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# THE PROMISE OF GENOMICS AND BEYOND

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## Abstract

This review aims at providing a short synopsis of the history, advances and future of genomics sciences in various fields of research with focus on areas pertaining to forest tree species and ecosystems. It also provides a broader outlook of the place of genomics and other 'omics' approaches like transcriptomics, proteomics and metabolomics in future biology research at various levels of organization, particularly on system level knowledge at organismal and ecosystem scales. Specifically, emphasis is placed on new sequencing technologies that open the gate for thorough exploration of the DNA and RNA world from a single cell and individual to population and ecosystem scales. These and other technological innovations are holding great promise for advanced breeding, deeper knowledge of developmental and physiological mechanisms and better understanding of the processes occurring at ecosystem levels.

**Key words:** Systems biology, genetics, functional genomics, next-generation sequencing.

## Brief History and Definitions

Genomics is a field of science dealing with a large number, if not all, genes and, more recently, the DNA sequence of the entire genome. This is in sharp contrast with preceding endeavors focusing typically on one or a few genes at a time. The dawn of genomics dates back to the late 1980s. Yet, the idea of sequencing and understanding the genetic information in its entirety is not new and is probably as old as the realization that DNA is the carrier of the hereditary information. However, it was only in the mid to late 1980s when the convergence of several major technological advances provided the foundations for the modern genomics field. These included recombinant DNA (Jackson et al. 1972), restriction enzymes (Luria and Human 1952), reverse transcriptase, polymerase chain reaction (Saiki et al. 1985) and Sanger sequencing (Sanger et al. 1977). The latter, followed by the automation of the method (Martingallardo et al. 1992), was the catalyst of the ensuing explosion of genomics sequencing data, resulting in complete sequences of initially prokaryotic and simple eukaryotic genomes (Blattner et al. 1997, Fleischmann et al. 1995, Goffeau et al. 1996), then closely followed by several more complex eukaryotic genomes like *Caenorhabditis elegans* (Maupas, 1900, *C. elegans* Sequencing Consortium 1998), *Drosophila melanogaster* (Meigen, 1830, Adams et al. 2000), *Arabidopsis thaliana* (L.) Heynh. (Kaul et al. 2000), and culminating in the publication of the human genome at the turn of the millennium (Lander et al.

2001, Venter et al. 2001). Particularly instrumental in the sequencing of these genomes and gene discovery in organisms with more complex and large genomes is the development and application of the Expressed Sequence Tag (EST) technology in the early 1990s (Adams et al. 1991). ESTs represent random partial sequences of small stretches of complementary DNA (cDNA) (derived from mRNA through reverse transcription) that are typically captured in plasmid vectors constituting what is known as cDNA libraries. The EST technology has been the workhorse of gene discovery in a number of species, including forest trees, for many years (Allona et al. 1998, Hertzberg et al. 2001, Kirst et al. 2003). Because of the complexity of conifer genomes, even for current sequencing capacities, ESTs have allowed gathering of useful genomics data by reducing the complexity and size of the sequencing effort that would have been impossible to afford by a whole-genome approach. ESTs have also proved extremely useful for 'training' the models for gene prediction, following whole-genome sequencing. Early realizations that the *de novo* gene prediction (based purely on sequence inferences) was extremely error-prone made the ESTs an invaluable resource to validate and 'educate' gene prediction algorithms (Martingallardo et al. 1992). Lessons learned from assembly of ESTs also led to the computational approaches used for assembly of whole-genome sequencing using the shotgun approach that is now accepted as a standard for whole-genome sequencing.

The know-how learned during the sequencing of the human genome opened the floodgates for sequencing of an array of other eukaryotic genomes from every nook and cranny of life. Plant genomes were among the first to be sequenced starting with the model *Arabidopsis thaliana* (Kaul et al. 2000), and even preceding the release of the human genome. This was closely followed by the publication of the rice genome (Goff et al. 2002) and ultimately the first tree and third plant genome: this of a poplar tree (*Populus trichocarpa* Torr. & A.Gray – Nisqually1 clone from the Pacific Northwest of the United States) (Tuskan et al. 2006).

## Breaking the Confines of Model Organisms

Despite the significant advances in sequencing and assembly of genomes, these ventures remained confined to single genotypes and species of significance as model organisms or of significant economic, environmental and epidemiological importance. It is not coincidental that the first three sequenced eukaryotic genomes were of such organisms – *Saccharomyces cerevisiae* (Meyen & Hansen, 1883) (yeast), *Caenorhabditis elegans* (nematode), *Drosophila melanogaster* (fruit fly) and *Arabidopsis thaliana* (Arabidopsis, mouse-ear cress). In fact, one of the definitions of a model organism used to encompass (among other attributes) a small and simple genome. The main obstacle in whole-genome sequencing was the prohibitively high cost driven by the relatively low throughput of the traditional Sanger sequencing.

Sanger sequencing is based on dideoxynucleotide chemistries, with one read per reaction. The throughput and ease of Sanger was significantly increased through the introduction of capillary columns and the automation of the process

by Applied Biosystems (Martingallardo et al. 1992), but the basic shortcoming of the process – one read per reaction – remained unchanged and imposed a ceiling to the throughput of this technology. The understanding of this limitation led to the realization that a quantum leap in the process is necessary to meet the ever-increasing glut for sequence information. A massive search for new sequencing technologies resulted in several conceptually new sequencing methods. The majority of these methods employ the concept of massive, parallel sequencing of arrayed DNA fragments simultaneously using various light emitting chemistries (Mardis 2008, Metzker 2010) Thus, instead of sequencing one DNA template per reaction, tens of millions of DNA fragments could now be sequenced at the same time. These technologies are now known as next-generation sequencing (next-gen). The evolution of new sequencing methods is still ongoing. New technologies are coming online every year increasing sequencing accuracy, length and throughput, with seemingly no limits to the further improvement of the process.

The power of these technologies has brought democratization of genomics. Once an attribute and privilege of elite of a few species, today whole genome sequences are available for more than 180 eukaryotic organisms and genomic information is accumulating with unprecedented rate (Pagani et al. 2012). For example, the Joint Genomic Institute (JGI) of the US Department of Energy (DOE) has sequenced more than 40 plant genomes (phytozome.net). All of these are now easily and publicly available at the phytozome.net site, with powerful tools to compare genomics information among various linages of the plant evolutionary tree. In addition, genome sequencing is now expanding from the species to the individual level. A number of projects have been launched to sequence thousands of individual genomes from one species bringing genomics at the population level scales (Altshuler et al. 2012, Cao et al. 2011, Gan et al. 2011). This would enable unprecedented genome-based approaches in breeding, population genetics and conservation.

## Towards Systems Biology Understanding through Various 'Omics' Approaches

The ultimate purpose of genomics is to facilitate functional understanding and thus its eventual usefulness in various fields and endeavors (Fig. 1). As we start to rapidly realize that most biological processes are highly interconnected networks involving large numbers of genes, it is becoming evident that understanding the function of the genome would entail understanding the function of genes *en block*, as a system, rather than as isolated entities. This has spurred interest in what is now known as other 'omics' approaches. Most prominently and following the central dogma, these include transcriptomics, proteomics and metabolomics focusing on RNA, proteins and metabolites respectively.

Transcriptomics involves approaches for analyses of genome-wide gene expression. Methods for studying gene expression have been around for a long time (e.g., Northern blots and RT-PCR). However they are suitable for studying expression of one or a few genes. Monitoring the expression of every single gene in the genome is a daunting task.

The first breakthrough was the development of microarrays (Schena et al. 1995). This technology uses a nucleic hybridization approach. DNA probes, representing portions of the genes in the genome, are arrayed on solid surface. The RNA is converted into cDNA using reverse transcriptase, labeled with fluorescent dyes and, allowed to hybridize to the array, followed by washing to remove unspecific (non-homologous) hybridizations. The amount of fluorescence at each probe is proportional to the labeled cDNA that hybridized to the probe and thus provides an indirect measure of the quantity of the respective mRNA (gene expression) in the sample of interest. The advances of next-generation sequencing are quickly revolutionizing these fields through methods now known as RNA sequencing (RNA-seq, Malone and Oliver 2011, Wang et al. 2009). As with microarrays, mRNA is reverse-transcribed into cDNA, which is then intensively sequenced using one of the above mentioned next-generation platforms. In contrast to the indirect methods of expression estimation using hybridization signal as a surrogate, these methods allow direct counting of transcript abundance and thus truly quantitative estimation of differences in gene expression. Furthermore, microarrays were limited to the number of features on the array and were also contingent on available genome sequence or large EST datasets. RNA-seq does not require either. The sensitivity and inferences of transcriptomics approaches have been greatly enhanced by using highly specific targeted sampling of tissues and even cell types using techniques like laser micro capture (LMC) (Larisch et al. 2012) and fluorescent activated cell sorting (FACS) (Birnbaum et al. 2003).

Proteomics emerged in the late nineties, closely following the rise of genomics and focusing on identification and quantification of protein abundance at a genome-wide scale (James 1997). Proteomics approaches have historically lagged behind transcriptomics due to the technological difficulties of studying proteins in a high throughput fashion. Initially, this was achieved through two-dimensional (2D) electrophoretic separation of protein mixtures, followed by spot isolation and sequencing through proteolytic degradation (Rabilloud and Lelong 2011, Wilm et al. 1996). However, these approaches lack the throughput and resolution that are necessary to scale up the method to levels approaching genome coverage (Vanderschuren et al. 2013). For example, even in the Arabidopsis model plant and most advanced analytical tools, only approximately an estimated 70 % coverage of the whole proteome has been achieved (Mann et al. 2013). Recently, mass spectroscopy using Orbitrap instruments and Multidimensional Protein Identification Technology (MudPIT) has emerged as the leading technology for ‘omics’ level protein analysis (Yates et al. 2009). The results from a controlled proteolysis are analyzed via extremely sensitive liquid chromatography/mass spectroscopy instruments that allow separation of thousands of proteins and accurate sequence assignment based on mass spectroscopic identification using mass and charge characteristics (Yates et al. 2009).

Similar to proteomics, metabolomics involves separation of complex metabolic mixtures by liquid or gas phase chromatographies (LC, GC) followed by mass spectroscopy (MS) or other chemical analytical procedures (Goodacre et al. 2004). These basic techniques have been com-

bined in various configurations to achieve better separation and identification of the analyzed mixtures (Putri et al. 2013). The bottleneck of metabolomics is the poor knowledge about many metabolites which, although well-detected as molecular entities, remain 'unknown' as chemical structures and are thus uninformative with respect to the underlying metabolic mechanisms.

In addition to understanding the various levels of information flows (e.g., DNA-RNA-protein-metabolite), some novel approaches attempt to understand the dynamic nature of DNA, particularly related to its regulation. These include the study of the epigenome where the DNA histone chemical modifications (e.g., methylation, acetylation) patterns are studied on a genome scale using bisulfide sequencing (Frommer et al. 1992) and chromatin immune precipitation (ChIP)-seq technologies (Barski et al. 2007, Mikkelsen et al. 2007). These techniques involve selective isolation of modified DNA variants and their subsequent sequencing using one of the next-gen platforms (Brinkman et al. 2012). This allows mapping of different modifications onto the genome space and associations of modification patterns with various factors (e.g., developmental and physiological phases). Such techniques provide a glimpse into a new type of polymorphisms, one that may act beyond the changes in nucleotide sequences but are rather imprinted through DNA modifications. This allows a more plastic and faster adjustment to rapidly changing conditions that does not require generations and cycles of natural selection to fix/enrich the most adaptive variants. The importance of epigenetic polymorphism to phenotypic diversity and its potential role in plant evolution have been recently shown (Becker et al. 2011, Paszkowski and Grossniklaus 2011, Schmitz et al. 2011). For example, promoter hypermethylation of the FLOWERING WAGENINGEN (FWA) gene in Arabidopsis delays transition to flowering and is maternally expressed likely through genetic imprinting (Fujimoto et al. 2008). Intriguingly, epigenetic states can be environmentally induced and thus could be of significant value to adaptation, particularly under fast-changing conditions (Lang-Mladek et al. 2010). An epigenetic mechanism contributing to fast climatic adaptation and involving microRNAs has been described in spruce (Yakovlev et al. 2010).

The ultimate goal of all these and other 'omics'-scale endeavors is to create the foundations for integrated, comprehensive system-based approaches to understanding biological processes (Long et al. 2008, Weckwerth 2011, Fig. 1). These approaches require new methods and predictive algorithms and models to accommodate the disparate nature of the data streams and the uncertainties about the underlying causative relations and mechanisms (Keurentjes et al. 2011, Saetzler et al. 2011).

## Applications of Genomics in Forestry Research

Advances in genomics have been extremely empowering in many fields of research, including forestry. Early genomics efforts encompassed generation of EST resources in species of economic and environmental significance from the genera *Pinus*, *Picea*, *Eucalyptus*, *Quercus* and *Populus*. These efforts were tailored towards discovery of genes in various regulatory and metabolic processes of interest.

For example an intensive area of research was the wood-forming and particularly the lignin biosynthetic pathway. Although these first genomics projects did not extensively characterize the respective genomes, they provided important information about critical genes and enabled translation approaches based on homologies to model systems like Arabidopsis, where the processes were much better understood (Allona et al. 1998, Kirst et al. 2003). ESTs also empowered the generation of the first cDNA microarrays that allowed simultaneous interrogation of large numbers of genes involved in various processes in trees (Brinker et al. 2004, Hertzberg et al. 2001). The information generated through these EST projects in *Populus* was instrumental in the assembly and annotation of the first poplar tree genome (Tuskan et al. 2006). Finally, ESTs became a rich source for discovery of polymorphic markers that can be used in Quantitative Trait Loci (QTL) and physical mapping research (Liewlaksaneeyanawin et al. 2004, Varshney et al. 2005).

