals are bound with the terrestrial mollusks. Accordingly, investigated mollusks may be invaded by representatives of these helminthes and the definitive host of these parasites can be animals preserved in Zoological Park. To prevent the distribution of trematodes, we recommend usage of already known medicines (Permetrine) that infect parasites and leave their host mollusks intact [Kurashvili et al., 1989].

It is known, that helminthes in invaded organisms changed their biochemical status, including quantity of proteins. To establish the total quantity of proteins in *Helix lucorum,* spectrophotometric method was used. In the liver of invaded specimens quantity of protein was equal to 0.1104±0.0062 mg/ml, in control test – 0.104±0.00144. Final results showed that total protein quantity in both groups of investigated mollusks didn't differ statistically ($P > 0,5$). This issue is explained by existing adaptation between parasite and host, but according to the literature data [Valli et al., 1980], even in case of no changes in proteins, fractional composition is changed. Further researches will allow proofing these hypotheses.

Aknowlegdements: We thank Gia Zarkua, Edisher Jankarashvili and Nato Archvadze for providing the study materials.

References:

Kurashvili B. *Helminths of hunting birds of Georgia.* Publ. AN SSSR, Moscow, 430 pp. 1957 (in Russian).

Kurashvili B., Eliava I., Japaridze L. *Helminths of poultry of Georgia.* Tbilisi, "Metsniereba" 242 pp. 1976 (in Georgian).

Kurashvili B, Rodonaia T., Matsaberidze G., Kvavadze E., Gogebashvili I.,Burtikashvili L., Eliava I., Japaridze L., Petriashvili L., Mikeladze L., Ramishvili N., Jankarashvili E. *The*

results of parasitological research of *animals of the Tbilisi Zoological Park.* Parasitological collection, Y, 96-113, Tbilisi, "Metsniereba", 1983 (in Georgian).

Kurashvili B., Matsaberidze G., Fidler J., Karachentseva I.. *Molluscocidity of Permetrine and its influence of fractional composition of lipids of snails.* Materials of the parasitological scientific conference of Georgia. Tbilisi, "Metsniereba", 26-28, 1989 (in Georgian).

Lowry et al. *Proten measurement with Folin phenol reagent.* J. Biol. chem., **193**, 265-275, 1951.

Mozgovoi A.A. *Ascaridata of Animals and Man and Diseases Provoked by them.* In: Basis of Nematology, vol. **II**, Moscow, "Nauka", 162-211, 1953 (in Russian).

Rodonaia T. *Helminths of hunting mammals of Georgia.* Tbilisi, "Metsniereba", 451 pp, 1971 (in Georgian).

Shults N.G. *Experimental study of the treatment of ascariodosis of Large Carnivora of the Moscow Zoological Park.* Proceedings of the Moscow Zoological Park, M., vol. **I**, 247-259, 1940 (in Russian).

Tsvetaeva N. P. *Diseases of animals of the Moscow Zoological Park.* Proceedings of the Moscow Zoological Park, M., vol. **II**, issue 2, 68-78, 1941 (in Russian).

Valli V.E.O., Mills J.N., Lumsden J.H., Rattray J.B., Forsberg C.M. *The quantitation of trypanosome congolense in calves. II Biochemical changes.* "Tropenmed. und Parasitol", **31**, N3, 288-298, 1980.

თბილისის ზოოლოგიური პარკის ცხოველების პარაზიტოლოგიური გამოკვლევა

მურვანიძე ლ., გოგებაშვილი ი., ნიკოლაიშვილი ქ., ლომიძე ც., კაკალოვი ე.

ზოოლოგიის ინსტიტუტი

(მიღებულია 07.04.2008)

რეზიუმე

თბილისის ზოოლოგიური პარკის გალიებსა და ვოლიერებში დაცული ცხოველებიდან კოპროლოგიურად გამოკვლეულია: 2003-2005 წლებში 6 სახეობის ფრინველი (44 ეგზ.) და 22 სახეობის ძუძუმწოვარი (77 ეგზ.); 2007 წელს 9 სახეობის ფრინველი (14 ეგზ.) და 12 სახეობის ძუძუმწოვარი (15 ეგზ.). გამოვლენილია პროტოზოული და ჰელმინთოზური დაავადებების გამომწვევი პარაზიტების ცისტები, კვერცხები და ლარვები. როგორც ფრინველებში, ისე ძუძუმწოვრებში ძირითადად გავრცელებულია ჰელმინთებით, კერძოდ, ნემატოდებით გამოწვეული ინვაზიები. ინვაზიის მაღალი პროცენტი აღინიშნა მტაცებელ ფრინველებში და მტაცებელ ძუძუმწოვრებში. დაცული ცხოველების გარდა, გამოკვლეულია აგრეთვე ზოოპარკის ტერიტორიაზე მობინადრე ტბის ბაყაყები და უხერხემლო ცხოველები (ხმელეთის მოლუსკები და ჭიაყელები), რომლებიც როგორც შუამავალი მასპინძლები, მნიშვნელოვან როლს ასრულებენ ჰელმინთების გავრცელებასა და ცირკულაციაში. აღინიშნა ბაღის ლოკოკინების (*Helix lucorum*) მასიური დაინვაზირება (92%) ტრემატოდების ლარვებით - ცერკარიებით. ჩატარებულია მათი ბიოქიმიური გამოკვლევა. დაცულ ცხოველებში ტრემატოდების გავრცელების თავიდან ასაცილებლად, მიზანშეწონილადაა მიჩნეული მოლუსკოციდური პირეტროიდული ჯგუფის პრეპარატების გამოყენება.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 1-2, 2008.

Plant Physiology

INFLUENCE OF ACIDIC PRECIPITATIONS ON THE INTENCITY OF CARBON ASSIMILATION AND NITROGEN METABOLISM IN GEORGIAN WHEAT

KACHARAVA N., CHKHUBIANISHVILI E., *CHANISHVILI SH., BADRIDZE G., KIKVIDZE M., MAZANISHVILI L., JANUKASHVILI N.

Tbilisi Botanic Garden and Institute of Botany, Agmashenebeli ave. 10 km.
E-mail: shchani@yahoo.com

(Received April 1, 2008)

Abstract

Effect of simulated acid precipitation on Georgian species of wheat: *Triticum macha* Dek. et Men., *T. monococcum* L., *T. timopheevi* Zhuk., *T. persicum* var. stramineum Zhuk., *T. persicum* var. fuliginosum Zhuk., and *T. persicum* var. rubiginosum Zhuk., and widely spread species of mild wheat – *T. aestivum* L. has been studied. Spraying with acid solution (H_2SO_4-HNO_3, in proportion 2:1, pH2.5) had either inhibitory or stimulating character on different physiological indices, according to species. Morphological damage of leaves was not observed among any of studied species. Stem growth was stimulated almost in all species. Activation of photosynthetic activity of leaves and chlorophyll synthesis was mentioned in two varieties of *T. persicum* (var. fuliginosum and rubiginosum). In all varieties of *T. persicum* along with inhibition of nitrate reductase activity, amount of total proteins in seeds was decreased. Effect of simulated acid rains on Georgian wheat is species-specific but none of the studied species appeared to be extremely sensitive to the given stress factor.

Key words: simulated acid rain, photosynthesis, total proteins, nitrate reductase, Georgian wheats.

Introduction

Atmospheric pollution caused by anthropogenic stressors represents a serious danger for stability of ecological systems. Rising of SO_2 and NO_2 concentrations results in acidification of atmospheric precipitations, which is a chemical stress for plants and in different ways is reflected on their life [Larcher, 1980; Dukhovski et al., 2003]. The polluting agents negatively influencing plants limit their tolerability towards other factors [Duek et al., 1994]. At the same time it was shown that small doses of any stressor applied for several times promote adaptation of plant to this factor and elevates resistance to other stressors too [Evans et al., 1982].

Literature data on effect of acid precipitations on plants are various. Forest and water ecosystems are better studied from this point of view, while agrobiocenosis are less investigated. According to the existed experimental results the biological effect of acid precipitations on plants is diverse and complex. It comprises external morphological damages and deep changes in plant

metabolism either [Johnson, 1985, Porter et al., 1989, Evans et al., 1982, Khan, Shikha, 2004; Munzuroglu et al., 2005; Wyrwicka, Sklodowska, 2006].

Georgian species of wheat are distinguished by many significant agricultural features, like resistance to diseases, pests and logging, easy threshing etc., which are dispersed among different varieties [Menabde, 1957; Gorgidze, 1977]. But no data exist on stability of Georgian wheat to acid precipitations. So the purpose of the made study was to investigate the effect of simulated acidic precipitations on Georgian species of wheat and to reveal those parameters, which would be of special importance in adaptation to this stress factor.

Material and Methods

Experimental work was carried out on Digomi experimental base of Tbilisi Botanical Garden and Institute of Botany. Following species of Georgian wheat were tested: *Triticum macha* Dek. et Men., *T. monococcum* L., *T. timopheevi* Zhuk., *T. persicum* var. stramineum Zhuk., *T. persicum* var. fuliginosum Zhuk., and *T. persicum* var. rubiginosum Zhuk., and widely spread species of mild wheat – *T. aestivum* L.

Spraying with acid solution (H_2SO_4-HNO_3, in proportion 2:1, pH 2.5) was performed in the phase of stem rising. Plants were sprayed four times with intervals of five days. Analyses were done one week later after the last spraying, in flowering phase.

Material for testing was picked from 10 plants of each species. Stem and ear length were measured in 15-20 plants.

Intensity of photosynthesis was studied radiometrically, using $^{14}CO_2$ [Voznesensky et al., 1965]. Chlorophyll content in leaves was determined in acetone extract spectrophotometrically (spectrophotometer SPECOL 11, Carl Zeiss, Germany). For calculation Wetschtein's formula was used [Gavrilenko et al., 1975]. Total content of proteins in grain was determined after Lowry (1956), activity of nitrate reductase was studied spectrophotometrically using sulphuric acid and α-naphtilamine [Mulder et al., 1959].

Analyses were performed in three replications. Results were treated using ANOVA (sigma stat 3.5 version). Mean values and their standard deviations are given in figures and table.

Results and Discussion

Experimental results have shown that the most intensive growth of stem was characteristic for *T. aestivum.* In *T. macha* and *T. persicum* (all varieties) this index was almost same (Fig. 1). Respond of plants to simulated acid precipitations was not equal. High acidity has stimulated growth ($P \leq 0.001$) almost in all studied species (exception were *T. persicum* var. fuliginosum and var. rubiginosum), especially in T. *macha* and *T. monococcum* (by 46 and 120% correspondingly).

Maximal length of ear was mentioned in *T. monococccum* and *T. timopheevi* (Fig. 2). Spraying with acid solution has not revealed statistically reliable results for this index ($P>0.05$). This may be the result of less sensitivity of ear growth to acidity.

According to literature data high acidity reduced stem growth in *T. aestivum* (var. Raj 3077) [Raj Sonia, 2003], while in our experiments pH 2.5 acidity had opposite effect (Fig.1). Some authors conclude that reaction of plant growth parameters and productivity to simulated acid rain, compared with other stressors, is slight [Porter et al., 1989]. This we can't say about Georgian wheats, according to different sensitivity to pH 2.5 of studied species.

Experimental results have shown that the photosynthetic activity of Georgian wheats was different (Table 1). The maximal level of carbon assimilation was mentioned in *T. timopheevi* and the minimal – in *T. persicum* var. fuliginosum. Plants reaction to acid spraying varied in different

species. High acidity (pH 2.5) mainly inhibited photosynthetic activity of experimental leaves, reducing CO_2 assimilation by 7-22% ($P<0.001$), while in *T. persicum* var. fuliginosum and var. rubiginosum photosynthesis in contrary, activated (Table 1).

Table 1. Influence of simulated acid rains on photosynthetic intensity and chlorophyll content of leaves of Georgian wheat species

Wheat species	Variant	Photosynthesis $imp \cdot min^{-1} \cdot 10^{-3} \cdot g^{-1}$	Chlorophyll, mg/g, fresh weight		a+b	a/b
			a	b		
Triticum aestivum L.	Control	268±37	2.1	1.4	3.5±0.07	1.5
	Sprayed	238±30	1.7	1.0	2.3±0.09	1.7
T. macha Dec. et Men.	Control	241±19	2.2	1.5	3.7±0.09	1.4
	Sprayed	216±28	1.6	0.9	2.5±0.12	1.7
T. monococcum L.	Control	275±11	1.8	1.2	3.1±0.11	1.5
	Sprayed	240±38	1.8	1.0	2.8±0.13	1.9
T. timopheevi Zhuk.	Control	355±20	2.1	1.2	3.4±0.17	1.5
	Sprayed	329±14	2.0	1.1	3.1±0.08	1.8
T. persicum var. stramineum Zhuk.	Control	274±5	1.6	1.1	2.6±0.18	1.4
	Sprayed	213±14	1.8	1.2	3.0±0.05	1.4
T. persicum var. fuliginosum Zhuk.	Control	122±29	1.4	1.0	2.4±0.12	1.4
	Sprayed	205±13	1.6	1.1	2.7±0.09	1.4
T. persicum var. rubiginosum zhuk.	Control	251±24	1.4	1.0	2.5±0.14	1.4
	Sprayed	290±33	1.7	1.3	2.9±0.06	1.4

Negative effect of acid rains on photosynthetic apparatus has been explained by limited use of light energy [Kreslavski et al., 2007; Meskauskiene et al., 2007]. Stressors change the structure of thylakoid membrane of chloroplast, thus damaging the photosynthetic apparatus [Gabara et al., 2003; Kreslovsky et al., 2007].

Following some authors, reactive oxygen, synthesized under the influence of stressor, inhibits the reduction of the photo system II, while significant changes in photo system I activity are not mentioned. An important role in stress resistance plays washing out of Ca from leaves. From its side, this diminishes stomatal conductivity and net photosynthesis. Increase of stomatal resistance, on the one hand, and decline of photosynthesizing pigments, on the other, is considered as the main reason for photosynthesis inhibition [Muthuchelian et al., 1994; Qui et al., 2001; Borer et al., 2005].

From the experimental results is clear that in control variants of Georgian wheats the sum of "a" and "b" chlorophylls varied in following species (Table 1). In *T. persicum* (all varieties) this index was low compared with other species, while a/b ratio remained the same. Spraying with acid solution changed content of chlorophylls: in all studied species, except *T. persicum,* amount of chlorophylls decreased ($P<0.05$). It may be supposed that sensitivity to acid stress was caused by degradation of the photosynthetic apparatus and consequent inhibition of reparative processes, which resulted in decline of photosynthesis intensity.

In sprayed varieties of *T. persicum* activation of chlorophyll biosynthesis was mentioned, followed by intensification of photosynthetic activity of leaves in varieties fuliginosum and rubiginosum ($P<0.05$) (table). So, stress factors affect the chlorophyll pull by increasing either its hydrolysis or biosynthesis [Pshibitko et al., 2004]. Taking into account the data given in table, experimental plants may be divided in two groups. In the first group of plants simulated acid precipitations induced photosynthesis decrease (*T. aestivum, T. macha, T. timopheevi, T. monococcum*), in parallel with chlorophyll concentration decline. Ratio a/b rose, compared with

control. In the second group of plants, formed by all studied varieties of *T. persicum* increase of photosynthetic activity took place, together with intensification of pigments synthesis; Ratio a/b chlorophylls remained the same.

According to literature data *T. aestivum* (var. Raj 3077) has demonstrated high tolerance to increased acidity (pH 1), but rising of acidity up to pH 0.5 caused significant changes in the content of proteins, carbohydrates and nitrogen [Raj Sonia et al., 2004]. Spraying of three varieties of *T. aestivum* with sulfuric acid has revealed different sensitivity of plants to acid stress [Johnson, Shriner, 1985].

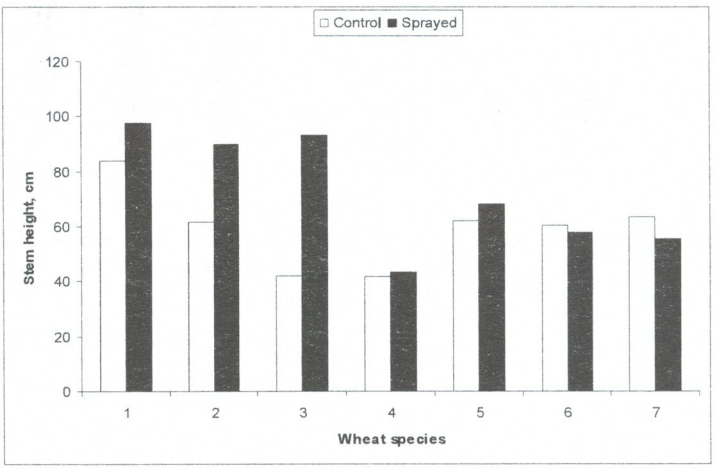


Fig. 1. Influence of acid precipitations (pH 2.5) on stem length of Georgian wheat species. 1. *T. aestivum* L., 2. *Triticum macha* Dek. et Men., 3. *T. monococcum* L., 4. *T. timopheevi* Zhuk., 5. *T. persicum* var. stramineum Zhuk., 6. *T. persicum* var. fuliginosum Zhuk., 7. *T. persicum* var. rubiginosum Zhuk.

Fig. 2. Influence of acid precipitations on length of ear of Georgian wheat species. 1. *T. aestivum* L., 2. *Triticum macha* Dek. et Men., 3. *T. monococcum* L., 4. *T. timopheevi* Zhuk., 5. *T. persicum* var. stramineum Zhuk., 6. *T. persicum* var. fuliginosum Zhuk., 7. *T. persicum* var. rubiginosum Zhuk.

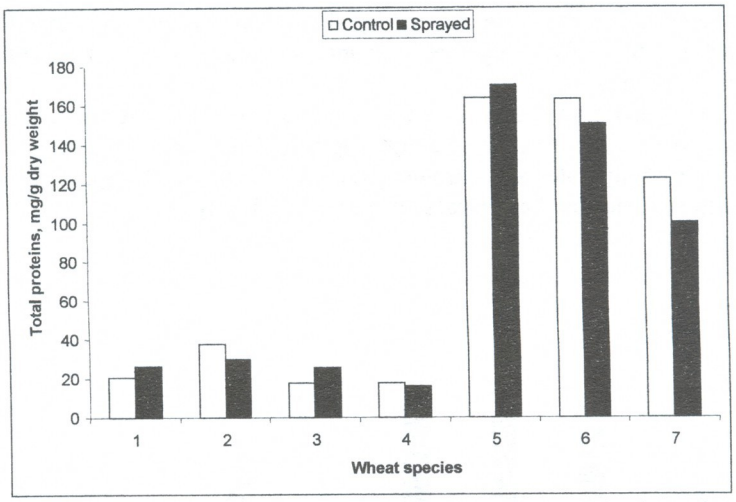


Fig. 3. Influence of acid precipitations on the content of total proteins in seeds of Georgian wheat species. 1. *T. aestivum* L., 2. *Triticum macha* Dek. et Men., 3. *T. monococcum* L., 4. *T. timopheevi* Zhuk., 5. *T. persicum* var. stramineum Zhuk., 6. *T. persicum* var. fuliginosum Zhuk., 7. *T. persicum* var. rubiginosum Zhuk.

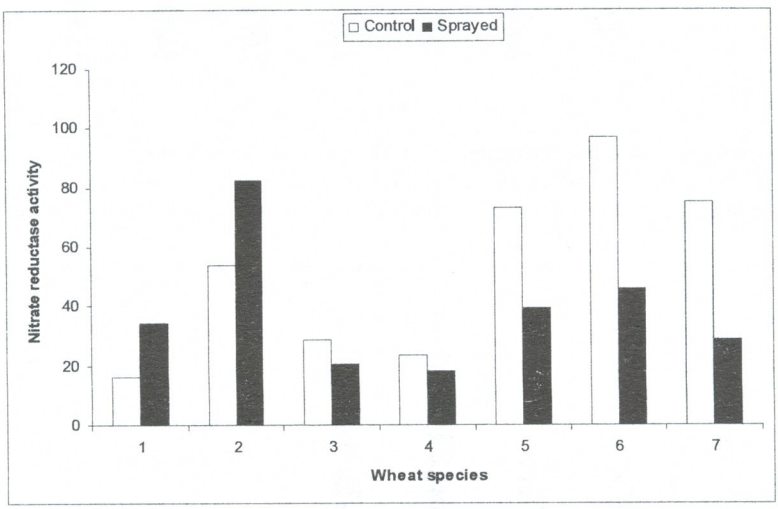


Fig.4. Influence of acid precipitations on the activity of nitrate reductase in leaves of Georgian wheat species (NO_2 μg in 30 min, fresh weight). 1. *T. aestivum* L., 2. *Triticum macha* Dek. et Men., 3. *T. monococcum* L., 4. *T. timopheevi* Zhuk., 5. *T. persicum* var. stramineum Zhuk., 6. *T. persicum* var. fuliginosum Zhuk., 7. *T. persicum* var. rubiginosum Zhuk.

Following to our experimental data accumulation of total proteins in grain indicates different sensitivity of the studied species to acid stress (Fig. 3). In all three varieties of *T. persicum* content of total proteins was higher compared with other species.

Spraying with acid solution caused activation of protein synthesis in some species (T. *aestivum, T. monococccum, T. persicum*, var stramineum), while in others *(T. macha, T. timopheevi, T. persicum*, var. fuliginosum and var. rubiginosum) abating of the index was mentioned. It may be supposed that the last species reveal high sensitivity to acid stress.