The collection of EST genomic information logically further escalated into whole-genome sequencing. This was first accomplished in *Populus* (Tuskan et al. 2006). The poplar genome was relatively small, well-mapped and abundant EST resources were available. The convergence of these factors allowed its successful sequencing, assembly and annotation. Since the originally published draft, the poplar genome has been updated several times to include some unresolved gaps and incorporate previously unmapped scaffolds (DNA fragments comprising tens of thousands of base pairs) into the genome sequence. The home of the poplar genome is the phytozome.net site, and it includes a genome browser and a rich resource for comparative genomics with other woody and herbaceous plants. The sequencing of the poplar genome is now followed by a number of other whole genome sequencing efforts of forest trees. These include eucalypt, pine and spruce (Mackay et al. 2012, Neale and Savolainen 2004, Nystedt et al. 2013). In addition, several species of trees and vines with importance to the fruit industry, like peach, apple and grapevine, were also sequenced (Jaillon et al. 2007, Velasco et al. 2010, Verde et al. 2013).

The genomics resources generated through these projects are empowering in several ways and have been tailored to facilitate breeding and biotechnological approaches to improve biomass yield, quality and resistance to biotic/abiotic stresses (Brinker et al. 2010, Guerra et al. 2013, Quesada et al. 2010, Ralph et al. 2006, Wegrzyn et al. 2010). Because sequencing projects were funded through public sources, the majority of this data is publicly available through online outlets (Table 1). The primary goal was and still is the detection of polymorphisms that can be used to assist breeding efforts by providing genotypic predictions to phenotypic variability in traits of economic and/or environmental significance. Initially, this was achieved using QTL and more recently association genetics (Brown et al. 2003, Gonzalez-Martinez et al. 2007, Quesada et al. 2010). The genome-wide availability of markers has also spurred interest in applying genome-wide selection approaches (Harfouche et al. 2012, Meuwissen et al. 2001, Resende M.D. et al. 2012, Resende M.F. et al. 2012)

In addition to the purely applied aspects of genomics research in breeding, there has been considerable interest in using genomics information for gaining more

fundamental understanding of processes that are typical for woody perennials and that are difficult or impossible to approach in herbaceous annual systems. These include wood formation, bud dormancy and some symbiotic interactions like mycorhizal associations (Brunner et al. 2004, Jansson and Douglas 2007). These studies are predominantly performed in poplar because of an efficient transformation system, which is not available or is difficult in any of the other trees species with significant genomics resources (Busov et al. 2005a, 2005b). Because of this, poplar has emerged as a 'model' taxon for understanding woody perennial development (Wullschleger et al. 2002). The importance of transformation as a functional tool in poplar has been reviewed elsewhere (Busov et al. 2005a, 2005b; Flachowsky et al. 2009). Briefly, the transformation can generate dominant lesions through using upregulation or gene-silencing mediated suppression of gene activity (e.g., RNAi or amiRNA). These dominant approaches bypass the long generation time and outcrossing system in trees, which makes performing traditional genetics, requiring rounds of selfing or close-relative mating to expose recessive mutations, an impractically long process. Several recent studies have demonstrated the power of these approaches in poplar (Bohlenius et al. 2006, Du et al. 2009, Hsu et al. 2006, Leple et al. 2007, Pilate et al. 2002, Plett et al. 2010, Rohde et al. 2002, Ruttink et al. 2007). In addition to validation function of known and/or candidate genes (reverse genetics), these approaches could also be used to find genes *de novo* through forward genetics by generating a population of T-DNA insertional mutants (Busov et al. 2010; Busov et al. 2005a, 2005b; Busov et al. 2003; Fladung et al. 2004; Fladung and Polak 2012; Harrison et al. 2007; Plett et al. 2010; Yordanov et al. 2010). The availability of genome sequence makes these approaches the fastest and most straightforward path to linking phenotypic to genotypic variation. The presence of known sequence (T-DNA) that affect gene function allows rapid recovery of sequences flanking the T-DNA and thus positioning the lesion in the genome sequence and providing the link between phenotypic changes and their genetic bases. The combination of transgenic manipulations with other 'omics' approaches, like for example transcriptomics, allows unprecedented insight into the regulatory mechanisms that underlie diverse biological processes and traits (Fig. 2).

The use of genomics has also recently been extended outside the traditional genetics area, into the ecology research arena. Genomics approaches in ecology have been known as metagenomics (beyond genomics) (Riesenfeld et al. 2004, Singh et al. 2009, Venter et al. 2004). This is an extremely promising and yet still under-employed area of research. In a nutshell, the presence of DNA from specific organism is indicative of its occurrence in a particular ecosystem (Simon and Daniel 2011). This is particularly interesting in elucidating the structure of microbial communities, which in many instances are impossible to study otherwise because cannot be readily cultured (Schloss and Handelsman 2005) and thus their presence and abundance is difficult to impossible to track with conventional culturing methods. These approaches not only provide estimates of presence/absence but also can quantify the composition, as well as shifts in the microbial communities in response to various experimental factors. Furthermore, RNA-seq (see above) or proteomics approaches applied to the same systems can

go a step further and not only identify the microbes in a sample but also what they are engaged in with respect to, for example, metabolic activities (Carvalhais et al. 2012, Simon and Daniel 2011, Wilmes and Bond 2006). This provides unprecedented insights into the function of the ecosystems at a new level that has been previously nearly ignored because of the lack of tools to approach it. Several recent studies have shown the power of this approach. A study in the root system of *Arabidopsis* plants has shown that specific soil samples drive particular endophytic communities associated with these samples (Lundberg et al. 2012). The development of these communities showed predictable and repeatable patterns, suggesting the existence of established and evolutionary conserved microbial-plant interactions.

## The Road Ahead

One of the grand challenges in the 'post-genomic' area is the integration of all data streams into biological coherent information. This will require novel approaches for analysis, visualization and modeling. Ultimately, these integrative approaches will result with what is now known as virtual plant, best exemplified in the iPlant project (http://www.iplantcollaborative.org/). An even further-reaching project known as KBase (http://kbase.science.energy.gov/) is attempting to extend these integrative approaches to ecosystem scales. This will require novel software and analytical tools but also unprecedented computational power that can accommodate the demands of the analyses.

Enormous technological steps have made genomics, transcriptomics, proteomics and metabolomics approaches that are scalable to full genome scale. In stark contrast, and despite its prominent role in the system biology approaches (Fig. 2), genetics has remained a low throughput and generally a bottleneck. Therefore major technological breakthroughs are needed so that genetics measures to the power and throughput of the other 'omics'

**Table 1. Publicly available genomics resources for various tree species.**

| Species/Genera/Family | Web site |
|---|---|
| Various european conifer and decidious tree species | http://www.evoltree.eu/index.php/e-resources |
| *Quercus* spp. | https://w3.pierroton.inra.fr/QuercusPortal/index.php?p=OAK_GENOME_SEQUENCING |
| *Eucalyptus* spp. | http://web.up.ac.za/eucagen/ |
| Various hardwood tree species of North America | http://www.hardwoodgenomics.org/ |
| Tree species of Fagaceae family | http://www.fagaceae.org/home |
| *Populus* spp. | http://popgenie.org/ |
| *Populus* spp. | http://treesbio.com/ |
| Tree and herabceous species of Rosaceae family | http://www.rosaceae.org/ |
| Various conifer species and *Populus* spp. | http://www.arborea.ulaval.ca/ |
| Various conifer species | http://www.treenomix.ca/ |
| *Pinus taeda* | http://pinegenome.org/pinerefseq/ |
| Various conifer species | http://congenie.org/ |

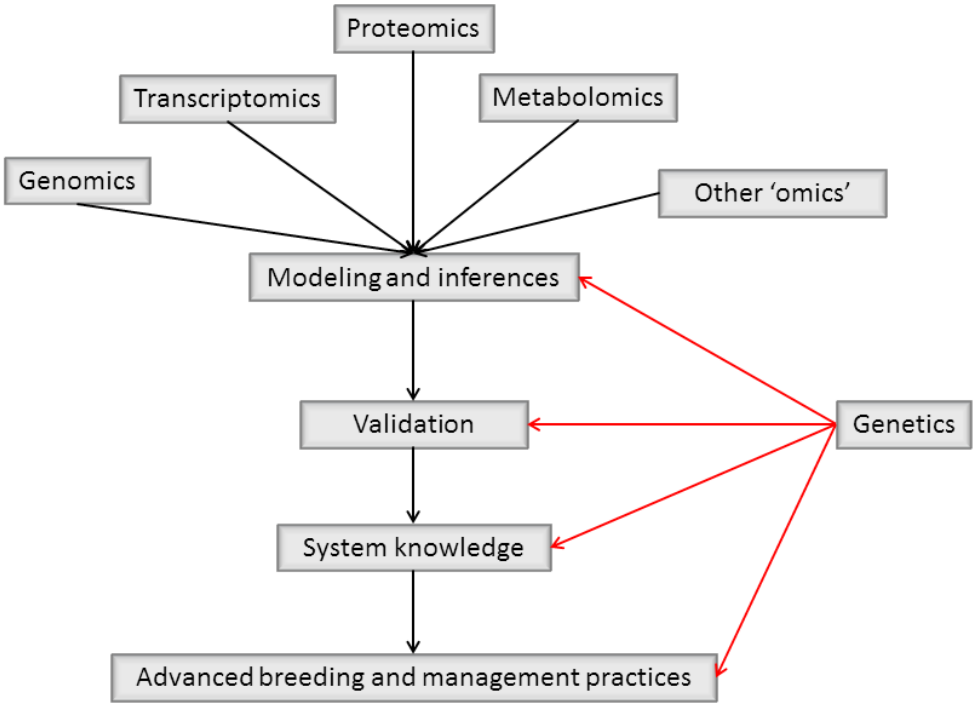


**Fig. 1. Role of 'omics' approaches in generation of system level knowledge about biological systems.**

Note the central place of genetics (indicated by red arrows) at various levels of generating, validating and implementing system knowledge. Genetics indicate both traditional and transgenic approaches for genetic analyses.

technologies. This is particularly important in the generation strong gain and loss-of-function mutations that are most effective in elucidation the gene function. Among plant species, it is only Arabidopsis that has mutagenized populations that approach genome coverage (Alonso et al. 2003). Such populations are not feasible in many species and thus require innovative approaches to generate viable alternatives. Some innovations involving zinc finger nucleases show promise but their utility across many species remains to be proven and are by no means are approaching even moderate throughout scale (Zhang et al. 2010).

Ultimately and most importantly, genomics has to yet decisively enter real mainstream life. Despite the technological democratization, genomics is still a privilege of science laboratories that can afford monetarily and intellectually the demands of such investigations. Although, the cost per base pair has been steadily and dramatically decreasing, these costs are based on high sample volumes requiring significant investments worth thou-

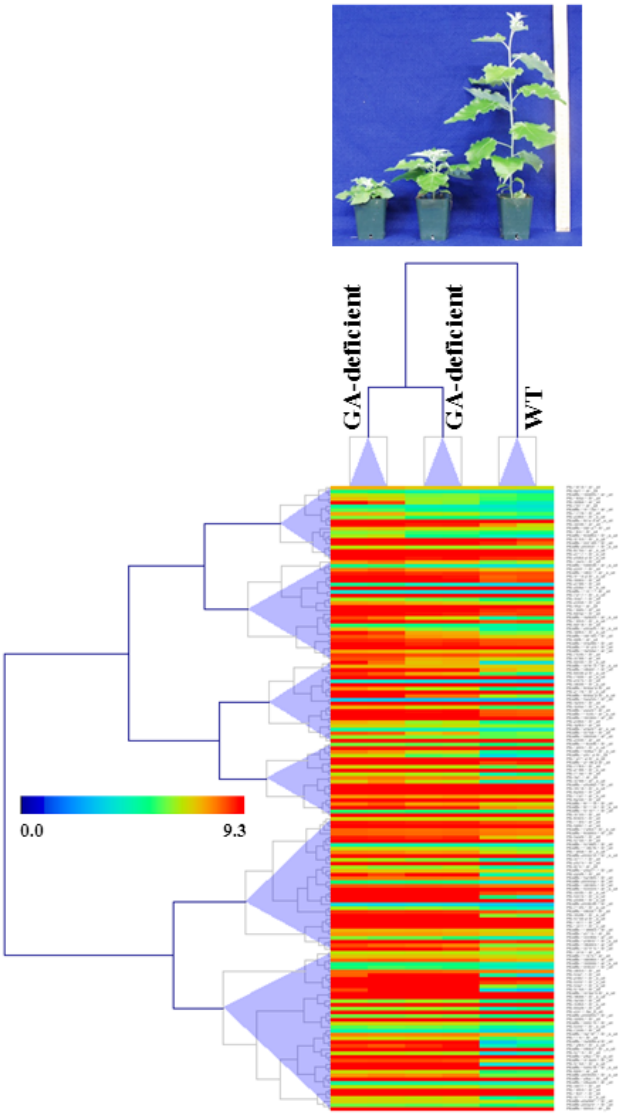


**Fig. 2. Transgenics and 'omics' approaches provide a powerful system for molecular dissection of traits and processes.**

GA-deficient and insensitive transgenic poplars are shown on the picture (Fig. 2). Note the characteristic dwarf phenotype of the GA-modified plants (left and middle) when compared to WT (right). A total of 1600 differentially regulated genes were found in the GA-insensitive and deficient poplars when compared to WT. Generation and characterization of the transgenics was previously described (Busov et al. 2006; Busov et al. 2003; Elias et al. 2012; Gou et al. 2010; Zawaski et al. 2011a, 2011b). The figure shows 168 upregulated

genes in the leaves of the GA-deficient and GA-insensitive poplar transgenics compared to WT. The picture at the top shows representative plants of each genotypic class as specified at the branches of the cluster and positioned above the respective columns showing the differentially regulated genes in each genotype. The figure was generated using hierarchical clustering. Colors indicate levels of expression as shown in the scale bar. Blue highlights indicate groups of genes and/or genotypes. WT=wild type.

sands of dollars. Therefore genomics has yet to become intellectually and economically approachable. This would require better genomics education, data sharing and significant expansion of 'genomics' service industry. In addition, genomics faces significant ethical problems related to intellectual property rights and the patentability of sequence data. Therefore one of the grand challenges in the future is making genomics and other 'omics' approaches intellectually, economically and sociably feasible and acceptable.