In the metabolism of proteins and nitrogen pull nitrate reductase is a key enzyme for nitrogen assimilation. Thus enhancement of nitrogen containing compounds in atmosphere must influence the enzyme's activity. Activation of nitrate reductase is one of the mechanisms of rising plant resistance to stress by detoxification of harmful substances. Investigation of the catalytic activity of the enzyme in 26 species of plants has revealed three different groups of plants by stability to atmospheric pollution. In resistant plants enzyme's activity increased for 200% [Sergeichik, 1988]. Moreover, experimental results show that resistance to stress depends on the activity of antioxidative system too [Chkhubianishvili et al., in press].

Among the investigated species of Georgian wheat the highest activity of nitrate reductase was revealed in varieties of *T. persicum* (Fig. 4). Acid spraying caused activation of the enzyme in *T. macha* and *T. aestivum*. In parallel with Abating of nitrate reductase activity in *T. timopheevi* and *T. persicum* (40-60%), diminishing of protein synthesis in seeds of these species may indicate that detoxifying activity of nitrate reductase is not on a high level in these plants, which would be guarantee for their resistance to stress.

Analyzing the experimental results it may be concluded that the effect of simulated acid rains on Georgian wheat species was diverse and had either inhibitory or stimulating influence on different physiological indices, according to species. Morphological damage of leaves was not discovered among any of studied species. Stem growth was stimulated almost in all species. Activation of photosynthetic activity of leaves and chlorophyll synthesis was mentioned in two varieties of *T. persicum* (var. fuliginosum and rubiginosum). *T. timopheevi* appeared to be comparatively stable to acid stress: all studied indices increased or changed slightly after acid spraying. The mild wheat *T. aestivum*, taken as a comparative species has revealed resistance to acid stress by the studied indices.

According to obtained results it may be concluded that sensitivity of Georgian wheat species to acid precipitations has specific character. If resistance of some species depends on high activity of nitrate reductase, in others stress tolerance is conditioned by activation of antioxidative system.

In spite of spraying with acid solution during the most period of vegetation, none of the studied species of wheat appeared to be extremely sensitive to the given stress factor.

References:

Borer C. H., Schaberg P. G., Dehayes D. H. *Acid Mist Reduces Foliar Membrane-associated Calcium and Impairs Stomatal Responsiveness in Red Spruce.* Tree Physiol., **25,** 673-680, 2005.

Chkhubianishvili N., Kacharava N., Chanishvili Sh., badridze G., Mazanishvili L., Janukashvili N. *Influence of Simulated Acidic Precipitations on Antioxidative Substances in Leaves of Georgian Wheats.* J. Biophysics and Biochem (in press).

Dueck T. E., Bossen M. E., Elderson I. *The Combined Influence of O_2 and NH_3 on Growth, Physiology and Drought Stress of Scots Pine (Pinus sylvestris L.).* In: Air Pollution and Multiple Stress. Eds. Cox et al. Fredericton. PIMSAPEFE, 95-112, 1994.

Duchovskis P., Juknis R., Brazaytite A., Zhukauskayte I. *Plant reaction on complex influence of natural and anthropogenic stress factors.* Russian J. Plant Physiol., **50**, 2, 165-173, 2003.

Evans L. S., Gmur N. F., Mancini D. *Effects of Simulated Acid Rain on Yields of Raphanus sativa, Triticum aestivum and Medicago sativa.* Envir. Exp. Botany, **22**, 4, 445-453, 1982.

Evans L. S. *Biological effects of acidity in precipitation on vegetation.* Envir. Exp. Botany, **63**, 155-169, 1982.

Gabara B., Sklodowska M., Wyrwicka A., Glinska S., Gabinska M. *Changes in Ultrastructure of Chloroplasts and Mitochondria and Antioxidant Enzyme in Lycopersicum esculentum Mill. Leaves Sprayed With Acid Rain.* Plant Sci., **164**, 4, 507-516, 2003.

Gavrilenko V. F., Ladigina M. E., Xandobina L. M. *Practical Handbook in Plant Physiology.* Moscow, "Visshaia Shkola", 127-132, 1975 (Russian).

Gorgidze A. D. *Phylogeny of Georgian Endemic Wheats.* Tbilisi, "Metsniereba", 1977 (Russian).

Johnson J. W., Shriner D. S. *Responses of Three Wheat Cultivars to Simulated Acid Rain.* Env. Exp. Botany EEBODM, **25**, 4, 349-353, 1985.

Khan T. I., Shikba D. *Physiological and Biochemical Effects of Simulated Acid Rain on Phaseolus vulgaris var. HUR-15.* In: The Environmentalist. Springer, Netherlands, **24**, 223-226, 2004.

Kreslavski V. V., Karnentier R., Klimov V. V., Murata N., Alla S. I. *Molecular mechanism of stress resistance of the photosynthetic apparatus.* Biomembranes, **24**, 3, 195-217, 2007 (Russian).

Larcher W. *Physiological Plant Ecology.* Springer, 303, 1980.

Lowry O. H. Methods of Enzymology. **2**, 448-450, 1956.

Mazanishvili L. A. *Phylogenesis of the wheat Dika – Triticum persicum Vav. Candidate diss.,* p.119. Tbilisi, 1995 (Russian).

Menabde V. L. *Wheats of Georgia.* Tbilisi, 1957.

Meskauskiene R., Klaus A. *The genetic basis of singlet oxigen-mediated signaling of stress responses in plants.* World Conference of Stress Hans Selye. Budapest, 2007.

Mulder E. G., Boxma R., Van Veen W. Z. *The effect of molibdenium on nitrate reduction in plant tissues.* Plant and Soil, **10**, 4, 335,1959.

Munzuroglu O., Erdal O., Fikket N., Sule Yuksel T. *Effect of simulated Acid Rain on Vitamins A, E, C in strawberry (Fragaria vesca).* Pakistan J. Nutr. **4**, 6, 402-406, 2005.

Muthuchelian K., Nedunchezhian N., Kulandavelu. *Acid Rain: Acidic Mist-Induced Response in Growth and Photosynthetic Activities on crop Plants.* Archives of Environmental Contamination and Toxicology, **26**, 4, 521-526, 1994.

Porter P. M., Banwart W. I., Zeigler E. L., Vasilas B. L., Hasset J. J. *Effects of Simulated Acid Rain on growth Parameters and Yield Components of Two Soybean Cultivars.* New Phytologist, **113**, 79-83, 1989.

Pshibitko N. L., Kalitukho L. N., Javoronkova N. B., Kabashnikova L. F. *Condition of Chloroplast Pigments in Different Age Seedlings of Barley Under the Influence of Heat Shock and Water Deficiency.* Russ. J. Plant Physiol., **51**, 1, 20-26, 2004.

Qui D. L., Lui X. H., Guo S. Z. *Effects of Simulated Acid Rain on Langan Photosynthesis.* ISHS Acta Horticulturae 558: I International Symposium on Litchi and Lagan. **1**, 301-304, 2001.

Raj S., Devpura Sh., Solomon D. M., Khan T. I. *Simulated acid Rain Exposure on triticum aestivum var. Raj 3077 and Its Impact on growth and Pgysiological Parameters Dimentions.* Pollen, **2**, 87-94, 2003.

Sergeichik S. A., Sergeichik A. A. *Influence of Industrial Gas Toxicants on Peroxidase and Nitrate Reductase Activity of Wood Plant Leaves.* Ecotoxicology and Protection of Nature. Proceedings of Training, Jurmala.158-161, 1988 (Russian).

Voznesenski V. L., Zalenski O. V., Semikhatova O.A. *Photosynthesis and Respiration Studying Metods.* M., "Nauka", 1968 (Russian).

Wyrwicka A., Sklodowska M *Influence of Repeated Acid Rain Treatment on Antioxidative Enzyme Activities and Lipid Peroxidation in Cucumber Leaves.* Envir. Exp. Botany, **56**, 2, 198-204, 2006.

მჟავე ნალექების გავლენა ნახშირბადის ასიმილაციასა და აზოტოვან ცვლაზე ქართულ ხორბლებში

კაჭარავა ნ., ჩხუბიანიშვილი ე., ჭანიშვილი შ., ბადრიძე გ., მაზანიშვილი ლ., ჯანუაშვილი ნ.

თბილისის ბოტანიკური ბაღი და ბოტანიკის ინსტიტუტი

(მიღებულია 01.04.2008)

რეზიუმე

შესწავლილია სიმულირებული მჟავე ნალექების გავლენა ხორბლის ქართულ სახეობებზე: *Triticum macha* Dek. et Men., *T. monococcum* L., *T. timopheevi* Zhuk., *T. persicum* var. stramineum Zhuk., *T. persicum* var. fuliginosum Zhuk., and *T. persicum* var. rubiginosum Zhuk.; აგრეთვე ფართოდ გავრცელებულ რბილ ხორბალზე *T. aestivum* L. მჟავას ხსნარით შესხურება (H_2SO_4-HNO_3, პროპორციით 2:1, pH 2.5) მასტიმულირებელი ან მაინჰიბირებელი აღმოჩნდა სხვადასხვა ფიზიოლოგიურ მაჩვენებლებზე სახეობების მიხედვით. არც ერთ შესწავლილ სახეობაში ფოთლების ხილული დაზიანება არ აღინიშნა. ღეროს სიგრძეში ზრდამ თითქმის ყველა სახეობაში მოიმატა. *T. persicum*-ის ორ ფორმაში var. fuliginosum და rubiginosum) აღინიშნა ფოთლების ფოტოსინთეზური აქტივობის და ქლოროფილების შემცველობის მატება. *T. persicum*-ის ქვესახეობების ფოთლებში ფერმენტ ნიტრატრედუქტაზას აქტივობის დაქვეითებასთან ერთად თესლში შემცირებულია საერთო ცილების ბიოსინთეზი. სიმულირებული მჟავე წვიმების ზემოქმედების ეფექტი სახეობრივ სპეციფიკას ექვემდებარება, მაგრამ არც ერთ სახეობას არ გამოუმჟღავნებია უკუდურესი მგრძნობიარობა მაღალი მჟავიანობის მიმართ.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 1-2, 2008.

Zoology

TETRANICHOID MITES (*ACARI: TETRANYCHOIDEA*) FAUNA OF GEORGIA

ARABULI T.

Institute of Zoology, Chavchavadze ave, 31, 0179, Tbilisi, Georgia
E-mail: tea_arabuli@yahoo.com

(Received February, 20, 2008)

Abstract

On the basis of analysis of literature data and latest systematic material on Tetranichoid mites it was found that at present Tetranichoid fauna of Georgia is presented by 97 species which are associated in 3 families and 24 genera.

Key words: *Tetranychoidea,* Mite, Georgia, Host Plant.

Introduction

Tetranichoid mites are widespread and diverse in all areas where higher plants occur. Tetranichoid superfamily includes 5 families: *Tetranychidae, Aallochetophoridae, Tuckerellidae, Linotetranidae & Tenuipalpidae*, among them *Tetranychidae & Tenuipalpidae* families are distinguished with species diversity. All over the world more than 2500 species of Tetranichoid mites are known.