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# SPECIES COMPOSITION OF THE ICHTHYOFAUNA OF SOME TRIBUTARIES OF THE MARITZA RIVER

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## Abstract

Study of fish fauna of the rivers Topolnitsa, Luda Yana, Stryama, Chepinska and Vucha all tributaries of the Maritza River was carried out. The research was conducted in the autumn of 2006 and 2007. The material was collected by electrofishing using unpuled direct current (DC). In the studied rivers the 11 sampling areas were marked and explored. During the investigation in these rivers 17 fish species belonging to 5 families were found. The family Cyprinidae was the most representative in the sample. The fish fauna composition was predominated by two reofilic species – *Barbus cyclolepis* and *Squalius orpheus*. Among the species found in this study there was three alien fishes for the local fish fauna composition were found – *Oncorhycus mykiss, Lepomis gibbosus* and *Pseudorazbora parva.* The present study identified four species endemic to Aegean watershed – *Gobio bulgaricus*, *Chondrostoma vardarense*, *Squalius orpheus* and *Vimba melanops*. The author was found also two species endemic to Balkan Peninsula – *Cobitis strumicae* and *Sabanejewia balcanica* and one species endemic to Maritza River basin – *B. cyclolepis*.

The estimated composition of the fish fauna in this study was composed of species characteristic mainly for the middle zone of the rivers. The present study showed some changes in the species composition of fish fauna have occurred in recent years. The species like *G. bulgaricus* which were prevalent before today are found in much smaller quantities. At the same time in the fish fauna composition the number of the species such as *Lepomis gibbosus, Pseudorazbora parva, Carassius gibelio* and *Perca fluviatilis* have increased. The author found some rarer species like *Rhodeus amarus* while the species that was common before as *Carassius carassius* was not established.

**Key words:** Maritza (Evros/Meriç) River basin, ichthyofauna, species composition.

## Introduction

Among the inland rivers in Bulgaria Maritza River has the largest catchment area of up to 21,100 km$^2$ to the Bulgarian-Greek border (Tsatchev et al. 1977). The Maritza catchment area is entirely in the south of the main watershed of Bulgaria – Stara Planina Mountains. The ichthyofauna of Maritza River is different from the one of Northern Bulgaria and includes some endemic species for Southern Bulgaria (Chichkoff 1935, Heckel 1837, Kottelat and Economidis 2006). The majority of the Maritza River runoff is formed by tributaries located in Sredna Gora Mountains as well as Rhodopes Mountains.

The first partial information on the species composition of the River Maritza's ichthyofauna was reported by Heck-

el in 1837. After examining material from the Maritza River Heckel listed 17 fish species belonging to 5 families (Table 3). Particularly valuable in this publication was the first description of the barbel from the Maritza River. Kovatcheff (1921) has published the results of a survey of the ichthyofauna of the Maritza River, conducted in the period 1914–1915, and reported 25 fish species belonging to 11 families, among which there was also a representative of the family Petromyzonidae. The next available data on ichthyofauna of the Maritza River and its tributaries fishfauna composition were reported by Drensky (1928, 1930, 1951) and Morov (1931). In 1935 Chichkoff published an overview of fish fauna of the Aegean watershed with particular attention to the ichthyofauna of Maritza River.The author mentioned the presence of 26 fish species for Maritza River from 9 families, including species living in fresh and marine water as *Acipenser sturio* (Linnaeus, 1758), *Proterorhinus marmoratus* (Pallas, 1814) and *Platichthys flesus* (Linnaeus, 1758).

A three year investigation on the fish fauna of the whole course of the Maritza River was carried out by Mihaylova (1965). The material for this study was collected by sweep-nets and fishing rods. The author explored 970 specimens of fish from the main stream of the Maritza River and from its tributaries, including rivers Toponitsa, Luda Jana and Chepinska. Mihaylova (1965) established 18 fishes belonging to 8 families. She has shown the proportion of each species in the sample (Table 3). The largest share in her sample had *B. cyclolepis*, followed by *Gobio bulgaricus*. As the number of individuals in the Mihaylova's (1965) sample *Sq. orpheus* ranked third. The amount of captured *B. cyclolepis, G. bulgaricus* and *Sq. orpheus*, taken together, constituted over 50 % of the total catch cited in this study. In the sample from the Maritza River between towns Septemvry and Pazardzhik and from the Maritza's tributaries Toponitsa, Luda Jana and Chepinska Mihaylova (1965) established even predominance of *G. bulgaricus* in front of *B. cyclolepis* and *Sq. orpheus*. Since the author has explored all along the Maritza River in Bulgaria, in its data presented species typical of the lower area as *Esox lucius, Silurus glanis, Sander lucioperca* and *Tinca tinca*. She recorded also the presence of two non native species *Onc. mykiss* and *Gambusia holbrooki* in Maritza River. The author mentioned that *G. holbrooki* was introduced in the Maritza River near the town of Pazardzhik to combat mosquitoes. Mihaylova (1965) indicated that the species composition of the ichthyofauna of the Maritza River was significantly impoverished compared to the 30$^{s}$ of the twentieth century. The author pointed out that there were no species in the catch: *Eudontomyzon* sp., *Acipenser sturio, Barbus barbus, Abramis brama Sabanejewia balcanica* and *Platichthys flesus*, mentioned before that.

Dikov et al. (1994) published the results of a survey of fish stocks in inland rivers of Bulgaria. They reported data on the Arda River, a tributary of the lower course of the Maritza River. Authors calculated the average abundance and biomass of the four species – *G. bulgaricus, B. cyclolepis, Phoxinus phoxinus* and *Sq. orpheus* in the Arda River. Velcheva and Mehterov (2005). a study on the fish fauna of the Maritza River. They found 18 different species from 6 families, one of them non native.

Stefanov and Trichkova (2006) made an overview of the species composition of the fish fauna of the Rhodope Mountains. They cited the results of the previous studies on the ichthyofauna composition of some tributaries of the Maritza River. In this publication authors sowed also the results of there investigation on the fish fauna composition of some reservoirs in the Maritza River basin: Dospat, Beglica, Golyam Beglik, Toshkov Chark, Shiroka Polyana, Teshel, Vucha and Batak. They explored the fish fauna composition also in the upper and middle zone of the rivers: Dospat, Trigradska, Devinska and Vucha. Stefanov and Trichkova (2006) reported two imported species *Huho huho* and *Salmo salar* in the reservoirs: Dospat, Golyam Beglik and Batak. According to the authors in rivers and reservoirs in the basin of the Maritza River found a total of 19 fish species from the 7 families. One of this species was catadromus *Anguilla anguilla* and four of them were non native for the Maritza River basin – *Lepomis gibbosus, Onc. mykiss, H. huho* and *S. salar*.

Partial information of the species composition of the fishfauna of Maritza River were reported also by Zashev (1961), Maitland (1987), Marinov (1989), Karapetkova and Zivkov (1995), Zivkov and Dobrev (2001), Kottelat and Freyhof (2007).

Studied in the present researches rivers were under strong anthropogenic influence. In all those rivers was identified pollution from domestic sewage water and industrial water (Mihaylova 1965, Tsatchev et al. 1977). Also, many dams were built along the Maritza River. Our study area contained six dams – three on the watercourse of the Topolnitsa River and three on the Vucha River. In the upper area of many of the tributaries of the Maritza River water abstractions are built. Such abstractions are built also on water courses of the rivers Luda Yana and Stryama.

## Purpose and Objectives

The aim of this study was to investigate the current status of the fish fauna of the rivers Topolnitsa, Luda Yana, Stryama, Chepinska and Vucha from the middle part of the basin of the Maritza River.

For this task it was necessary to establish:

– species composition of fish fauna, including the presence of endemic species, introduced and invasive species in the studied rivers;
– percentage composition of fish fauna;
– most represented species in number.

## Materials and Methods

The study area included five tributaries of Maritza River: Topolnitsa, Luda Yana and Stryama from the Sredna Gora Mountains as well as Yadenitsa, Chepinska and Vucha in the Rhodope Mountains (Figure 1). The study was focused on reofilic fish communities.

Topolnitsa River is a left tributary of the Maritza River with total length 154.8 km. Topolnitsa River is formed by the confluence of the stream of Shirine and Kriva, which sprigs from the peaks Bunaya and Bogdan, in Sredna Gora Mountains. The catchment area of Topolnitsa River is 1,789 km$^2$. There are three dams: Dushantsi, Zhekov Vir and Topolnitsa. Topolnica River flows into Maritza River west of the town of Pazardzhik.

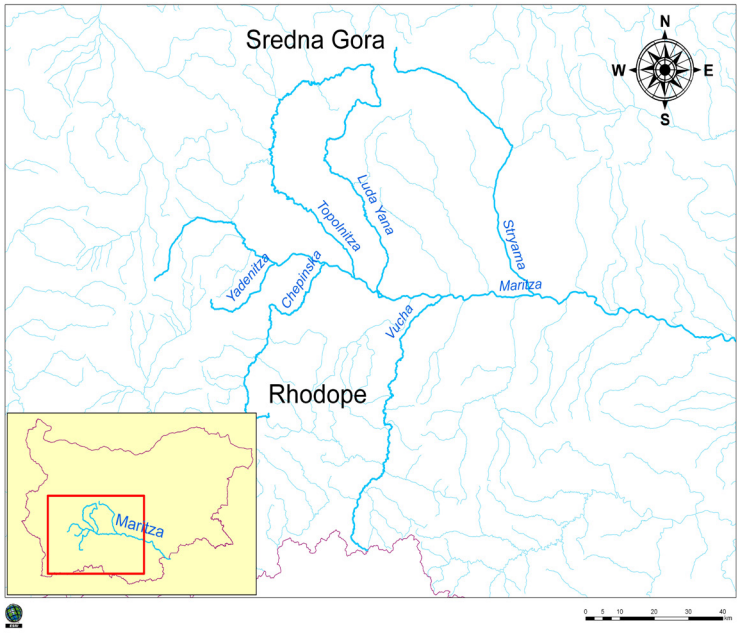


**Fig. 1. Location of studied rivers: Maritza River; Topolnitsa River; Luda Jana River; Sryama River; Chepinska River; Vucha River, Arc Map 10.0 (ESRI).**

The Luda Yana River sprins from Bich Peak of Sredna Gora Mountains. It is also a left tributary of Maritza River. The length of Luda Yana River is 74 km and its catchment area is 685.3 km$^2$. Near the village Sinitovo it flows into Maritza River.

Stryama River springs east of peak Vezhen in the Middle Stara Planina Mountains and flows between Mountains: Stara Planina, Sredna Gora and Sarnena Gora. Stryama is a left tributary of the Maritza River. Its length is 110 km with catchment area of 1,789 km$^2$. The river flows into Maritza River near the village of Manole.

Yadenitsa River is a right tributary of the Maritza River originated from the saddle of Yundola. The River runs through the deep carved valley and forming the boundary between the mountains of Rila and Rhodopes. Its length is 26 km and its catchment area 137.9 km$^2$. Near the town of Belovo the Yadenitsa River flows into Maritza River.

Chepinska River is a tributary of Maritza River, which flows from peak Mala Syutkya in Rhodope Mountains. The length of this river is 83 km. Its catchment area is 899.6 km$^2$. Near the village Zlokuchane Chepinska River flows into Maritza River.

Vucha River is a tributary of Maritza River springs from Rhodopes Moutains. The river originates from stream Buynovska. Vucha River is 111.5 km long. Its catchment area is 1,645 km$^2$. Vucha River flows into Maritza River west of the village of Kadievo. The Vucha River water quantity is strongly depends on stunts of Dospat-Teshel-Devin-Vucha.

The study material was collected by electrofishing. The electrofishing was conducted with unpuled direct current (DC). We used the backpack electrofisher SAMUS 725G – (Samus special electron-

ics, Poland), powered by a 12 V accumulator battery with 75 Ah capacity. The electrofisher converter provides DC impulses with frequency ranged between 5 and 100 Hz, duration 0.03–3 ms and maximum power of 650 W. The electrofisher is suitable for water resistance from 25 to 1000 Ω. The amperage in load condition is from 5 to 65 A. The collection of the material was made most commonly using operation U1, where the Output Voltage was 640 V, the used Output Frequency was of 50 Hz and Output Power reached to 200 W.