In Georgia Tetranichoid mites are presented by the following 3 families: *Tetranychidae, Tenuipalpidae & Tuckerellidae,* and as for species number, it consists of only 97 species.

There are a lot of literature data about Tetranichoid mites fauna [Reck, 1941, 1941, 1941, 1947, 1948, 1948, 1948, 1950, 1959, 1976; Tskitishvili 1982, 1998, 2000], though in the viewpoint of taxonomy many changes are recorded in the last years. Hence, we decided to compose the new checklist, which will be in line with modern literature and help scientists in future researches.

Material and Methods

To compile faunistic list we have acquainted with all literature data existed on Tetranichoid mites of Georgia [Reck, 1941, 1941, 1947, 1948, 1949, 1950, 1959, 1976; Tskitishvili, 1998, 2000] and also with the latest data [Migeon and Flechtmann 2004; //insects.tamu.edu/research/ollection/hallan/acari/Tetranychidea.txt; //insects.tamu.edu/research/collection/hallan/acari/Tenuipalpidae.txt; //insects.tamu.edu/research/collection/hallan/acari/tuckerellidae.txt; Spider mites web]. As a result of analysis of mentioned material compliance of old and new data was realized and the new checklist of Tetranichoid mites for Georgia was composed.

In the annotated list species synonyms, distribution areas within Georgia, host plants are indicated. The following abbreviations are used: Synonym - Syn; East Georgia - E.G.; West Georgia - W.G.; Host Plants - H.P.

Results and Discussion

Tetranichoid mites distributed in Georgia were united in 17 genera [Reck, 1976); Tskitishvili 1982, 1998, 2000], after our analysis - in 24 genera, which is caused by replacement of many species into the new genera. Namely: *Bryobia longisetis* is moved into genus *Pseudobryobia*; *Petrobia shirakensis* - into *Aplonobia; Petrobia samgoriensis - into genus Neopetrobia; Tetranychus savenkoae & Tetranychus viennensis* - into genus *Amphitetracychus; Schizotetranychus carpini, Sch. fagi, Sch. fraxini, Sch. pomeranzevi, Sch. populi, Sch. Pruni, Sch. rajae, Sch. rubiphilus, Sch. tiliarium* - into genus *Eotetranychus.* As for *Schizotetranychus georgicus*, according to new data it belongs to *Mononychellus*, and *Brevipalpus mespilis, B. platani, B. pseudospinosus, B. pulcher & B. quadricornis* to *Cenopaplus.*

In acarological catalogue of Reck [Reck, 1976] for Georgian fauna and Tskitishvili works [Tskitishvili 1982, 1998, 2000] 102 species of Tetranichoid mites are given. Today their number is reduced, because some species is introduced in synonym. Those species are: *Tetranychu atlanticus* McGregor, 1941, which is the synonym of *T. urticae* Koch,1836. In the checklist instead of *Bryobia redikorzevi* Reck, 1947 is given synonym *B. rubrioculus* (Scheuten, 1857). Instead of *Tetranycopsis hostilis* Reck, 1956, is entryied *T. horrida* (Canestrini & Fanzago, 1876). About this species even Reck[Reck, 1976] noted that it is the synonym of *T. Horrida;.Schizotetranychus bakurianensis* Reck, 1948 is the synonym of *Eotetranychus rubiphilus* Reck, 1948. As for *Oligonychus biotae* (Reck, 1953), *O. rollowi* (Reck, 1956), *O. pini* Tuttle, Baker & Abbatiello, 1976 all of them are synonym of *Oligonychus ununguis* Jacobi, 1905.

It should be noted that two species described by Reck: *Eotetranychus aceri & E. coryli* are considered as valid species in literature, but they were not included in the given checklist because Reck [Reck, 1959] considered them as synonyms of *Eotetranychus pruni* (Oudemans, 1931).

Thus, at present Tetranichoid mites fauna of Georgia is presented by 97 species.

Sistematic List of the Superfamily
Tetranychoidea Donnadieu, 1985
Family-*Tetranychidae*
Subfamily- *Bryobiinae* Reck, 1950
Tribe-*Bryobiini*
Genus-*Bryobia* C.L. Coch,1835

1. *B. angustisetis* Jakobashvili, 1958
Distribution: E.G. Tbilisi (Botanical Garden).
H.P.: *Corylus colurna.*

2. *B. artemisiae* Bagdasarian, 1951
Distribution: E.G. Tbilisi (Botanical Garden), Kojori, Trialeti, Shiraki, Akhaldaba, Borjomi, Akhaltsikhe, Akhalkalaki, Aspindza.
H.P.: *Artemisia sp.*

3. *B. borealis* Oudemans, 1930
Distribution:E.G. Bakuriani; W.G. Mestia (high mountainous zone).
H.P.: *Lonicera caucasica*

4. *B. graminum* (Schrank, 1781)
Syn: *Acarus graminum* Schrank, 1781; *Bryobya amygdale* Reck, 1947
Distribution:E.G. Tbilisi (Khudadovi Forest), Mtskheta, Rustavi, Samgori, Shiraki, Lagodekhi.
H.P.: *Aegilops, Amygdalus communis, Hedera helix, H. colchica, Lolium temulentum.*

5. *B. kakuliana* Reck, 1956

Distribution: E.G. Shiraki (Eldaris lowland).
H.P.: *Medicago sativa, Trigonella spicata.*

6. *B. kissophila* Eyndhoven, 1955
Distribution:E.G. Tbilisi (Botanical Garden), Mtskheta, Saguramo, Rustavi, Lagodekhi Reserve, Alazani Riverbank, Sioni.
H.P.: *Hedera colchica, H. caucasica, H. helix.*

7. *B. lagodechiana* Reck, 1953
Distribution:E.G. Tbilisi (Mtatsminda), Kojori, Bakuriani (Kokhta Mountain), Lagodekhi; W.G. Oni (Glola, Shovi), Mestia.
H.P.: *Campanula alliariifolia, C. lactiflora, C. argunensis, C. tridentate, C. aucheri, Festuca djimilensis, Galium sp., Myosotis aloestris, Thymus sp.,*

8. *B. lonicerae* Reck, 1956
Distribution: E.G. Tbilisi (Botanical Garden, Mama Daviti Mountain), Mtskheta, Kojori, Gori, Norio, Gardabani, Shiraki, Pantishari canyon, Lagodekhi, Akhaldaba, Bakuriani.
H.P.: *Lonicera iberica, L. tatarica.*

9. *B. osterloffi* Reck, 1947
Distribution: E.G. Tbilisi (Kus Tba, Mtatsminda), Mtskheta, Samgori, Kojori, Rustavi, Gardabani, Shiraki, Borjomi, Vardzia;
H.P.: *Astragalus caucasicus.*

10. *B. parietariae* Reck, 1947
Distribution: E.G. Tbilisi (Mtatsminda, Mama Daviti Mountain), Samgori, Rustavi, Mtskheta, Kojori, Lagodekhi Reserve, Aspindza, Vardzia.
H.P.: *Campanula argunensis, Galium verum, Mentha sp., Parietaria judaica.*

11. *B. rubrioculus* (Scheuten,1857)
Syn: *B. redikorzevi* Reck, 1947
Distribution: All over Georgia.
H.P: representatives of *Rosaceae* family.

12. *B. tiliae* Bagdasarian, 1957
Distribution: E.G. Tbilisi surrounding area, Saguramo, Akhaldaba, Borjomi.
H.P.: *Tilia sp.*

13. *B. ulmophila* Reck, 1947
Distribution: E.G. Tbilisi, Mtskheta, Saguramo, Akhaldaba, Borjomi, Lagodekhi.
H.P.: *Ulmus sp.*

14. *B. vasiljevi* Reck, 1953
Distribution: E.G. Kojori, Shiraki, Borjomi,, Akhaldaba, Lagodekhi Reserve, Bakuriani.
H.P.: *Campanula alliariifolia, Festuca djimilensis, Poa pratensis, Triticum durum.*

Genus-*Pseudobryobia* McGregor, 1950

15. *P. longisetis* (Reck, 1947)
Syn: *Bryobia longisetis* Reck, 1947
Distribution: E.G. Tbilisi (Kus Tba, Mtatsminda, Mama Daviti Mountain), Samgori, Vaziani, Soganlugi, Kojori, Rustavi, Manglisi, Surami, Norio, Shiraki, Lagodekhi Reserve, Aspindza, Vale, Akhaltsikhe;
H.P.: *Salvia viridis, S. nemorosa, S. aethiopis, S. verticillata.*

Tribe-*Hystrichonychini*

Genus-*Aplonobia* Womersiey, 1940

16. *A. shirakensis* (Reck, 1956)
Syn: *Petrobia shirakensis* Reck, 1956
Distribution: E.G. Shiraci, Eldari steppe.
H.P.: *Agropyrum sibircum, A. pectinatum.*

Genus-*Hystrichonychus* McGregor,1950

17. *H. nepetae* (Bagdasarian, 1951)

Distribution: E.G. Tbilisi suburbs, Kojori surrounding area.

H.P.: *Mentha, Teuctium polium, T. chamaedrys.*

Genus-*Neopetrobia* (*Reckia*) Wainstein,1956

18. *N. samgoriensis* (Reck, 1949)

Syn: *Mesotetranychus samgoriensis* Reck, 1949; *Petrobia samgoriensis* (Reck, 1949)

Distribution:E.G. Tbilisi suburbs (Kus Tba, Samgori steppe, Lilo), Gardabani, Mtskheta, Kojori, Saguramo.

H.P.: *Eryngium campestre, E. coeruleum.*

Genus-*Tetranycopsis* Canestrini, 1890

19. *T. horrida* (Canestrini & Fanzago, 1876)

Syn: *T. hostilis* Reck, 1956

Distribution: All over Georgia.

H.P.: *Corylus avellana, Carpinus caucasica.*

20. *T. hystriciniformis* Reck, 1956

Distribution: E.G. Tbilisi, Kojori surrounding area, branches of the Trialeti mountain ridge.

H.P.: *Eryngium campestre, Potentilla reptans.*

21. *T. matikashviliae* Reck,1953

Distribution: E.G. Tbilisi suburbs, Norio, Sioni, Martkopi, Akhaldaba, Saguramo.

H.P.: *Prunus spinos, P. dulcis.*

22. *T. spiraeae* Reck,1948

Distribution: E.G. Tbilisi (Mtatsminda, Shavnabada, Kus Tba), Samgori steppe, Kojori, branches of the Trialeti mountain ridge, Omalo (Mountainous zone of Tusheti Region), Bakuriani.

H.P.: *Ephedra procera, Spiraea hypericifolia.*

Tribe-*Petrobiini*

Genus-*Petrobia* (*Mesotetranychus*) Reck, 1948

23. *P. vachushtii* (Reck, 1948)

Syn: *Mezotetranychus vachushtii* Reck, 1948

Distribution: E.G. Tbilisi (Mtatsminda, Shavnabada), Samgori steppe, Shiraki, Kojori, Gori.

H.P.: *Ephedra procera.*

Genus-*Petrobia (Petrobia)* Murray, 1877

24. *P. brevipes* Reck & Bagdasarian, 1949

Distribution: E.G. Tbilisi suburbs, Shiraki.

H.P.: *Artemisia sp., Astragalus caucasicus, Kochia prostrate, Malva silvestris.*

25. *P. latens* (Muller,1776)

Syn: *Acarus latens* Muller,1776; *A. praegnans* Schrank, 1781; *Petrobia erevanica* Reck & Bagd., 1949; *P. cephae* Sayed, 1946; *Tetranychina tritici* Ewing, 1921.

Distribution: All over Georgia.

H.P.: Grass and shrubby plants.

Genus-*Petrobia* (*Tetranychina*) Banks, 1917

26. *P. harti* (Ewing, 1909)

Syn: *Neopetrobia spectabilis* Reck, 1941

Distribution:E.G., Lagodekhi Reserve. W.G. Sukhumi, Batumi, Tchakvi, Poti.

H.P.: *Malus orientalis.*

27. *P. zachvatkini* (Reck & Bagdasarian, 1949)

Syn: *Tetranychina zachvatkini* Reck & Bagdasarian, 1949

Distribution: E.G. Tbilisi suburbs, Shiraki.

H.P.: *Kochia prostrata, Consolida divaricata, C. orientalis.*

Subfamily-*Tetranychinae* Donnadieu, 1876

Tribe-*Eurytetranychini*

Genus- *Eurytetranychoides* Reck, 1950

28. *E. thujae* (Reck, 1947)

Syn: *Eurytetranychus thujae* Reck, 1947

Distribution:All over Georgia.

H.P.: *Biota orientalis, Juniperus sp.*

Genus- *Eurytetranychus* Oudemans, 1931

29. *E. buxi* (Garman, 1935)

Syn: *Neotetranychus buxi* Garman, 1935

Distribution: All over Georgia.

H.P.: *Buxus colchica.*

30. *E. recki* Bagdasarian, 1948

Distribution: E.G. Tbilisi, Samgori, Rustavi, Kojori, Shiraki.

H.P.: *Astragalus caucasicus, Medicago sativa, M. glutinosa, M. coerulea, Thymus sp., Teucrium chamaedrys, Spiraea sp.*

Tribe-*Tenuipalpoidini*

Genus-*Tenuipalpoides* Reck & Bagdasarian, 1948

31. *T. zizyphus* Reck & Bagdasarian, 1948

Distribution: E.G. Tbilisi, Vashlovani, Shiraki.

H.P.: *Caragana sp., Halimodendron halodendron.*

Tribe-*Tetranychini*

Genus-*Amphitetranychus* Oudemans, 1931

32. *A. savenkoae* (Reck, 1956)

Syn: *Tetranychus savenkoae* Reck, 1956

Distribution: E.G. Tbilisi, Mtskheta, Gori, Lagodekhi, Telavi.

H.P.: *Quercus sp.*

33. *A. viennensis* (Zacher, 1920)

Syn: *Tetranychus viennensis* Zacher, 1920

Distribution: All over Georgia.

H.P.: representatives *of Rosaceae* family. *Quercus sp., Tilia sp.*

Genus-*Eotetranychus* Oudemans, 1931

34. *E. carpini* (Oudemans, 1905)

Syn: *Schizotetranychus carpini* (Oudemans, 1905)

Distribution: E.G. Tbilisi (Kus Tba, Bagebi), Manglisi, Saguramo, Sioni, Gori, Lagodekhi Reserve, Telavi. W.G. Svaneti.

H.P.: *Carpinus caucasica, C. orientalis, Corylus avellana,Quercus iberica.*

35. *E. fagi* (Zacher, 1922)

Syn: *Schizotetranychus fagi* (Zacher, 1922)

Distribution: All over Georgia.

H.P.: *Fagus orientalis.*

36. *E. fraxini* Reck, 1948

Syn: *Schizotetranychus fraxini* Reck, 1948

Distribution: E.G. Tbilisi, Samgori, Saguramo, Sioni, Gombori, Telavi, Tsinandali.

H.P.: *Fraxinus excelsior.*

37. *E. pomeranzevi* (Reck, 1956)

Syn: *Schizotetranychus pomeranzevi* Reck, 1956

Distribution: E.G. Tbilisi (Vazisubani), Shiraki, Eldari Lowlend.

H.P.:*Agropyrum sibiricum.*

38. *E. populi* (Koch, 1838)

Syn: *Schizotetranychus populi* (Koch, 1838);

Distribution: E.G. Tbilisi, Samgori, Saguramo, Sioni, Gombori, Gori, Borjomi.

H.P.: *Populus canadensis, P. pyramidalis, Salix wilhelmsiana.*

39. *E. pruni* (Oudemans, 1931)

Syn: *Schizotetranychus pruni* (Oudemans, 1931); *Sch. viticola* Reck, 1948; *Sch. aceri* Reck, 1948; *Sch. ulmicola* Reck, 1948; *Sch. aesculi* Reck, 1950; *Sch. coryli* Reck, 1950.

Distribution: All over Georgia.

H.P.: *Acer campestre, A. negundo,A, platanoides, Aesculus hippocastanum, Celtis, Malus domestica, Prunus domestica, P. spinosa,, Vitis vinifera.*

40. *E. rajae* Wainstein, 1956

Syn: *Schizotetranychus rajae* Wainstein, 1956

Distribution: E.G. Tbilisi, Rustavi.

H.P.: *Ulmus foliacea.*

41. *E. rubiphilus* Reck, 1948

Syn: *Schizotetranychus bakurianensis* Reck, 1948, *Sch. rubiphilus* Reck, 1948.

Distribution: All over Georgia.

H.P.: *Rubus sp., Alchemilla erythropoda.*

42. *E. tiliarium* (Hermann, 1804)

Syn: *Schizotetranychus tiliarium* Hermann, 1804, *Sch. telarius* (Hist,1920)

Distribution: All over Georgia.

H.P.: *Tilia sp.*

43. *E. ulmicola* Reck, 1948

Syn: *Sch. ulmicola* Reck, 1948

Distribution: E.G. Tbilisi, Rustavi, Gardabani, Saguramo, Sioni, Gombori, Borjomi, Bakuriani.

H.P.: *Ulmus campestris, U. Montana*

Genus-*Mononychellus* Wainstein, 1971

44. *M. georgicus* (Reck, 1948)

Syn: *Apotetranychus georgicus* Reck, 1948; *Schizotetranychus georgicus* (Reck, 1948)

Distribution: E.G. Tbilisi, Samgori, Rustavi, Gardabani, Saguramo, Sioni, Gombori.

H.P.: *Ramnus pallasii.*

Genus-*Neotetranychus* Tragardh,1915

45. *N. rubi* Tragardh,1915

Distribution: East Georgia.

H.P: *Rubus ideaus.*

Genus- *Oligonychus* Berlese, 1886

46. *O. brevipilosus* Zacher, 1932

Syn: *Paratetranychus brevipilosus* Zacher, 1932

Distribution:E.G. Tbilisi, Mtskheta, Saguramo, Sioni, Akhaldaba, Borjomi.

H.P.: *Pinus eldarica.*

47. *O. buschi* (Reck, 1956)

Syn: *Paratetranychus buschi* Reck, 1956

Distribution:E.G. Tbilisi, Saguramo, Sioni, Gombori.

H.P.: *Quercus sp.*

48. *O. caucasicus* (Reck, 1956)

Syn: *Paratetranychus caucasicus* Reck, 1956

Distribution:E.G. Lagodeckhi Reserve.

H.P.: *Carpinus betulus, C. caucasica, Corylus avellana.*

49. *O. coffeae* (Nietner,1861)

Syn: *A. coffeae* (Nietner,1861)

Distribution:W.G. Ozurgeti, Anaseuli, Batumi.

H.P.: *Thea sinensis.*

50. *O. kobachidzei* (Reck, 1947)

Syn: *Paratetranychus kobachidzei* Reck, 1956

Distribution: : E.G. Tbilisi, Samgori, Saguramo, Sioni, Lagodekhi, Telavi.

H.P.: *Platanus orientalis, Corylus avellana, Juglans regia.*

51. *O. lagodechii* Livshits & Mitrofanov, 1969

Distribution: : E.G. Lagodekhi Reserve; W.G. Batumi (Botanical Garden).

H.P.: *Biota orientalis, Cupressus sp., C. lawsoniana, Cryptomeria japonica.*

52. *O. longiclavatus* (Reck, 1953)

Syn: *Paratetranychus longiclavatus* Reck, 1956

Distribution: E.G. Tbilisi, Lagodekhi.

H.P: *Carpinus caucasica, Quercus iberica.*

53. *O. piceae* (Reck, 1953)

Syn: *Paratetranychus piceae* Reck, 1956

Distribution: E.G. Tbilisi (Kus Tba, Mtatsminda), Kojori, Akhaldaba, Borjomi, Bakuriani.

H.P.: *Pinus sosnovskyi, Picea orientalis.*

54. *O. tshimkenticus* (Wainstein, 1956)

Syn: *Paratetranychus tshimkenticus* Wainstein, 1956;

Distribution: E.G. Tbilisi, Rustavi.

H.P.: *Ulmus sp.*

55. *O. ununguis* (Jacobi,1905)

Syn: *Paratetranychus ununguis* Jacobi,1905, *Oligonychus biotae* (Reck, 1953), *O. rollowi* (Reck, 1956), *O. pini* Tuttle, Baker & Abbatiello, 1976,

Distribution: All over Georgia.

H.P.: *Biota orientalis, Cryptomeria japonica, Cupressus sp., Juniperus sp., Picea orientalis, Picea excelsa, Pinus hamata, Pinus silvestris, Ttuja*

Genus-*Panonychus* Yokoyama, 1929

56. *P. citri* (McGregor, 1916)

Syn: *Tetranychus citri* McGregor, 1916; *Paratetranychus citri* (McGregor, 1916); *Metatetranychus citri* Reck, 1941.

Distribution: All over Georgia.

H.P.: *Amygdalus communis, Citrus paradisi, C. nobilis, C. sinensis, C. limon, Laurocerasus officinalis, Malus domestica, Menyanthes trifoliate, Rosa sp.*

57. *P. hadzhibejliae* (Reck, 1947)

Syn: *Metatetranychus hadzhibejliae* Reck, 1947

Distribution: All over Georgia.

H.P.: *Ficus carica.*

58. *P. ulmi* (Koch,1836)

Syn: *Tetranychus ulmi* Koch,1836;

Distribution: All over Georgia.

H.P.: *Elaeagnus, Morus alba, Ulmus sp; Pterocarya fraxinifolia, Tilia sp; Celtis sp; Robinia pseudoacacia* and *Rosaceae* family.