**Table 1. Sampling areas in rivers of Topolnitsa, Luda Yana, Stryama, Chepinska and Vucha.**

| No | Date | River | N | E | Altitude | Location |
|---|---|---|---|---|---|---|
| 1 | 29.10.2006 | Topolnitsa | 42°12'25,24'' | 24°17'44,77'' | 214 m | Near the bridge on the road Pzardzhik-Septemvry |
| 2 | 04.11.2006 | Topolnitsa | 42°21'34,87'' | 24°05'44,22'' | 288 m | Near the village of Lesichevo |
| 3 | 05.11.2006 | Topolnitsa | 42°24'39,77'' | 23°59'55,44'' | 346 m | Near the village of Muhovo below the dam of Topolnitsa |
| 1 | 05.11.2006 | Topolnitsa | 42°12'25,24'' | 24°17'44,77'' | 214 m | Near the bridge on the road Pzardzhik-Septemvry |
| 4 | 10.11.2006 | Luda Yana | 42°11'39,43'' | 24°23'55,35'' | 209 m | Near the bridge on the road Pzardzhik-Plovdiv |
| 5 | 10.11.2006 | Luda Yana | 42°16'24,63'' | 24°23'25,95'' | 235 m | Near the village of Chernogorovo next to the road bridge |
| 6 | 16.11.2006 | Srtyama | 42°15'07,29'' | 24°50'21,05'' | 174 m | Near the bridge of the road Plovdiv-Rakovski |
| 7 | 17.11.2006 | Stryama | 42°33'39,11'' | 24°47'45,81'' | 302 m | Near the town of Banya next to the fish farm |
| 8 | 27.11.2006 | Chepinska | 42°11'09,67'' | 24°09'45,07'' | 240 m | Near the bridge of the road Belovo-Lozen |
| 8 | 04.10.2007 | Chepinska | 42°11'09,67'' | 24°09'45,07'' | 240 m | Near the bridge on the road Belovo-Lozen |
| 9 | 04.10.2007 | Chepinska | 42°07'42,78'' | 24°07'21,71'' | 417 m | Near the spa baths of the village of Varvara |
| 8 | 05.10.2007 | Chepinska | 42°11'09,67'' | 24°09'45,07'' | 240 m | Near the bridge on the road Belovo-Lozen |
| 10 | 12.10.2007 | Vucha | 42°06'59,28'' | 24°33'06,89'' | 182 m | Near the village of Yoakim Gruevo |
| 11 | 12.10.2007 | Vucha | 42°01'25,87'' | 24°28'22,49'' | 209 m | Near the town of Krichim |
| 4 | 13.10.2007 | Luda Yana | 42°11'39,43'' | 24°23'55,35'' | 209 m | Near the bridge on the road Pzardzhik-Plovdiv |

Fish sampling was undertaken in the autumn of the 2006 and 2007. The fish were collected from 11 sampling areas (Table 1). From every sampling area more than 100 individuals were caught. The protected fish species were returned alive back into the water. The rest of the material was used for the author's investigations, not showed in the present work.

The identification of the species was made according to Kottelat and Freyhof (2007). The classification of families was made according to Kottelat and Freyhof (2007). The percentage composition of fish species in the studied rivers (Table 2) was calculated on the basis of total material, collected in the period 2006–2007.

## Results

From the studied rivers 3880 fish were caught in the period 2006–2007. 17 species belonging to 5 families were recorded (Table 3).

**Table 2. Present data of the fish fauna composition of rivers Topolnitsa, Luda Yana, Stryama, Chepinska and Vucha.**

| No | Family and species | Fishes found in present study | Fishes described in the literature | First reference for the Maritza River | Percentage composition of fish species | |
|---|---|---|---|---|---|---|
| | | | | | Present study, 2006–2007 | Michaylova, 1965 |
| | **Fam. Petromyzonidae** | | | | | |
| 1 | *Eudontomyzon* sp. | – | + | Kovatcheff, 1921 | – | – |
| | **Fam. Acipenseridae** | | | | | |
| 2 | Acipenser sturio | – | + | Kovatcheff, 1921 | – | – |
| | **Fam. Anguillidae** | | | | | |
| 3 | Anguilla anguilla | – | + | Kovatcheff, 1921 | – | – |
| | **Fam. Cyprinidae** | | | | | |
| 4 | Rhodeus amarus | + | + | Heckel, 1837 | 0.00 | 6.91 |
| 5 | Gobio bulgaricus | + | + | Drensky, 1930 | 1.19 | 18.24 |
| 6 | Pseudorasbora parva | + | + | Karapetkova & Živkov, 1995 | 0.75 | – |
| 7 | Barbus barbus | – | + | Kovatcheff, 1921 | – | |
| 8 | Barbus cyclolepis | + | + | Heckel, 1837 | 52.84 | 22.37 |
| 9 | Barbus petenyi | – | + | Kovatcheff, 1921 | – | – |
| 10 | Carassius carassius | – | + | Heckel, 1837 | – | – |
| 11 | Carassius gibelio | + | + | Drensky, 1930 | 0.72 | – |
| 12 | Cyprinus carpio | – | + | Heckel, 1837 | – | – |
| 13 | Abramis brama | – | + | Morov, 1931 | – | – |
| 14 | Alburnus alburnus | + | + | Kovatcheff, 1921 | 1.88 | 10.52 |
| 15 | Aspius aspius | – | + | Kovatcheff, 1921 | – | – |
| 16 | Chondrostoma vardarense | + | + | Drensky, 1930 | 0.77 | 2.47 |
| 17 | Phoxinus phoxinus | + | + | Kovatcheff, 1921 | 0.21 | 2.16 |
| 18 | Rutilus rutilus | + | + | Heckel, 1837 | 2.84 | 0.52 |
| 19 | Scardinius erythrophthalmus | – | + | Heckel, 1837 | – | 1.95 |
| 20 | Squalius orpheus | + | + | Kovatcheff, 1921 | 34.90 | 16.70 |
| 21 | Vimba melanops | + | + | Heckel, 1837 | 0.30 | 3.60 |
| 22 | Tinca tinca | – | + | Kovatcheff, 1921 | – | 0.10 |
| | **Fam. Cobitidae** | | | | | |
| 23 | Cobitis strumicae | + | + | Drensky, 1930 | 1.75 | 4.54 |
| 24 | Sabanejewia balcanica | + | + | Drensky, 1930 | 0.03 | – |
| | **Fam. Siluridae** | | | | | |
| 25 | Silurus glanis | – | + | Chichkoff, 1935 | – | 0.62 |
| | **Fam. Esocidae** | | | | | |
| 26 | Esox lucius | – | + | Heckel, 1837 | – | 1.24 |
| | **Fam. Salmonidae** | | | | | |
| 27 | Oncorhyncus mykiss | + | + | Mihaylova, 1965 | 0.05 | 0.10 |
| 28 | *Salmo* sp. | + | + | Kovatcheff, 1921 | 0.23 | – |
| | **Fam. Poecilidae** | | | | | |
| 29 | Gambisia holbrooki | – | + | Mihaylova, 1965 | – | 7.22 |
| | **Fam. Centrarchidae** | | | | | |
| 30 | Lepomis gibbosus | + | + | Karapetkova & Živkov, 1995 | 0.82 | – |
| | **Fam. Percidae** | | | | | |
| 31 | Perca fluviatilis | + | + | Heckel, 1837 | 0.72 | – |
| 32 | Sander lucioperca | – | + | Kovatcheff, 1921 | – | 0.21 |
| | **Fam. Cobiidae** | | | | | |
| 33 | Proterorhinus marmoratus | – | + | Kovatcheff, 1921 | – | 0.52 |
| | **Fam. Pleuronectidae** | | | | | |
| 34 | Platichthys flesus | – | + | Kovatcheff, 1921 | – | – |
| | **Total** | | | | **100.00** | **100.00** |

**Table 3. Distribution of fish species in rivers of Topolnitsa, Luda Yana, Stryama, Chepinska and Vucha during the period of 2006–2007.**

| No | Species | Presence/Absence | | | | |
|---|---|---|---|---|---|---|
| | | T | LY | Str | Ch | Vch |
| | **Fam. Cyprinidae** | | | | | |
| 1 | *Rhodeus amarus* Bloch, 1782 | – | – | – | + | – |
| 2 | *Gobio bulgaricus* Drensky, 1926 | – | + | + | + | + |
| 3 | *Pseudorasbora parva* Temminck & Schlegel, 1846 | + | + | – | – | – |
| 4 | *Barbus cyclolepis* Heckel, 1837 | + | + | + | + | + |
| 5 | *Carassius gibelio* Bloch,1782 | + | + | – | + | – |
| 6 | *Alburnus alburnus* Linnaeus, 1758 | + | + | + | + | – |
| 7 | *Chondrostoma vardarense* Karaman, 1928 | + | – | + | + | – |
| 8 | *Phoxinus phoxinus* Linnaeus, 1758 | + | – | + | – | – |
| 9 | *Rutilus rutilus* Linaeus, 1758 | + | + | + | + | – |
| 10 | *Squalius orpheus* Kottelat & Economidis, 2006 | + | + | + | + | + |
| 11 | *Vimba melanops* Heckel, 1837 | + | – | + | + | – |
| | **Fam. Cobitidae** | | | | | |
| 12 | *Cobitis strumicae* Karaman, 1955 | + | + | + | + | + |
| 13 | *Sabanejewia balcanica* Karaman, 1922 | + | – | + | – | – |
| | **Fam. Salmonidae** | | | | | |
| 14 | *Oncorhyncus mykiss* Walbalm, 1792 | – | – | + | + | – |
| 15 | *Salmo* sp. | + | + | + | + | + |
| | **Fam. Centrarchidae** | | | | | |
| 16 | *Lepomis gibbosus* Linnaeus, 1758 | + | + | + | + | – |
| | **Fam. Percidae** | | | | | |
| 17 | *Perca fluviatilis* Linnaeus, 1758 | – | + | + | + | + |

Legend: + = presence, – = absence, T = Topolnitsa; LY = Luda Yana; St = Stryama; Ch = Chepinska; Vch = Vucha.

The most representative family in the studied rivers was the cyprinid family, including 11 species. The remaining families were represented with only one or two species of fish.

Pursuant to classification of Kottelat and Freyhof (2007) four of the identified species – *G. bulgaricus*, *Chondrostoma vardarense*, *Sq*. *orpheus* and *Vimba melanops* are endemic to Aegean watershed, two of them – *Cobitis strumicae* and *Sabanejewia balcanica* are endemic to Balkan peninsula and one species – *B*. *cyclolepis* is endemic to Maritza River basin.

In the investigated rivers one non native species – *On*. *mykiss* and two invasive species – *Ps*. *parva* and *L*. *gibbosus* were found.

*B*. *cyclolepis* predominated with 54.8 % of the sample, followed by *Sq*. *orpheus* with 34.9 % (Table 2), both of them are native and reofilic.

## Discussion

This study found that the species composition of fish fauna of the studied tributer-

ies of the Maritza River – Topolnitsa, Luda Yana, Stryama, Chepinska and Vucha, was predominated by *B*. *cyclolepis* and *Sq*. *orpheus* (Table 3), two species typical of middle zone.

Reconciliation of the species composition of the catch in this study with other authors (Michaylova 1965, Dikov et al. 1994) showed that the *B*. *cyclolepis* was among the dominant species in fish community all along the Maritza River (Table 3). This study and the data of Michaylova (1965), showed that fish community from Maritza River basin, were dominated by the fishes of the family Cyprinidae, which were most numerous.

In the sample area over 400 m altitude in the gorge of the river Chepinska, where the rate of water flow is greater and where the riverbed is covered with stones, the ichthyofauna was represented almost exclusively by *B*. *cyclolepis*. At the bottom of the river, near the confluence in to the Maritza River, where there is an accumulation of sand and organic, the composition of fish fauna was very diverse and was dominated by *Sq*. *orpheus*.

The present study showed that in rivers Topolnitsa, Luda Yana, Stryama, Chepinska and Vucha the number of *G. bulgaricus* decreased greatly. According to Mihalova (1965) *G*. *bulgaricus* was one of the most numerous species in the Maritza River basin. In the present study this species accounted for only 1 % of the fish composition. *G*. *bulgaricus* was even absent in the Topolnitsa River (Table 3), one of the most polluted river in the Maritza River basin (Tsatchev et al. 1977). As the species of the genus Gobio are sensitive to water pollution was very likely that it was the main cause of reducing the number of *G*. *bulgaricus* in studied rivers over the past decades.

*Rhodeus amarus* was recorded only in Chepinska River (Table 3). The habitat is characterized by almost no water current, a sand-gravel bottom and availability of necessary mussels of the genus Unio.

The author established *Carassius gibelio* in the tree of five studied rivers (Table 3). However will merely be noted that *C*. *gibelio* was found only near the confluence of the tributaries to the main course of the Maritza River. In previous investigation *C*. *gibelio* was found in the Maritza River only by Velcheva and Mehterov (2005). Stefanov and Trichkova (2006) reported the species for the reservoirs: Dospat, Beglica, Golyam Beglik, Toshkov Chark, Teshel, Shiroka Polyana and Vucha, situated in the watershed of the Maritza River.