Genus-*Schizotetranychus* Tragardh, 1915

59. *Sch. avetjanae* Bagdasarian, 1954

Distribution:E.G. Tbilisi suburbs, Kojori area.
H. P.: *Spiraea, Hypericum androsaemum.*

60. *Sch. bambusae* Reck, 1941
Distribution: E.G. Tbilisi (Botanical Garden); W.G. Kobuleti, Tsikhisdziri, Tchakvi, Batumi, Sukhumi.
H.P.: *Phyllostachys sp., Arundinaria sp.*

61. *Sch. ibericus* Reck, 1947
Distribution:E.G. Tbilisi, Samgori, Mtskheta, Sioni, Gombori.
H.P.: *Quercus iberica.*

62. *Sch. jachontovi* Reck, 1953
Distribution: E.G. Tbilisi, Kojori, Saguramo, Mtskheta, Sioni, Lagodekhi, Telavi region (Tetritsklebi, Lapotis area, Didi-khevi).
H.P.: *Quercus sp.*

63. *Sch. saba-sulchani* Reck, 1956
Distribution: E.G. Shiraki, Eldari lawland.
H.P.: *Cynodon dactylon.*

64. *Sch. schizopus* (Zacher, 1913)
Distribution:E.G. Manglisi, Tbilisi, Saguramo, Sioni, Gori, Lagodekhi, Napareuli.
H.P.: *Salix sp.*

65. *Sch. tbilisiensis* Reck, 1959
Distribution: E.G. Tbilisi, Gardabani.
H.P.: *Lolium temulentum, L. persicum.*

Genus-*Tetranychus* Dufour,1832

66. *T. ludeni* Zacher, 1913
Distribution: E.G. Tbilisi (Botanical Garden).
H.P.: *Ricinus communis.*

67. *T. nikolskii* Reck, 1953
Distribution: E.G. Tbilisi suburbs.
H.P.: *Salvia aethiopis, Botrychium lunaria.*

68. *T. przhevalskii* Reck, 1956
Distribution: E.G. Shiraki, Eldari lawland.
H.P.: *Agropyrum sibiricum, A. pectiniformes, Arrhenatherum elatius, Dactylis glomerata, Phleum pretense, Triticum dicoccu, Zea maysi.*

69. *T. urticae* Koch,1836
Syn: *T. atlanticus* McGregor, 1941;
Distribution: All over Georgia.
H.P.: *Polyphagus.* Does not feed by *Cpore* and *Gymnodamae* plants.

Family-*Tenuipalpidae* Berlese,1913

Genus- *Aegyptobia* Sayed, 1950

70. *A. pavlovskii* (Reck, 1951)
Syn: *Brevipalpoides pavlovskii* Reck, 1951, *Pentamerismus pavlovskii* (Reck, 1951)
Distribution: E.G. Tbilisi suburbs (Shavnabada), Kojori, branches of the Trialeti mountain range, Vashlovani.
H.P.: *Ephedra procera.*

71. *A. xerophilus* (Reck, 1953)
Syn: *Brevipalpoides xerophilus* Reck, 1951, *Pentamerismus xerophilus* (Reck, 1951)
Distribution: E.G. Tbilisi suburbs, Kojori, branches of the Trialeti mountain range, Shiraki, Aspindza surrounding area. Registered only in Georgia.

H.P.: *Acantholimon lepturoides, Cerastium argenteum.*

72. *A. zaitzevi* (Reck, 1951)

Syn: *Brevipalpoides zaitzevi* Reck, 1951, *Pentamerismus zaitzevi* (Reck, 1951)

Distribution: E.G. Tbilisi suburbs, Samgori steppe, Gardabani, Shiraki.

H.P.: *Thymus sp, Atraphaxis spinosa.* Endem of Caucasia.

Genus- *Brevipalpus* Donnadieu, 1875

73. *B. bagdasariani* Livshitz & Mitrofanov, 1970

Distribution:E.G. Tbilisi.

H.P.: *Fraxinus excelsior.*

74. *B. californicus* (Banks, 1904)

Syn: *Tenuipalpus californicus* Banks, 1904

Distribution: E.G. Tbilisi (Botanical garden); W.G. Batumi (Botanical Garden).

H.P.: *Trachycarpus excelsa, Thea sinensis, T. assamica, Citrus limon.*

75. *B. carpini* Livshitz & Mitrofanov, 1967

Distribution: E.G. Tbilisi, Saguramo, Sioni, Borjomi.

H.P.: *Fagus orientalis.*

76. *B. lewisi* McGregor, 1949

Distribution:W.G. Zestafoni, Sakara (Zestafoni region).

H.P.: *Vitis vinifera.*

77. *B. lineola* (Canestrini & Fanzago, 1876)

Syn: *Cenopalpus lineola* (Canestrini & Fanzago, 1876); *Cenopalpus kalandadzei* Reck, 1951

Distribution: All over Georgia.

H.P.: *Pinus hamata, P. sp.*

78. *B. obovatus* Donnadieu, 1875

Distribution: All over Georgia.

H.P.: Polyphagus.

79. *B. phoenicis* (Geijskes, 1939)

Syn: *Tenuipalpus phoenicis* Geijskes, 1939

Distribution: E.G. Tbilisi, (Botanical Garden).

H.P.: *Matthiola incana, Hevea brasiliensis, Yuglans regia, Citrus limon, C. reticulata.*

80. *B. populi* Livshitz & Mitrofanov, 1967

Distribution: E.G. Tbilisi, Mtskheta, Saguramo. Registered only in Georgia.

H.P.: *Populus gracilis.*

81. *B. russulus* (Boisduval, 1867)

Syn: *Acarus russulus* Boisduval, 1867

Distribution: E.G. Tbilisi (Botanical Garden), W.G: Batumi (Botanical Garden).

H.P.: Representatives of the *Cactaceae* family.

82. *B. thelycraniae* Livshitz & Mitrofanov, 1967

Distribution: E.G. Tbilisi, Samgori, Mtskheta, Borjomi. Registered only in Georgia.

H.P: *Thelychrania australis.*

Genus- *Cenopalpus* Pritchard & Baker, 1958

83. *C. mespili* (Livshitz & Mitrofanov, 1967)

Syn: *Brevipalpus mespilis* Livshitz & Mitrofanov, 1967

Distribution: E.G. Tbilisi surrounding area.

H.P.: *Malus domestica, Mespilus germanica.*

84. *C. platani* (Livshitz & Mitrofanov, 1967)

Syn: *Brevipalpus platani* Livshitz & Mitrofanov, 1967

Distribution: E.G. Tbilisi surrounding area.

H.P.: *Platanus orientalis.*

85. *C. pseudospinosus* (Livshitz & Mitrofanov, 1967)
Syn: *Brevipalpus pseudospinosus* Livshitz & Mitrofanov, 1967
Distribution: W.G. Adjara seaside, Batumi surrounding area.
H.P.: *Potentilla reptans, Fragaria vesca.*
86. *C. pulcher* (Canestrini & Fanzago, 1876)
Syn: *Caligonus pulcher* Can. & Fanz., 1876; *Brevipalpus pulcher* Can. & Fanz., 1876;
Distribution: All over Georgia.
H.P.: Mainly bushes and trees of *Rosacea* family. Dangerous for frut cultures.
87. *C. quadricornis* Livshitz & Mitrofanov, 1967
Syn: *Brevipalpus quadricornis* Livshitz & Mitrofanov, 1967
Distribution: W.G. Adjara seaside, Batumi surrounding area.
H.P.: *Fragaria vesca.*

Genus- *Pentamerismus* McGregor, 1949

88. *P. erythreus* (Ewing, 1917)
Syn: *Tenuipalpus erythreus* Ewing, 1917)
Distribution: W.G. Abkhasia seaside.
H.P.: *Libocedrus decurrens, Cyperus sp., Juniperus, Pinus sp, Tuja, Chamaecyparis lawsoniana.*
89. *P. juniperi* (Reck, 1951)
Syn: *Brevipalpoides juniperi* Reck, 1951
Distribution: All over Georgia.
H.P.: *Juniperus sp.*
90. *P. oregonensis* McGregor, 1949
Distribution: All over Georgia.
H.P.: *Biota orientalis, Cupressus, Libocedrus decurrens, Tuja occidentalis. Juniperus sp.*

Genus- *Tenuipalpus* Donnadieu, 1875

91. *T. baeri* Reck, 1956
Distribution: E.G. Tbilisi surrounding area, Shiraki, Eldari steppe, Pantishari canyon, Lekistskali.
H.P.: *Mimosa sp.*
92. *T. cheladzeae* Gomelauri,1960
Distribution: E.G. surrounding area of the Abastumani observatory (Mountain Kanobili). W. G. Batumi (Botanical garden).
H. P.: *Abies firma, A. numidica, Taxus baccata.*
93. *T. dubinini* Reck, 1951
Distribution: E.G. Tbilisi suburbs, Shavnabada Mountain, Mtskheta, Kojori, branches of the Trialeti mountain ridge, Shiraki, Pantishari,Vardzia surrounding area. W.G. Shovi.
H.P.: *Ephedra procera.*
94. *T. kobachidzei* Reck,1951
Distribution: E.G. Tbilisi, Vaziani, Samgori steppe, Kojori.
H.P: *Calamintha chinopodium, Mentha sp,Thymus sp.*
95. *T. punicae* Pritchard & Baker, 1958
Distribution: All over Georgia.
H.P.: *Punica granatum.*
96. *T. zhizhilashviliae* Reck, 1953
Distribution: W.G. Seaside in Abkhasia and Adjara.
H.P.: *Diospyros kaki.*

Family-*Tuckerellidae* Baker & Pritchard, 1953

Genus- *Tuckerella* Womersley, 1940

97. *T. pavoniformis* (Ewing, 1922)

Syn: *Parabriobia aenigmatica* Reck, 1952

Distribution:W.G. Poti, Tchakvi, Makhinjauri, Batumi (Botanical Garden), Sukhumi, Eshera, Lidzava.

H.P.: *Chamaecyparis lawsoniana, Cryptomeria japonica, Sequoia sempervir, Sequoiadendron giganteum, Rosmarinus officinalis, Thea sinensis, Hippophae sp., Erica sp., Citrus limon, C. sinensis, C. nobelis.*

References:

Aleksidze N. and Lekishvili T. *Web Spider Mites of Vine.* Telavi, 5-16, 1938 (in Gorgian).

Migeon A. and Flechtmann C.H.W. *Fierst additions and corrections World catalogue of the Spider Mite family* (*Acari: Tetranychidae*). Intewrnational Journal of Acarology, **30** 2, 143-152.2004.

Pritchard A. E., Baker E. W. *A Revision of The Spider Mite Family Tetranychidae* Memoirs Series Volume **2**, 1-475, 1955.

Reck. G. F. *New genus and species of spider mites in Georgia (Tetranychidae, Acari).* Bull. of the Ac. Sci. of Georgian SSR, **2**, 8, 749-753, 1941.

Reck G.F. *A new Schizotetranychus species (Tetranychidae, Acari).* Bull. of the Ac. Sci. of Georgian SSR, **2**, 5, 449-453, 1941.