*Carassius carassius* mentioned by some authors before (Chichkoff 1935, Velcheva and Mehterov 2005) (Table 2) now was not detected in this study confirming the claim (Zivkov and Dobrev 2001) that the species occurs much less frequently in recent years.

It should be noted that two reofilic species – *Ch*. *vardarense* and *V*. *melanops*, found in large quantities in the midle area of the rivers Mesta and Struma (Apostolou 2005, Apostolou et al. 2010) in most of surveyed tributaries of Maritza River were presented in very low quantity (Table 3). Both fish species were caught only in the lower area of the tributaries just before their infusion into Maritza River. The small number of both species was determined by hydrological particularity of the studied watercourses: the speed of water flow, shallow, covered with rocks and sand riverbed, lack of submerged vegetation in the water. On the other hand these species were absent in the Luda Yana and Vucha, whose flow rate is very low and variable in summer.

Of all studied rivers Vucha is characterized by the poorest species composition of the fish fauna. Frequent changes in the flow of the river Vucha, resulting from the operation of stunts of Dospat-Vucha and Devin-Teshel probably had an effect and its fish fauna.

The cited data and the present data showed that a widespread species in the rivers Struma and Mesta (Apostolou et al. 2010) *Alburnoides bipunctatus* was not found in the rivers of the watershed of the Maritza River.

According to Karapetkova and Zivkov (1995) and Stefanov (2007) *Onc*. *mykiss* have been introduced in Bulgaria in 1934. In the rivers of Stryama and Chepinska. *On*. *mykiss* were caught in areas near fishfarms, where the fish most likely escaped from (Table 3).

As per to Golemanski and Bozkov (2003) *Ps. parva* was recorded for the first time in Bulgaria in 1977 in the fish farm near the village of Mechka, Russe region, while *L. gibbosus* was found for the first in Bulgaria in the marsh of the town of Svishtov in 1920. Both species have a proven negative effect on local fish fauna (Karapetkova and Zivkov 1995; Zivkov and Dobrev 2001). The two species was first reported in the research of Velcheva and Mehterov (2005) (Table 2). In the present study *L*. *gibbosus* and *Ps*. *parva* were found in the lower part of the tributaries before inflowing into Maritza River. *L*. *gibbosus* was present in all studied rivers with the exception of Vucha (Table 3). *L*. *gibbosus* was also reported by Stefanov and Trichkova (2006). Probably the two species *L*. *gibbosus* and *Ps*. *parva* have been in widespread in the studied rivers of the Maritza River basin in recent decades. *L*. *gibbosus* however, had a wider distribution.

*C*. *strumicae* was first reported by Heckel (1937) quent and almost all cited authors (Table 2). Obviously, the species was widespread throughout the basin of the Maritza River. In the present study a large number of *C*. *strumicae* was established in all studied rivers whereas *S*. *balcanica* was more rarely found. The author caught a few speciments of *S*. *balcanica* in the rivers Stryama and Topolnitsa (Table 3). In the rivers of the watershed of Rhodope Mountains *S*. *balcanica* was not established. Kottelat and Freyhof (2007) considered that *C*. *strumicae* usually associated with fine substrate, while *S*. *balcanica* prefers gravel bottom, this is there are more places with sandy and muddy bottom in the studied rivers. In the Maritza River *S*. *balcanica* was first reported by Drensky (1930) and later by Chichkoff (1935) (Table 2). Absence of *S*. *balcanica* in the later study of Mihailova (1965) perhaps was due to the way of collecting material for this study (sweep-nets and fishing rods), the tendency of species to be buried in the substrate (Kottelat and Freyhof 2007) and at its lower number (Table 2).

In the present study *Perca fluviqtilis* was established in all most of studied rivers (Table 3) near the infusion in the Maritza River. It should be noted that in many of the earlier studies this species have not been registered (Kovatcheff 1921, Michaylova 1965, Velcheva and Mehterov 2005). It was likely that the distribution of *P*. *fluviqtilis* have not been very widely as it is now.

The estimated composition of the fish fauna in this study was predominated of species characteristic mainly for the middle zone of the rivers. In the current study were not found species such as the *Acipenser sturio*, *Silurus glanis*, *Sander lucioperca*, *Tinca tinca*, *Esox lucius*, *Aspius aspius*, characteristic of the lower

zone and reported by other authors for the Maritsa River.

## Conclusions

The cyprinid family was the most representative in the species composition in rivers Topolnitsa, Luda Yana, Stryama, Chepinska and Vucha fishfauna.

The ichthyofauna of the rivers Topolnitsa, Luda Yana, Stryama, Chepinska and Vucha is predominated by two species: *B. cyclolepis* and *S. orpheus*.

Invasive species *L. gibbosus* was widespread in the rivers Topolnitsa, Luda Yana, Stryama and Chepinska.

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# RATING AND MAPPING FIRE HAZARD IN THE HARDWOOD HYRCANIAN FORESTS USING GIS AND EXPERT CHOICE SOFTWARE

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## Abstract

Fire can be a destructive ecological factor, but a good plan can provide appropriate tools for ecosystem management. In this connection fire prevention must be paid special attention. For locating fire susceptible zones as a first step in this research, effective factors for forest fires were determined by studying different sources, and then we used weighed criteria to take advantage of experts' views. Maps of slope gradient, exposure, forest type, hydrography, topography, distance from roads and distance from settlements were studied. Results from the survey show that vegetation coverage allocates the greatest weight to itself. From the results it can be seen that, 11.7 % of the area is too adverse; it means that fire hazard is too high in these areas, 40.68 % is adverse, 14.48 % is average, 8.37 % is quite favorable, 18.72 % is favorable, and 6.05 % is very good. In general, we can say half of the local area is susceptible to forest fire, and we should plan the access to different areas for controlling probable fires, regarding the existing facility conditions and roads.

**Key words:** spatial distribution, forest fire, fire prevention, fire hazard factors, fire risk management.

## Introduction

Forest fires cause many negative effects in various aspects of life such as natural environment, economics and health (Herawati et al. 2006).

Fire can be a destructive ecological factor, but a good plan can provide appropriate tools for ecosystem management. In this connection fire prevention must be paid special attention. People can play an important role in forest fire reduction by their cultural education concerning forest fire prevention, appropriate management, providing necessary facilities for fire fighting, forest protection during critical seasons by means of monitoring by trained people, and use of new tools such as remote sensing and GIS systems (Farahi et al. 2011).

Darmawan et al. (2001) integrated remote sensing techniques with GIS to create a model of forest fire hazard in East Kalimantan, Indonesia. Moreover, a forest fires risk model for West Kutai District in East Kalimantan Province, Indonesia

was developed by Hadi (2008) using GIS, remote sensing and Multi-criteria Analysis (MCA).

Currently, data mining techniques are also applied in modeling forest fire risk. Stojanova et al. (2006) built predictive models based on geographic data, meteorological ALADIN data and MODIS satellite data.

The rate of change in communities after fires is influenced by severity, destruction power, fire period and season (Wright and Bailey 1982), also other factors such as raining cycle (Moore et al. 2006), and "why" patterns. Fires can influence natural ecosystems by vegetation destruction, changing sequence patterns, changing vegetation sources such as timber, branch, leaves, and wildlife habitat (Debano et al. 1998). Most of the world forests with different climates have experienced fires with different destruction power. The return time in temperate deciduous forests varies from decades (or less) to centuries (or more) (Sanford et al. 1985). Fires in the northern part of Iran are mainly surface ones and their flame height rarely exceeds 10–30 cm at normal fuel and humidity conditions. Annually 300 to 400 hectares of Iran north forests burn (Banj Shafiei et al. 2010). Having information about the natural fire effects can make us increase our understanding of the impact of fires on forests and help us make management decisions (Laughlin et al. 2004).

There are two main reasons for each fire: a natural reason, an unnatural reason (Naebi 2003). Fire is a natural power that affects vegetation communities over time and as a natural process has a significant effect on preserving special ecosystems health. Since twentieth century, increasing fires due to human activities turned fires to a major threat for forests (Nasi et al. 2002). Forest fires are started mainly by human factors, inattention and ignorant people.

In European countries, fires are one of the main causes for forest destruction, so that in Southern Europe 10 billion hectares of forests have been ruined by fires for the last two decades. An important factor that leads to fires over different areas is the road development. Besides, passengers' and workers' inattention imposes irreversible damage to forests. In 2005 the International Food and Agriculture Organization reported that 0.06 % of Iran forests burn every year. FAO (1998) reported that in an 18-year period up to 1998, on average 42,100 arsons had been committed every year in Europe and the annual average of burnt areas is estimated to be 2 billion hectares. Amin Amlashi et al. (2010) reported that in the period 1977–1979 the large fires in the forests of Gilan were 37 and 285 hectares of the forests of Gilan were burnt as a result. According to statistical reports from 1993 to 2010 a total of 711 arson fires occurred in the forests and pastures of the Gilan province, 2063 ha of forests and 114 ha of pastures, and a total area of 2717 ha have been affected by fire (Department of Natural Resources, Gilan province 2010). The development of various models based on simulation and mathematics in developing countries such as Iran and Turkey should be considered, because traditional methods are now slowly fading and other ancient philosophies are less fit to the current world situation (Zeki and Keles 2005). That is why much research in the field with predicted fire behavior modeling has been performed in GIS environment (Giglio et al. 2006). In connection with forest fires, remote sensing can provide useful information about the environment before and after fires. Particularly widely used are the techniques for monitoring of current fires

(Roy et al. 1999). In this study we intend to examine and analyze the spatial distribution and characteristics of fires in Gilan, in order to identify areas prone to fires and by reviewing the process and its cycling to provide the necessary steps to deal with it.

## Materials and Methods

The study area of 3559 hectare is located within the watershed number 7 in Northern Iran (Fig. 1). This forest is located between 48°44'36" and 48°49'58" latitude and between 37°37'23" and 37°42'31" longitude and the altitude is ranging from 280 to 2120 m.

The study area is mainly in rainy regions and according to the climatic classification its climate is humid. Most of the year it rains and the maximum rainfall is in September and October.

Tavalsh Hashtpar city is an administrative center adjacent to this forest situated 10 km away from the Nav Asalem. Because of Nav river, this forest has been named Asalem Nav forest.

### Rating of layers

At first we rated the effective factors. In this rating method, both inner rating of layers and outer rating of layer have been made by using of experts views to combining this factors in GIS.

### A. Internal rating of layers

Using various sources listed in the article, 6 factors were selected as factors affecting the forest fires in the Hyrcanian forests, namely as follows:

1. Forest type;
2. Distance from roads (meters);

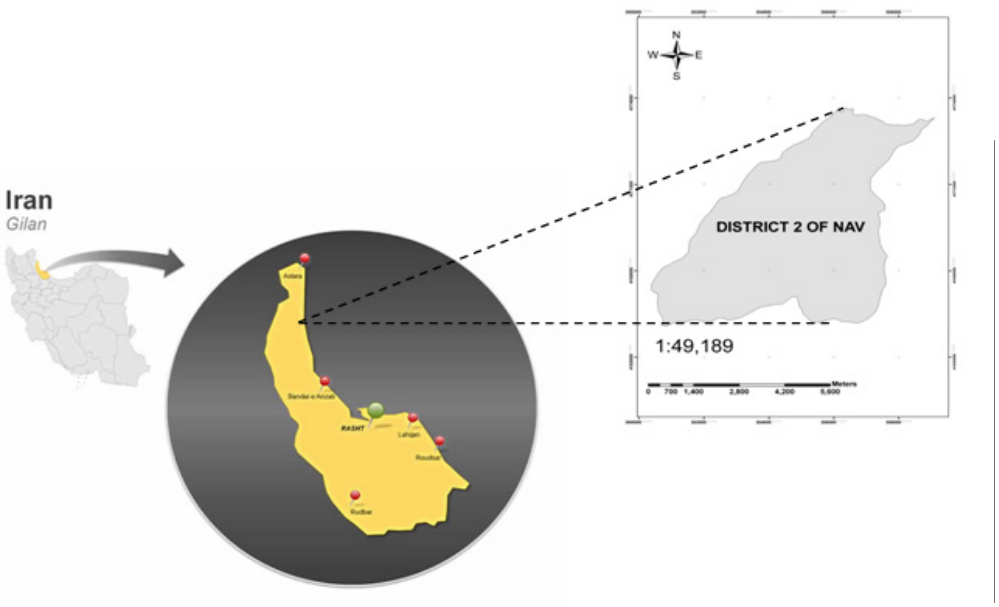


**Fig. 1. Study area.**

3. Slope gradient (%);
4. Distance from rivers (meters);
5. Distance from settlements (meters);
6. Exposure.

Each of these layers, on the basis of the value for the utility of fire, can be classified and rated internally (fire can be useful for growing some grasses). This rating was consistent with Gilan University Professors because of their acquaintance to this area. The rating levels within each layer are between 1 and 9. The parts of this region in the event of a fire that had more importance were given more value (number 9) and the less important areas go down with their values (down to number 1). The steep slopes increase, fire risk also increases. Now we will describe each item separately.

**1. Forest type**: forest trees type affects the severity or reduction of fire. The traditional field survey for determining the type of fuel which is detailed and labor intensive, is practicable for small areas. But forest fire prediction model needs to have information about fuel material types over large areas. Remote sensing successfully manages with this task. (Darmawan et al. 2001). Factors evaluation within each layer is shown in Table 1.

**2. Distance from roads**: close proximity to a passing road also increases the human impact on forests and the likelihood of fire.