Reck. G. F. *New species of Tetranychid mites from Georgia (Tetranychidae, Acari).* Bull. of the Ac. Sci. of Georgian SSR, **8**, 7, 471-475, 1941.

Reck. G. F. G*enus Bryobia Koch (Tetranichidae) described on the data material from Georgia.* Bull. of the Ac. Sci. of Georgian SSR, **8**, 9-10, 653-660, 1947.

Reck. G. F. *Descriptions of species of the genus Schizotetranychus (Trag.) from Georgia (Tetranychidae, Acari).* Bull. of the Ac. Sci. of Georgian SSR **9**, 7, 445-452, 1948.

Reck. G. F. G*enus Schizotetranychus (Tetranychidae, Acari) described on the data material from Georgia.* Bull. of the Ac. Sci. of Georgian SSR **9**, 6, 369-376, 1948.

Reck. G. F. *Fauna of Spider mites (Tetranychidae, Acari) from Georgia.* Proceedings of Institute of Zoology. Ac. Sci. of Georgian SSR, **8,** 175-185, 1948.

Reck. G. F. *Spider mites fauna from Georgia (Tetranychidae, Acari).* Proceedings of Institute of Zoology. Ac. Sci. of Georgian SSR, **9,** 117-134, 1950.

Reck. G. F. *A Key to the Tetranychoid mites. Fauna Trans. Caucasia.* Bull. of the Ac. Sci. of Georgian SSR, 1-152, 1959.

Reck. G. F. *Catalogue of Acarofauna of Georgia.* Bull. of the Ac. Sci. of Georgian SSR, 1-152, 1976.

SpiderMitesWeb: http://insects.tamu.edu/research/collection/hallan/acari/Tetranychidae.txt

http://insects.tamu.edu/research/collection/hallan/acari/Tenuipalpidae.txt

http://insects.tamu.edu/research/collection/hallan/acari/tuckerellidae.txt

Tskitishvili M. *The Fauna of Insects and Mites in Saguramo-Gombori Raing.* Tbilisi, "Metsniereba", 99-106, 1982.

Tskitishvili M. *Catalogue of Tetranychoidea Ticks in Georgia.* Proceedings of the Institute of Zoology. Vol. **XX**, 95-100, 2000.

Tskitishvili M. *List of Tetranychoid (Tetranychoidea* Reck, 1952*) Ticks in Georgia.* Proceedings of the Institute of Zoology. Vol. **XXI**, 96-102, 2002.
Wainshtein B.A. *Tetranixovie Kleshi Kazaxistan.* Alma-Ata, 3-275, 1960.

საქართველოს ტეტრანიხისებრი ტკიპების (*Acari: Tetranychoidea*) ფაუნა

არაბული თ.

ზოოლოგიის ინსტიტუტი

(მიღებულია 20.02.2008)

რეზიუმე

საქართველოში გავრცელებული ტეტრანიხისებრი ტკიპების ფაუნის შესახებ ლიტერატურული მონაცემებისა და უახლესი სისტემატიკური მასალის ანალიზის შედეგად ცნობილი გახდა, რომ დღეისათვის საქართველოს ტეტრანიხების ფაუნა წარმოდგენილია 97 სახეობით, რომლებიც გაერთიანებულია სამ ოჯახსა და 24 გვარში.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 1-2, 2008.

Letter to Editor
Entomology

RENEWAL OF POPULATION OF THE MAIN CARRIER OF MALARIA *ANOPHELES MACULIPENNIS* IN TBILISI (GEORGIA)

GUGUSHVILI G., ZEREKIDZE L.

Virsaladze Institute of Medical Parasitology and Tropical Medicine

(Received March 6, 2008)

Abstract

The paper deals with the observations of renewal of population of the main carrier of malaria, *Anopheles maculipennis* in Tbilisi area. Phenology, durations of vital activity and transmission seasons of this species are studied. Risk factor of malaria introduction and its spreading is dangerous in this area. It is necessary to carry out preventive measures against malaria carrier

In 70s of 20th century in Georgia malaria was liquidated, but due to rich fauna of disease carriers and favorable climatic conditions potential threat of epidemic activation existed. In Tbilisi area the number of *An. maculipennis*, the main malaria carrier was high in 20-50s of last century. In Georgia epidemiological situation with respect to malaria was progressively worsened from 1996. From 2001 single cases of malaria were registered. Up to 2003 *An. maculipennis* was not recorded. Substantial factors leading to reactivation of malaria in Georgia are declining in living standards, absence of prophylactic measures, global warming, unsatisfied conditions of irrigational systems, growth of number of disease carrier populations, etc.

Registration-systematization of pre-imago phases of biotopes was conducted during 2003-2006 within Tbilisi area. The main test subjects were mosquito of genus Anopheles and mosquito of other genera inhabited in their biotopes. Material was mainly collected in Saburtalo district of Tbilisi. Corresponding biotopes were chosen for material collection. Observations were conducted once in every 10 days for collection of pre-imago and imago phases. From every biotope 5-10 samples were taken. For identification of species quantitative and qualitative detection of larva and pupa according to metamorphosis phases was carried out using the general methods [Beklemishev, 1949; Pavlovski, 1959; Shipitsina, 1957]. Physiological state of *An. maculipennis* was established through sectioning of females [Detinova, 1962].

While observations upon exophilic species the following species were revealed: *An. maculipennis, Ae.vexans, Ae. caspius, Culex pipiens pipiens* and *Culex pipiens mofestus.*

To study phenology of *An. maculipennis* observations were carried on spring, summer and autumn populations.

An. maculipennis was registered within Saburtalo (Nutsubidze street) and Digomi (plot of research farming) districts. Endophilic, as well as exophilic species were registered in both natural and artificial biotopes. Exophilic species of the main carrier of malaria, *An. maculipennis* were not revealed. This fact indicates that biotopes characteristic for them are limited on researched area. Biotopes of non-malaria genera - Culex and Aedes are in abundance. Numbers of *Ae.vexans, Ae. caspius* and *Culex pipiens pipiens* are rather high.

Period of vital activity of *An. maculipennis* in the territory of Tbilisi lasts 8-8.5 months (March-October). Transmission season of malaria is 5-5.5 months. Fly out of females is noted when average diurnal temperature reaches 7^0C. Within Tbilisi area this period comes on I-II decades of March. Fly out of females of first generation is significant in epidemiological point of view, as the beginning of the first cycle of sporogonium is related with this period, which according to our studies in noted in III decade of April and I decade of May. In epidemiological viewpoint for prolonging the transmission season of malaria females being in gonotropic dissociation are essential, which in our case under influence of thermal regime was registered at the end of III decade of October and in I decade of November. Mass cessation of bloodsucking by females and mass diapause was recorded in II decade of November.

After 80 years recruitment of population of the main carrier of malaria in Tbilisi area indicates the changes occurring in ecosystem, which are caused by changes of hydrological and thermal regimes. Potential danger of transmission resumption and emergence of epidemiological outbreaks on the territory of Tbilisi still exist. Basic factors leading to reactivation of malaria are the following: mass migration of population in the zones of intensive transmission of infection, global warming, unsatisfied conditions of irrigational systems causing broadening of reservoir areas – hatching areas of Anopheles, growth of number of disease carrier populations, increase of their contacts with human, etc. In recent years substantial factor for resumption of malaria, besides declining in living standards and absence of prophylactic measures, are emigrational processes.

Al those factors have influence on increasing of population of bloodsucking arthropods and on the process of circulation of pathogenic agents.

It is necessary to carry out preventive measures against malaria carriers. It is advisable to use ecologically safe biological agents, such as larva-phages and entomopathogenic nematodes to control disease carriers.

Literature:

Beklemishev V.N. *Manual of medical entomology.* M., "Medizdat", 1949 (in Russian).

Detinova T.S. *Methods of determination of age composition of dipterans significant for medicine.* Geneva, pp. 1303, 1962.

Pavlovski E.N. *Laboratory practical work of medical parasitology.* M., 1959 (in Russian).

Shipitsina N.K. *Phenological preconditions for efficiency of terms of measures against malaria and effective terms of measures in USSR.* M., 439-461, 1957 (in Russian).

მალარიის ძირითადი გადამტანის *Anopeles maculipennis* პოპულაციის განახლება თბილისის ტერიტორიაზე

გუგუშვილი გ., ზერეკიძე ლ.

ს. ვირსალაძის სახ. სამედიცინო პარაზიტოლოგიისა და ტროპიკული მედიცინის ინსტიტუტი

(მიღებულია 06.03.2008)

რეზიუმე

მოცემულია თბილისის ტერიტორიაზე მალარიის ძირითადი გადამტანის, *Anopeles maculipennis* პოპულაციის აღდგენასთან დაკავშირებული დაკვირვებების შედეგები. შესწავლილია ამ სახეობის ფენოლოგია, აქტიური ცხოვრებისა და ტრანსმისიის სეზონის ხანგრძლივობა. ქალაქის ტერიტორიაზე მალარიის შემოტანის და გავრცელების რისკ-ფაქტორი მეტად საშიშია. აუცილებელია მალარიის გადამტანის წინააღმდეგ პრევენციული ღონისძიებების გაძლიერება.

Proc. Georgian Acad. Sci., Biol. Ser. B
Vol. 6, No. 1-2, 2008.

Letter to Editor
Botany

SOME BIOLOGICAL CHARACTERISTICS OF GROWTH AND DEVELOPMENT OF TWO SPECIES OF CORK TREE (*PHELLODENDRON AMURENSE*, *PH. AMURENSE* VAR. *LAVALLEI*) IN AJARA

VARSHANIDZE N., IAROSH E., BERISHVILI L., LOMTATIDZE N., ALASANIA N., MANVELIDZE Z.

Batumi Rustaveli State University

(Received March 31, 2008)

Abstract

Some biological features of growth and development of *Phellodendron amurense* Rupr. and *Ph. amurense* var. *lavallei* (Dog) Spraque were studied. Two ways of propagation - by seeds, as well as by root cuttings are proposed. It was shown that cultivation of these two species of cork tree is possible in Ajara region (West Georgia).

Plants from genus Phellodendron imported in the last century are abundant within parks and Botanic gardens of Black Sea Coast of Caucasus. In this region, and namely in Batumi Botanic Garden 5 species of cork tree occur [Pilipenko, 1978; Trees and shrubs of Batumi Botanic Garden, 1987].

Phellodendron amurense Rupr. is diecious deciduous tree with height up to 25m from the family Rutaceae. Areas of its distribution: Manchuria, the Primorye, Sakhalin, riv. Aleur basin. Lifetime - 250-300 years. In the North part of its distribution it is grown as shrubbery. It occurs on flat slopes, along river valleys. It belongs to protected plants. Hence, this species is not used as row material for preparation of medicines. In this viewpoint attention was paid to another species of cork tree - *Ph. amurense* var. *lavallei* (Dog) Spraque, which may be cultivated [Otriashenkova et al., 1987, Glizin, Otriashenkova, 1979].