**3. Slope gradient**: western and southern slopes are more sensitive to fire. Because of their sun exposure, they are drier and hotter. In contrast, these slopes have generally less fuel (Parsakhoo et al. 2009).

**4. Distance from rivers**: if the distance from a river and levels of wet lands increase, dry land will certainly increase, and this factor will cause more fires.

**5. Distance from settlements**: with increasing distance to the village, vegetation increases and the risk of fire decreases.

**6. Exposure**: Exposure, directly or indirectly, affects the other properties of the area. In the northern hemisphere southern and western slopes are set to sun longer compared to northern and eastern slopes, thus they are drier and more prone to fire. This also predetermines the difference between the vegetation density and the distribution of plants and plant communities on southern and western slopes and these on northern and eastern slopes. Thus, on western and southern slopes due to the greater solar radiation, evaporation and transpiration are higher and humidity is

**Table 1. Inner rating of factors.**

| Forest type | Value | Distance from roads, m | Value | Slope gradient,% | Value | Distance from rivers, m | Value | Distance from settlements, m | Value | Exposure | Value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dense | 9 | <100 | 5 | >35 | 5 | <15 | 5 | <1000 | 4 | South | 4 |
| Plant | 8 | 100–200 | 4 | 35–25 | 4 | 15–30 | 4 | 1000–2000 | 3 | West | 3 |
| Woodland | 7 | 200–300 | 3 | 25–10 | 3 | 30–100 | 3 | 2000–3000 | 2 | East | 2 |
| Residential forest | 6 | 300–400 | 2 | 10–5 | 2 | 100< | 2 | 3000< | 1 | North | 1 |
| Semi-thin | 3 | 400< | 1 | 5> | 1 | | | | | | |
| Sparse | 2 | | | | | | | | | | |

lower, respectively vegetation percentage is lower (Sagheb-Talebi and Yazdiyan 2005).

Rating of layers, respectively, described in Table 1 based on specified criteria.

**B. External rating of layers**

Now we are going to describe the external rating of layers, for which we used the EC software as it is explained in details below.

Expert choice software and Analytical Hierarchy Process (AHP)

Analytical Hierarchy Process is a flexible, robust and simple method, and is used for deciding when the conflicting criteria make it difficult to choose between options. This multi-criteria evaluation method, first time was proposed in 1980 by Thomas L. Saaty. AHP is one of the most effective methods for determining the criteria significance and the relative relationship between the criteria (Saaty 1980). In this method the importance of each criterion is measured relatively to other criteria. Then, based on a scale of 1 to 9 that indicates the factors average significance relative to each other, all factors are given weights. Coefficients are then calculated and if the conflict is less than 0.1 it is acceptable and already it has numerous functions in various sciences. In this study Analytical Hierarchy Process was used for rating the layers due to the simplicity, flexibility, qualitative and quantitative criteria utilization and the ability to simultaneously examine the consistency in judgments. This process is usually done by Expert choice software.

Three components create a suitable environment for fires, namely fuel, topography, and weather. These three components have mutual influence on each other and the sum of these interactions will determine the fire behavior. Although some sources consider weather, fuel, inflammation causes and human activities that affect the factors in fire activity (Johnson 1992). In this research effective factors for forest fires were determined by studying different sources and then taking advantage of the experts' views in order to attribute weighting. Twenty five questionnaires were designed and distributed among Department of Natural Resources experts of different provinces, Fire and AHP professionals, some professors of the Faculty of Natural Resources and Industries of Iran. After an explanation of the purpose and general principles of this technique, they were asked for their opinion about the importance of nine criteria considered to be comparable to the couple of parameters. After collecting the questionnaires, their data were entered into the Expert Choice (EC) software and the inconsistency rate was calculated for each questionnaire.

Now for more specific cases, we can take advantage of ArcGIS9.2 software. In fact we performed a schematic analysis for 6 factors, using their specific maps.

**Used maps**

The maps used for the study can be conditionally divided into two categories – existing maps and maps that had to be prepared.

**1. Existing maps**

These maps include area maps topographic maps and existing roads maps that have been prepared by the Technical Office of Forest in order to performing forestry projects study. These maps are produced for use in ArcGIS9.2 software.

## 2. Prepared maps

### 2.1. Maps of distance from roads and distance from settlements

First, we prepared a "Distance from roads" map (Fig. 2) and a "Distance from settlements" map (Fig. 3) according to the data in Table 1.

The word "suitable" in the legends is used for the areas which are susceptible to fire. Then other maps were prepared, respectively of forest type, slope gradient, exposure. Also, hydrographic maps were extracted from the basic maps (using the ArcGIS 9.2 software) and the data presented in Table1 was collected.

### 2.2. Slope gradient and exposure maps

With respect to Digital Terrain Model of the map, slope map was prepared, valued, and divided into 5 categories according to the data in Table 1 (Fig. 4). Then, range of directions map was produced and classified into four main directions (Fig. 5).

### 2.3. Forest type map

Forest type is a very significant factor, from the standpoint of appropriate Species position for operation, extinction of species, and conditions that are genetically susceptible. In Iran, the type scheme consideration is based on the presence, abundance of species percentage and species dominant criterion. For mapping of types habitat, first the tree cover type information was extracted from the manual design book, and the accuracy was controlled by using a GPS device on the terrain. Finally, a variety of existing types was obtained by using Arc GIS 9.2 software (Fig. 6).

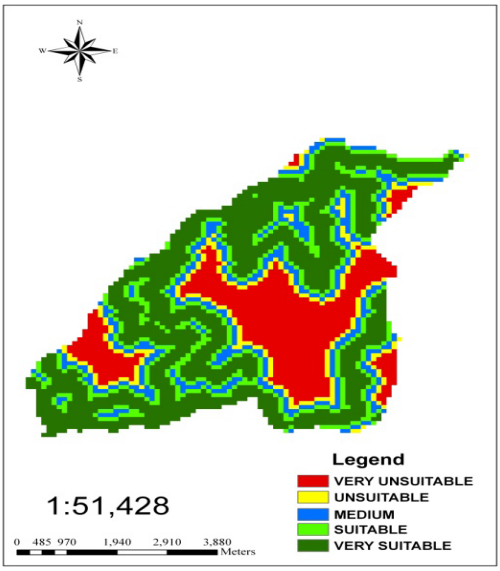


Fig. 2. Map of distance from roads.

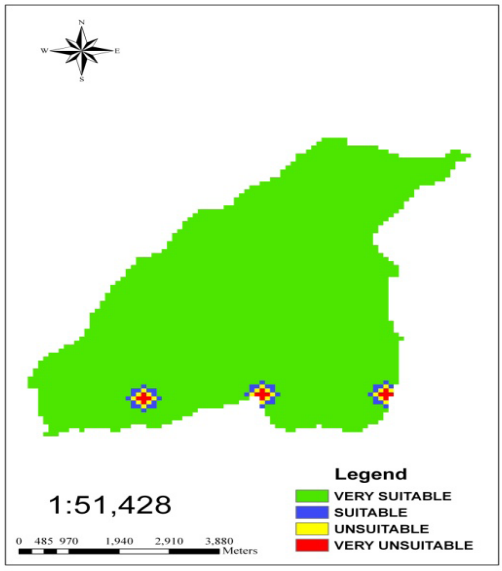


Fig. 3. Map of distance from settlements.

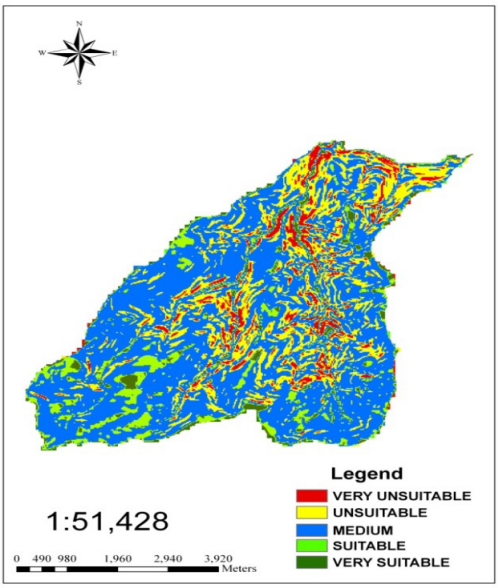


Fig. 4. Map of slope gradient.

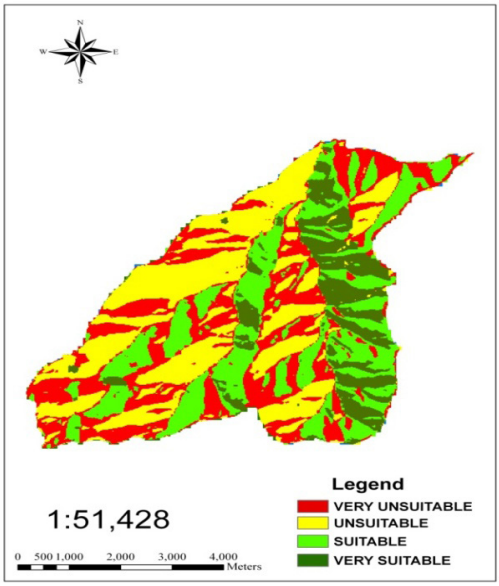


Fig. 5. Map of exposure.

### 2.4. Hydrologic map

DGN drawings, prepared by the State Geological Survey were used for preparing a Layer for waterways. Thus, using the DGN map, the desired area was separated, then DEM and TIN maps were created. Next, to determine the width of the rivers, Hill shade layer was prepared in the Arc GIS 9.2 software. In this way a hydrological layer was prepared and the area was divided and valued on the basis of fire desirability in the obtained map (Fig. 7).

## Results

The results from the Analytical Hierarchy Process survey are presented in Fig. 8. Obtained numbers represent the value or importance of priority. So, the highest weight (0.444) is assigned to vegetation and next we have the distance from settlements (0.219) and slope gradient (0.153), and finally – exposure (0.037). It

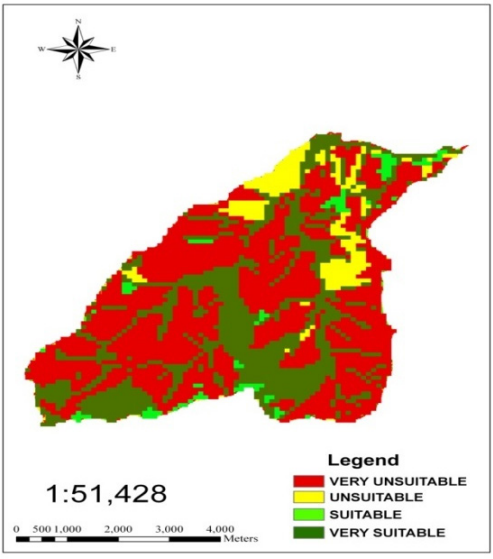


Fig. 6. Map of forest types

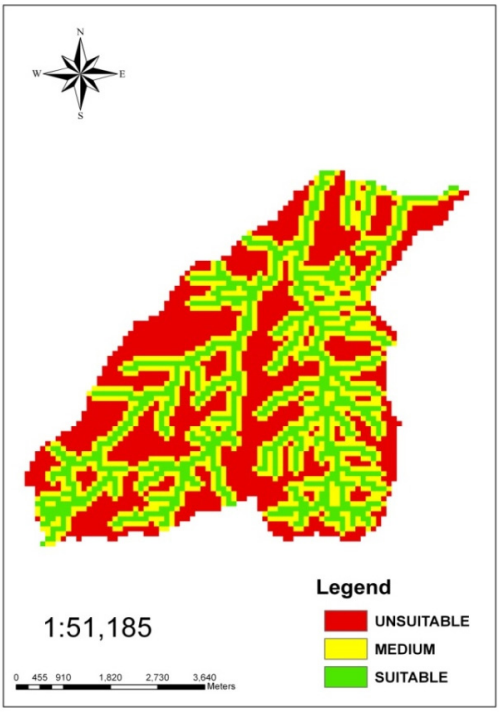


Fig. 7. Hydrologic map.

is worth noting that due to the inconsistency coefficient (0.05), that is smaller than (0.1), there is no need for revision of judgments.

## Final map of fire susceptible zones in the area

It is required to combine prepared maps in previous stages based on internal and external layer rating in order to prepare fire prone zones in the area. For this purpose the rated maps were overlaid. The resultant final map is shown as Fig. 9.

This means that from the coefficients of the layers in Fig. 8, an external layer rating was obtained. Then using these external layer coefficients a map overlay procedure was performed in GIS environment thus creating the final map.

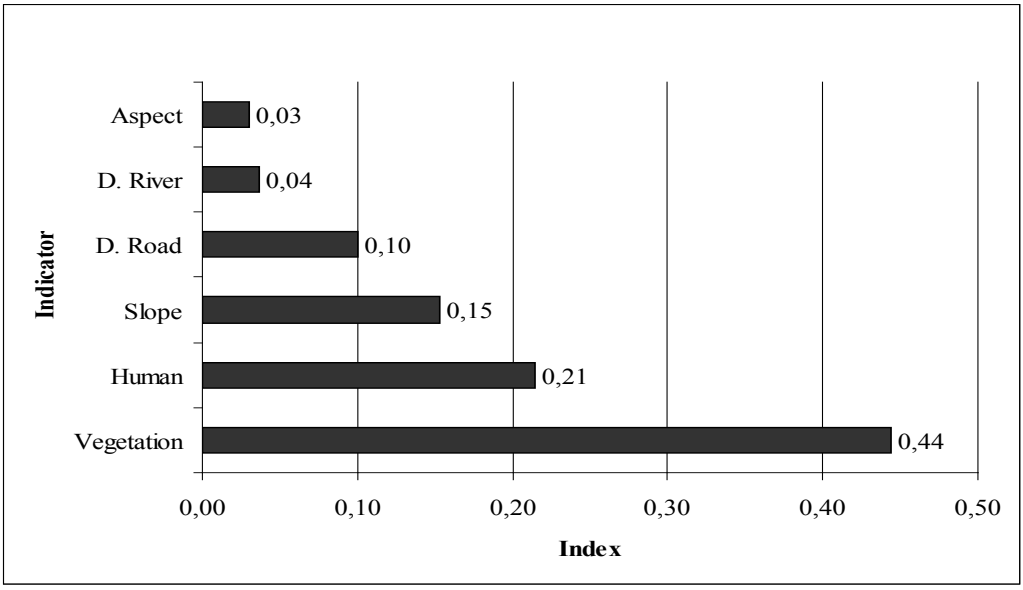


Fig. 8. Prioritization criteria that influence forest fires resulting from AHP.

## Discussion and Overall Conclusions

In the 25 years period between 1968 and 1992 more than 14 million saplings and 2 million trees have been destroyed in Iran because of forest fires (56,216 ha). In northern Iran, forest fires occurred 37 times and 285 ha of forest sites have been disturbed in 1998–2000 (Jazirei 2000).

Based on the above results, 11.70 % of the study area is very poor, with low value and high level of fire hazard. 40.68 % of the area is poor, 14.48 % is average, 8.37 % is quite valuable, 18.72 % is valuable, and 6.05 % is very good, it means that the value is high and fire hazard is low in these areas. In general we can say half of the area is prone to forest fires, and fire hazard is very high.

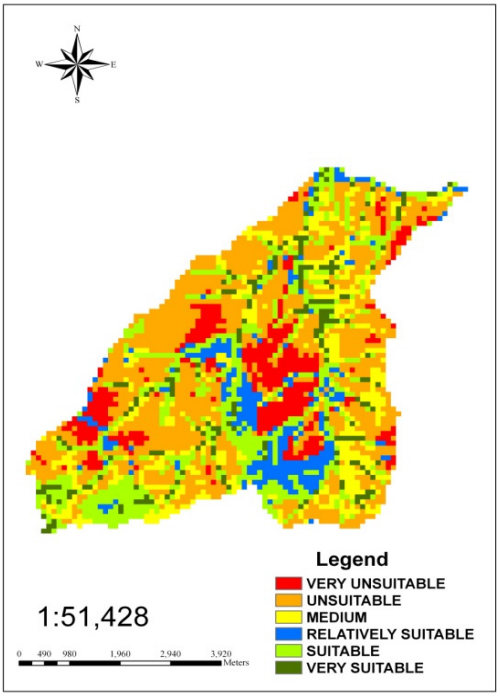


**Fig. 9. Final map of fire prone zones.**

## Suggestions

We propose: general cultural education and stimulating the public conscience to protect forests; organizing seminars, festivals and gatherings with influential people in rural areas especially the target areas; applied research for gathering required information; preparing educational tools, compact discs; exploration efforts and achieving a rapid and timely focus on forest fires; protecting forests in critical seasons with monitoring by trained personnel, and providing necessary facilities and logistics that can be effective in timely prevention and fire control. By regarding the divisions, Squads focus use in critical and semi critical areas is recommended According to a survey, conducted in the listed areas, establishing some posts is essential, and each post should be provided with some human resources, a car driver and firefighting facilities.

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# SEXUAL DIMORPHISM IN THE HEIGHT OF ANAL FIN OF MARITZA BARBEL (*BARBUS CYCLOLEPIS* HECKEL, 1937) IN THE MARITZA RIVER BASIN

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## Abstract

From the middle stretch of Maritza catchment area 229 sexually mature individuals *Barbus cyclolepis* Heckel, 1837 were examined. The height of anal fin in the two sexes was different. The anal fin of the female fishes was higher than that of males. In the ratio (percentage) between the height of the anal fin and standard length of the body there was a strong statistically significant difference between females and males. This ratio had also much higher value in females as compared to males.

**Key words:** anal fin, *Barbus cyclolepis***,** Maritza barbel, sexual dimorphism.

## Introduction

The height of anal fin is one of the systematic characteristics used to identify the species of genus *Barbus*. Anal fin is often with different height in the two sexes and is one of the few indicators of sexual dimorphism found in the species of genus *Barbus*. According to Kottelat and Freyhof (2007) in many species of this genus females have higher anal fin than males, probably in connection with excavating the substrate for spawning.

Chichkoff (1935) stated that the height of the anal fin of Maritza barbel vary from 17.3 to 20.0 % of the body length. Drensky (1951) claimed that in Maritza barbel the tip of anal fin reaches back to the base of the caudal fin.

Marinov (1964) examined 86 individuals obtained in catchment areas of the rivers: Mesta, Struma and Maritza. The author found that in all studied rivers female barbels had higher anal fin than males. Marinov (1964) calculated the ratio between anal fin height and standard length of the body and concluded that the difference between the two sexes in this indicator is even larger. Marinov (1964, 1989) found that this difference was statistically significant in each catchment area separately and for the three catchment areas pooled. The author concluded that the height of anal fin of female barbels is over 16.6 % of standard body length (between 16.6 and 23 %, usually more than 17 %), whereas for males it does not exceed 16.6 % (between 12.5 and 16.6 %).

According to Marinov (1989) the height of anal fin of *Barbus barbus* Linnaeus, 1758 is between 13.8 % and 19.8 % of standard length of the body.

The author concluded that in *Barbus meridionalis petenyi* Heckel, 1848 the height of the anal fin of females reaches to caudal origin, whereas for males surpasses this origin.

Sivkov (1991) did morphological characteristic of barbels, distributed in the watershed of the Danube River: Ogosta, Iskar, Vit, Yantra and Kamchia (Sivkov 1991). The author pointed out that the height of the anal fin is significantly greater in females than in males. He considered this as being the only sign of sexual dimorphism in barbels from these rivers.

According to Bianco (2009) the height of the anal fin of Maritza barbel is 23.8 % from the standard length of the body.

According to Kottelat and Freyhof (2007) *B. strumicae* occurs in the rivers Struma and Mesta, while *B. cyclolepis* is endemic to the basin of Maritsa River. The examination of the height of anal fin of barbels in the three streams taken together may lead to inaccuracies. Therefore, this character was examined in *B. cyclolepis* only in the basin of the Maritza River.

## Objective and tasks set

The aim of this study was to examine the difference in height of the anal fin in males and females of *B. cyclolepis* in two of the tributaries of Maritsa River.

For achieving this objective the following main tasks were set:

– to determine the variation in the height of anal fin in *B. cyclolepis* in both sexes;

– to calculate the ratio between the height of anal fin and standard length of the body of both sexes.

## Study Area

A large share of the runoff of Maritza River on territory of Bulgaria is formed by its tributaries in Sredna Gora Mountains and in Rhodopes Mountains. Therefore, two tributaries were included in the study: Stryama River coming from Sredna gora Mountains and Chepinska River – from the Rhodopes Mountains.

Stryama River rises east of peak Vejen in Zlatishko-Tetevenska Mountains, part of the Sredna Gora Mountains (Figure 1). Stryama is a left tributary of Maritza River, with a length of 110 km and catchment area 1,789 km$^2$. Stryama River flows into the Maritza River near the village of Manole.

Chepinska River is a tributary of Maritza River flowing from peak Mala Syutkya in Rhodopes Mountains. The length of this river is 83 km. Its catchment area is 899.6 km$^2$ and it flows into Maritza River near the village of Zlokuchane.

## Materials and Methods

Fish sampling was undertaken in the spring, summer and autumn of 2010 and 2011 and took place in 6 sampling areas (Table 1).

The material was collected by electrofishing, conducted with unpuled direct current (DC) and two upstream passes. We used the backpack electrofisher SAMUS 725G – (Samus special electronics, Poland), powered by a 12 V accumulator battery with 75 Ah capacity. The electrofisher converter provides DC impulses with frequency ranged between 5 and 100 Hz, duration 0.03–3 ms and maximum power of 650 W. It is suitable for water resistance

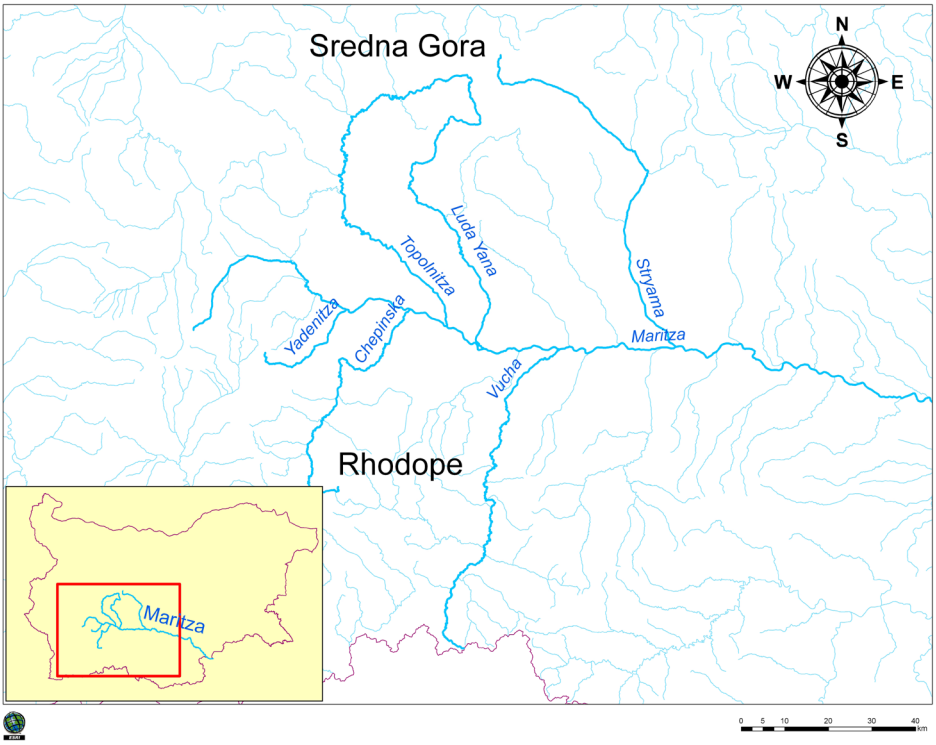


**Fig. 1. Location of studied rivers Sryama and Chepinska, Arc Map 10.0 (ESRI).**

**Table 1. Sampling areas in rivers Stryama and Chepinska.**

| No | Date | River | North latitude | East longitude | Altitude, m a.s.l. | Location |
|---|---|---|---|---|---|---|
| 1 | 13.06.2010 | Chepinska | 42°07‘42,02’’ | 24°07‘24,53’’ | 401 | Near the mineral baths of Varvara |
| 2 | 14.06.2010 | Chepinska | 42°06‘24,78’’ | 24°06‘20,11’’ | 465 | Neat the railway station M. Nikolov |
| 1 | 26.07.2010 | Chepinska | 42°07‘42,02’’ | 24°07‘24,53’’ | 401 | Near the mineral baths of Varvara |
| 3 | 27.07.2010 | Chepinska | 42°05‘43,81’’ | 24°04‘54,82’’ | 523 | Near the village of Draginovo |
| 4 | 19.11.2010 | Stryama | 42°33‘38,45’’ | 24°47‘54,11’’ | 302 | Near the fish farm of Banya |
| 2 | 08.04.2011 | Chepinska | 42°06‘24,78’’ | 24°06‘20,11’’ | 465 | Neat the railway station M. Nikolov |
| 4 | 09.04.2011 | Chepinska | 42°05‘43,81’’ | 24°04‘54,82’’ | 523 | Near the village of Draginovo |
| 5 | 17.04.2011 | Stryama | 42°32‘18,12’’ | 24°49‘22,78’’ | 283 | Near the town of Banya |
| 4 | 20.10.2011 | Stryama | 42°33‘38,45’’ | 24°47‘54,11’’ | 302 | Near the fish farm of Banya |
| 6 | 18.11.2011 | Chepinska | 42°11‘09,62’’ | 24°09‘45,65’’ | 240 | Near the village of Lozen |
| 4 | 20.11.2011 | Stryama | 42°33‘38,45’’ | 24°47‘54,11’’ | 302 | Near the fish farm of Banya |

**Table 2. The specimens of *B. cyclolepis* with minimum and maximum values of the height of anal fin and the standard length of their body in the sample of the rivers Stryama and Chepinska.**

| Sex/individuals | ♀/*n* = 98 | | ♂/*n* = 131 | |
|---|---|---|---|---|
| **River** | **min *h*, mm/ l, mm** | **max *h*, mm/ l, mm** | **min *h*, mm/ l, mm** | **max *h*, mm/ l, mm** |
| **Chepinska** | 17/134 | 45/204 | 12/77 | 20/104 |
| **Stryama** | 16/97 | 44/163 | 9/70 | 29/143 |

Legend: ♀ – females, ♂ – males, *h* – height of anal fin, *l* – standard body length in that specimen.

from 25 to 1000 Ω. The amperage in load condition was from 5 to 65 A. Besides in the present study, the material collected was used also for studying the fecundity of the species.