Ph. amurense var. *lavallei* (Dog) Spraque is deciduous tree introduced into Batumi Botanic Garden in 1954. Origin - Japan. Leaves - opposite, petiolar, imparipinnate-compound, spear-shaped, crenate, with odor nuisance, up to 60 cm. Flowers - small, diclinous, diameter - up to 12 mm. Inflorescence - paniculate. Stone-fruit - seedball or pear-shaped, diameter - up to 1 cm; black, lustrous, bitter, with odor nuisance. Fruit contains 4-6 dark-brown stones with meshwork surface [Vasiliev, 1957].

Leaves consist of essential oils, flavonoids, coumarins, tannins, saponins. Bark of branches contains saponins, coumarins, alkaloid berberin having choleretic action [Encyclopaedia of medicine plants, 1999].

Occurrence of valuable biological active compounds in the species of cork trees grown in Batumi Botanic Garden serves as beginning for study of these species with the aim to reveal the potential of their cultivation in mentioned region.

Our observations carried out within 2000-2005 have shown that both those species are cold resistant and do not damage at the lowest temperatures occurred in Ajara. In this region they preferably inhabit in moderate humid environment on fertile soils, do not grow on bogged soils. Cork trees are shade-enduring plants. They bloom and fructify at 5-6 years of life. Florescence occurs in May-June, maturation of fetus - in November-December.

In the conditions of Ajara cycling of occurrence of reproductive phases is observed. Florescence and fructification occur not every year - with intervals of one, or rarely two years. Weight of 1000 fruits is 300-440 g. Absolute weight of seeds - 10-11 g. From one mature tree (10-12 years old) 6-7 kg fruits with outcome of 50 000-55 000 seeds was obtained. After fruit collection we remove pericarp, dry and keep in closed vessels.

Propagation of both species by seeds, as well as by root cuttings was realized. Seed sawing was carried out in autumn. At spring sawing stratification of seeds is needed. Germinating ability of stratified seeds is 80-85%, and of unstratified ones - only 25%. Germination of seeds takes place at 15°C, mass germination was observed at 25th day.

Seed sawing was conducted in December-January with unstratified seeds, as they run natural stratification in soil in winter period. Seeds were put in soil on depth of 12 cm. Annual seedlings of cork tree were bedded in nursery with nutrient area of 30-15 cm. At the end of the first year of vegetation plants reached the height of 1 m and they were bedded into permanent habitat. Monaxial specimens developed up to 30 leaves with maximal length of 50 cm. At the second year they began to develop side shoots, which further form tree crown. At 6th year seedlings of cork tree usually reach the height of 3-4 m, at 10th year - more than 5 m, and at 30 years the height of the trees reaches 10-15 m. Generative period occurs at the age of 5-6 years.

Propagation with root cuttings was conducted in spring through replanting the seedlings from nursery into permanent habitat, when a lot of rootlets remain in the soil. They were grafted and sowed at the depth of 10-15 cm remaining 1-2 cm above the soil surface. Establishment of cuttings was 93-97%. At the end of vegetation the first order shoots reached 150-180 cm. After grubbing the seedlings a lot of plants were still developed from root offshoots, which further can be used.

Valuable biological characteristic of those plants is their ability to develop easily reproduction shoots while their planting into bole, as well as removal of boughs. After autumn trimming spring regrowth went very intensively. On the plants of the age of 6-8 years more than 50 reproduction shoots can be seen, which germ size during the vegetation period is 60-100 cm. With the plant aging intensity of reproduction of shoots is decreased, accordingly, the yield of leaf mass is decreased. Hence, for obtaining the leaf mass in industrial scale it can be recommended: trim of 10-12 years old trees at height of 20-25 cm above the soil surface, and also twofold – in the middle and at the end of vegetation - collection of leaves from young sprouts. This enables us to avoid partial drying of sprouts and abscise of crown leaves. At one-fold trimming portion of leaves in the whole phytomass composed 45%, at twofold trimming – 70%.

Harvest of leaf along with cuttings was carried out in July (sometimes - in August) manually. If trees are big branches should not be break. At least one third of leaves must be leaved on the tree. While trimming the branches bark of young branches was obtained, which consists of alkaloid berberin necessary for receiving the preparation "bisulphateberberin" having choleretic action [Medicine row material, 2006]. Both row material have been dried in aired houses or in dryer at 60-70°C. To dry in sunlight is not allowed. They were kept in dry place. Useful time of leaf is 3 years, and of bast - 4 years. Quality of used row material corresponded to scientific documentation: content of flacoside in leaves was more than 2.5%, and of berberin in basts - more than 0.5%.

Our studies have shown that the both species of cork tree - *Phellodendron amurense* Rupr. and *Ph. amurense* var. *lavallei* (Dog) Spraque - have high viability and plasticity in Ajara region.

This fact enables us to cultivate those species in this area and to develop the source of row materials for industry of preparation "Flacoside".

Literature:

Encyclopaedia of medicine plants and product of animal origin. St. Petersburg, "Specialnaia Literatura", p. 282, 1999 (in Russian).

Glizin V.I., Otriashenkova V.E. *Method of obtaining of fellavin.* Certificate of authorship N685345, May 21, 1979 (in Russian).

Medicine row material of plant and animal origin. Pharmacognosy. St. Petersburg, "Specialnaia Literatura", p. 474, 2006 (in Russian).

Otriashenkova V.E., Glizin V.I., Shreter G.K. *Flavonol glycoside from plants of genus Phellodendron.* "ХПС", Tashkent, N5, p. 662, 1976 (in Russian).

Pilipenko F.S. *Foreign trees and shrubs in the Black Sea Coast of Caucasus.* "Nauka", M., p. 220, N5, 1978 (in Russian).

Trees and shrubs of Batumi Botanic Garden. Ed. Sharashidze N., Tbilisi, "Metsniereba", p. 146, 1987 (in Russian).

Vasilieva A.V. *Flora of trees and shrubs of subtropics of West Georgia.* Proceedings of Sukhumi Botanic Garden, vol. III, issue X, p. 182, 1957 (in Russian).

კორპის ხის ორი სახეობის (*Phellodendron amurense, Ph. amurense* var. *lavallei*) ზრდა-განვითარების ზოგიერთი ბიოლოგიური თავისებურებანი აჭარაში

ვარშანიძე ნ., იაროში ე., ბერიშვილი ლ., ლომთათიძე ნ., ალასანია ნ., მანველიძე ზ.

ბათუმის რუსთაველის სახელმწიფო უნივერსიტეტი

(მიღებულია 31.03.2008)

რეზიუმე

შესწავლილია კორპის ხის ორი სახეობის - *Phellodendron amurense, Ph. amurense* var. *lavallei* – ზრდა-განვითარების ზოგიერთი ბიოლოგიური თავისებურებანი. შემოთავაზებულია გამრავლების ორი ხერხი: თესლით და ფესვის კალმებით. დადგენილია, რომ კორპის ხის ამ ორი სახეობის კულტივირება შესაძლებელია წარმატებით იქნეს განხორციელებული აჭარის რეგიონში.

ინსტრუქცია ავტორთათვის

სამეცნიერო ნაშრომი გამოიცემა ინგლისურ ენაზე, მას უნდა დაერთოს რეზიუმე ინგლისურ და ქართულ ენაზე, სამეცნიერო მიმართულება, სათაური, ავტორთა გვარები და მათი სამუშაო დაწესებულების დასახელება, საკვანძო სიტყვათა მოკლე (4-6) სია.

წერილის მოცულობა არ უნდა იყოს 5 გვერდზე ნაკლები და 12 გვერდზე მეტი. წერილი უნდა გაფორმდეს შემდეგი რუბრიკაციით: შესავალი და მიზნები (Introduction), მასალა და მეთოდები (Materials and Methods), შედეგები და მათი განხილვა (Results and Discussion), დამოწმებული ლიტერატურა. უკანასკნელი უნდა იყოს დალაგებული ანბანის მიხედვით, ხოლო ტექსტში წყაროების მითითება უნდა ხდებოდეს ფრჩხილებში ჩასმული ავტორის გვარითა და წლით [Lernmark, Hagglof 1981].

მითითებული ლიტერატურა წარმოდგენილი უნდა იყოს შემდეგნაირად:

ჟურნალის შემთხვევაში

Carvalho C., Pereira H., Pina C. *Chromosomal G-dark bands determine the spatial organization of centromeric heterochromatin in nucleus.* Mol. Biol. Cell, **12**, 5, 3563-3572, 2001.

წიგნის შემთხვევაში

Kuhn T.S. *The structure of scientific revolutions.* Chicago, IL, Chicago Press, 2000.

Brush S. *Flowing waters or teeming crowds*. In: Mental Models. D. Gentner (Ed.), Chicago IL., Chicago Press, 865-900, 2001.

მასალა რედაქციაში წარმოდგენილი უნდა იყოს ქაღალდზე ამობეჭდილი და დისკეტით (ან CD-ით). წერილი ერთი ფაილით უნდა იყოს შენახული (ცალკე ფაილად შეიძლება ილუსტრაციების წარმოდგენა), ხოლო ფაილის სახელწოდება წერილის პირველი ავტორის გვარს უნდა ატარებდეს.

ქართული ტექსტისთვის ოპტიმალური ფონტებია AcadNusx და AcadMtavr, ინგლისური ტექსტებისთვის - Times New Roman. შრიფტის ზომა - 12 პუნქტი, ინტერვალი - 1,5. ცხრილებში დასაშვებია უფრო მცირე ზომის შრიფტები. წერილი უნდა დაიბეჭდოს A4 ფორმატით, ზევით და ქვევით - 2,5 სმ., მარცხნივ – 3 სმ. და მარჯვნივ – 2სმ. დაშორებით. ცხრილები, გრაფიკები და დიაგრამები (მხოლოდ შავ-თეთრი) შესაძლებელია დამზადდეს როგორც Microsoft Word-ში, ისე Excel-ში, ფოტოსურათები მიიღება აგრეთვე ორიგინალების (არაელექტრონული) სახითაც.

ჟურნალის გამოცემა ავტორთა ხარჯებით ხორციელდება. თანხა რედაქციაში უნდა შემოვიდეს ნაშრომზე დადებითი რეცენზიის მიღებისთანავე. ნაშრომის რეცენზირება ანონიმურია და ავტორს აქვს უფლება მიიღოს ან არ მიიღოს რეცენზენტის შენიშვნები. უკანასკნელ შემთხვევაში ნაშრომი, დამატებით გაეგზავნება სარედაქციო საბჭოს ერთ-ერთ წევრს. მეორე უარყოფითი დასკვნის შემთხვევაში, ნაშრომი არ გამოქვეყნდება.

ნაშრომის ჩაბარება შეიძლება სამუშაო დღეებში, 12-დან 16 საათამდე, შემდეგ მისამართზე: თბილისი, რუსთაველის გამზირი 52, საქართველოს მეცნიერებათა აკადემია, ბიოლოგიის განყოფილება, IV სართული, 429 ოთახი, ტელ: 93-58-92, პასუხისმგებელი მდივანი – მაია გრიგოლავა.