Identification of the species was done according to Kottelat and Freyhof (2007) and Stefanov (2007). Sexual determination was done by examining the gonads by eye and under binocular microscope Carl Zeiss Jena with zoom 20–60 X. Measurement of the standard body length and height of the fins were performed by millimeter line with accuracy to nearest 1 mm.

The ratio between height of anal fin and standard length of the body was calculated using the formula (1).

$$R = 100 \cdot h \cdot l^{-1}, \quad (1)$$

where:

*R* is the ratio between height of anal fin and standard length of the body, %;

*h* is the height of anal fin, the distance from the base to the top of the spines of the anal fin, mm;

*l* is the standard length of the body, i.e. the distance from the top of the head to the end of scaly cover, mm.

Statistical data processing consisted of comparison among means using t-test and was done by using the software PAST (Hammer et al. 2001).

## Results

Total 229 sexually mature individuals of *B. cyclolepis* were caught in the studied rivers. The maximum value of the anal fin height of Maritza barbels in rivers of Stryama and Chepinska pooled was 45 mm for the females and 21 mm for males, respectively (Table 2). The lowest value of the anal fin height for females in the two studied rivers was 16 mm, while in the males it was 9 mm.

The results showed that the mean ratio between height of the anal fin and the standard body length of *B. cyclolepis* in the two studied rivers was 22.33 % for females and 16.34 % for males (Table 3). In females this ratio varied between 12.68 % and 26.99 %, and only in 5 females it was lower than 20 %. In the male Maritza barbels the ratio ranged from 11.2 % to 21.35 %. Only in three males this ratio was 20 % and more.

## Discussion

The results of the present study confirm the statement of Marinov (1964) that in females anal fin usually reaches the caudal origin, while in males often it does not reach it. The author's results support the assumption of Kottelat and Freyhof (2007) about the role of the anal fin in female barbels during the spawning period.

**Table 3. Ratio between height of anal fin and standard length of the body to the *B. cyclolepis* Heckel, from the rivers Stryama and Chepinska.**

| Sex/individuals and statistical index | ♀/*n*=98 | ♂/*n*=131 | *df* | *CI* | *p* | $S^2$ |
|---|---|---|---|---|---|---|
| Ratio between the height of anal fin from standard length of the body | 22.33 % | 16.34 % | 227 | 95 % | 0,00 | 4.07 |

Legend: ♀ – female, ♂ – males, *df* – degrees of freedom, *CI* – confidence limits, *p* – *p*-value, $S^2$ – pooled variance.

There was strong statistically significant difference between the two sexes in the ratio height of the anal fin: standard body lengthin Maritza barbel in two studied rivers (Table 3).

However, the percentage better indicates the difference in height of anal fin in both sexes than direct height comparison. The ratio height of the anal fin: standard body length better espressed the difference between the two sexes, than direct comparison of average heights anal fins.

The ratio of the height of the anal fin and the standard body length of *B. cyclolepis* reported by Bianco (2009) is similar to our data concerning the females.

In contrast to the results of Marinov (1964) present study included larger sample only from Maritza River Basin. The results showed that the ratio of the height of the anal fin and standard body length of *B. cyclolepis* varied widely in both sexes. However, there was a clear difference in the upper limit of this ratio, reaching up to 27 % in females, while in males it was no more than 21 %. In most cases, the ratio in males did not exceed 20 %, while in females it was more than 20 %. The obtained values of the studied ratio are greater than these in Marinov (1964), but confirmed the established sexual dimorphism in the height of the anal fin in *B. cyclolepis.* This difference could likely be due to the larger sample collected from only one catchment area.

## Conclusions

The height of anal fin in female individuals of *Barbus cyclolepis* from Maritza River was significantly greater than that of males.

The ratio of the anal fin to standard body length in female barbels was average 22 %, whereas in males the average value was 16 %.

Most often, the ratio between the height of the anal fin and the standard length of the body of males did not exceed 20 %, and for females was more than 20 %.

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# EFFECT OF SHADE AND FERTILIZER SUPPLEMENT ON SURVIVAL AND GROWTH OF AMERICAN CHESTNUT SEEDLINGS

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## Abstract

American chestnut (*Castanea dentata*) was once a dominant overstory species of eastern North American forests before it was decimated by chestnut blight. Blight resistant hybrid chestnuts share most morphological characteristics with *C. dentata*, so studying its establishment and early growth will help develop effective methods for establishing blight resistant hybrids. Supplementation with nutrients that are not sufficient in the soil likely will increase early *C. dentata* performance, but optimal amounts and combination of nutrients have not been determined. We examined the effect of three shade levels and a novel leaf spray fertilizer supplement on pure *C. dentata* seedlings. Shade levels were 0 %, 50 %, and 34 % overstory of sweetgum (*Liquidambar styraciflua*). After one growing season, fertilizer had a significant effect only on relative root collar diameter growth. However, due to interaction with shade, the growth was not different among the three fertilizer levels. Root collar diameter growth was about 53 % greater in the open than in stands with 50 % residual overstory. The effects were non-significant for relative height growth. By the end of the first growing season, seedling mortality was 57 %, 9 %, and 17 % in the open, light shade, and heavy shade, respectively. Planting of chestnuts in light shade had the most acceptable combination of survival and growth.

**Key words:** *Castanea dentata***,** shade, leaf spray fertilizer supplement.

## Introduction

American chestnut (*Castanea dentata* (Marsh.) Borkh.) was formerly a primary component of eastern North American forests. The chestnut blight (*Cryphonectria parasitica* (Murrill) Barr), an introduced fungal pathogen from Asia, decimated the species in the early part of the 20th century (Russell 1987). Over the last decades, breeding has been carried out of hybrids of *C. dentata* and Chinese chestnut (*Castanea mollissima* Blume) that retain most *C. dentata* characteristics but are resistant to the blight (Diskin et al. 2006).

Most seedling mortality occurs during the first years following planting, before the root system is well established. Ensuring the success of planting the first time requires less investment than replanting

an area many times (Keeton 2008). Initial treatment of soils before planting seedlings has a positive effect on survival and growth of a variety of species (Archibold et al. 2000, Karlsson 2002, Hewitt et al. 2004, Knapp et al. 2006, Rhoades et al. 2009). Fertilizing seedlings along with mechanically preparing the site could increase seedling survival (Hewitt et al. 2004).

Results from several studies on soil fertilization of *C. dentata* show that the growth effect is marginal. (Rieske et al. 2003) showed that weekly nitrogen fertilization does not significantly enhance the growth of *C. dentata* over control in greenhouse conditions. Additionally, treatment with controlled release fertilizer in greenhouse conditions resulted in higher mortality and lower growth rates compared to control seedlings, probably due to an increase in root disease (Herendeen 2007). The results from such greenhouse experiments are consistent with results from experiments with open grown seedlings planted on mine reclamation sites that had previously been fertilized. On these reclamation fields, *C. dentata* seedling growth on fertilized soils was not significantly different from growth on unfertilized soils (Herendeen 2007). The following four studies are the only ones that found any increase in growth or survival of *C. dentata* seedlings with soil fertilization: Latham (1992) observed greater growth with increasing soil nutrient availability, and increased growth was also found with greater availability of magnesium, potassium (McCament and McCarthy 2005), and nitrogen (Rieske et al. 2003, McCament and McCarthy 2005). It is likely that proper supplementation with nutrients that are limited on the site can increase *C. dentata* performance because it does so in other hardwoods (Trubat et al. 2008). However, in most experiments this has not been observed with *C. dentata*, possibly because the adequate combination or amounts have not been applied. Results with other chestnut species, however, consistently show an increase in growth with fertilization. Organic compound fertilizer increased *C. mollissima* sapling height growth about 1.5 times over control (Zeng et al. 2007). Similarly, fertilization increased shoot growth approximately 3 times over control in *C. sativa* seedlings (Kohen and Mousseau 1994).

The amount of fertilizer applied off-target can be reduced by using a fertilizer that is delivered directly onto the plant instead of through the soil. Such application method also prevents fertilization of neighboring competing plants, making the application process more efficient and possibly more cost effective. Avoiding fertilization of the competitors can be achieved if the seedlings are sprayed or dipped before outplanting, or if each of the outplanted target plants is sprayed individually, or if the entire planted area is sprayed at a time when the only vegetation present is the targeted seedlings. This type of delivery of the fertilizer is also possible for large trees. Successful fertilization generally increases tree vigor and its defenses against attacks by insects or fungi, including those that are new or exotic. Greater availability of resources to the tree allows it to increase its defensive compound production and improve its chance of surviving insect or fungal attacks (Sayler and Kirkpatrick 2003). Targeted fertilization through the leaves of *C. dentata* may also help to prevent the decline in root system vigor observed with standard fertilization (Sileshi et al. 2007). Information on the response of such fertilizer delivery is needed, and so is information on the possible interaction of shade levels and fertilizer treatments.

*C. dentata* has intermediate shade tolerance (Joesting et al. 2009). It responds well to release from the overstory and grows rapidly in full sun. However, it also survives well in the understory. When the main stem dies, the tree resprouts well and does so repeatedly, so seedling-sized trees in the understory may have 100-year-old root systems (Paillet 2002). Historical literature suggests that survival is greater under partial shade than in the open for the first two years and that this method was a good way of establishing *C. dentata* in the forest (Russell 1987). Examination of growth patterns in a Wisconsin stand suggest that planting blight-resistant chestnut hybrids in clumps in canopy gaps or after certain silvicultural treatments is a good method of re-establishing the species (McEwan et al. 2006). If such planting configuration is used, fertilizing the clumps of seedlings through foliar application would be easier than treating more scattered seedlings. The objectives of this study were to determine the effect of shade level and fertilizer supplement and their interaction on pure *C. dentata* seedling survival and growth. Knowledge about the first year survival and performance of American chestnut is crucial due to the frequently observed high levels of mortality during the first growing season after outplanting.

## Materials and Methods

### Study site

The study was conducted at the Alabama A&M University's Winfred Thomas Agricultural Research Station in Hazel Green, Alabama, on the southern Cumberland Plateau (34º53'50"N, 86º34'34"W). Stands of sweetgum (*Liquidambar styraciflua* L.), ca. 46 x 91 m, were planted February – March 1995 at 1.5 x 3.05 m spacing. In February 2009, the stands were thinned. One-third of each stand was left unthinned, one-third had approximately 50 % overstory removal, and the other one-third had approximately 66 % overstory removal. The thinning was from below with primary removal of trees from the overtopped and intermediate crown classes. The cut *L. styraciflua* trees resprouted, but the sprouts were not removed or treated in any way. Root and stump sprouts were fairly common by the end of the study. Soils are eroded, undulating, Decatur and Cumberland silty clay loams and silty clays. Soils are classified as fine, thermic Rhodic Paleudults and Paleudalfs (Soil Survey 2010).

### Experimental design

Four-hundred American chestnut bare root 1-0 seedlings purchased from the nearest available source, a nursery in Freesoil, Michigan, were planted in April 2009 at 1.5 x 1.5 m spacing in a modified randomized complete block split plot design inside the thinned portions of the *L. styraciflua* plantations and in the open. The seedlings were planted under three shade levels: open conditions (no overstory trees), 50 % residual overstory, and 34 % residual overstory. Each one of these three shade levels was replicated three times, and there were 44 seedlings planted in each of the 9 plots. The seedlings planted in three plots in the open were located to the south, east, and west, respectively, of the sweetgum stands at a distance of about 23 m from the stand

edge. All seedlings were planted with dibble bars.

The seedlings surviving after 8 weeks within each shade level were randomly assigned one of three fertilizer levels: no fertilizer, single application, and two applications during the growing season. The first fertilizer supplement application was done on August 13, 2009 and the second application on the two-application treatment was on September 30, 2009. Each fertilizer application consisted of Accele-Grow-M® fertilizer supplement (Accelegrow Technologies, West Point, Georgia) applied as a leaf spray. The fertilizer supplement is a mixture of a 3-0-3 fertilizer (NPK), preservative, stabilizer, activator, and carrier system that contains a large concentration of the amino acid sarcosine (Accelegrow Technologies 2008). Seedlings were sprayed until all the leaves were covered with fertilizer solution. We removed competitors from shrub, vine, and herbaceous species located up to 15 cm from the planting location of each seedling. All trees were sprayed regularly with Liquid Fence® deer and rabbit repellent (The Liquid Fence Company, Brodheadsville, PA) due to initial herbivory. Invasive species and other competitors within 15 cm of the seedlings were removed approximately ten times during the growing season.

We measured the root collar diameter (RCD) and height of all trees before and immediately after planting. The RCD was measured with digital calipers, and height was measured as vertical distance from the ground to the highest point on the stem with a tape measure. Before planting we also recorded the number of first order lateral roots (FOLR) with diameter over 1 mm at the proximal end. The roots were pruned to approximately 30 cm. Basal diameter and height of all living trees were measured again after the end of the 2009 growing season.

### Statistical analysis

We tested whether fertilizer supplement application, shade, and their interaction have an impact on the survival and on the absolute and relative seedling growth in height and RCD after the first growing season. Seedlings that died or were browsed were excluded from the analyses of seedling growth. Analysis of variance (ANOVA) was used to test if there were differences in the post-fertilization seedling mortality in each subplot. The different causes of seedling death were not considered in the analyses. ANOVA was used to test if seedling browse by 1) any herbivore, or 2) rabbit only, or 3) deer only, was different among the treatments. Deer browse was identified by missing parts of the main stem or branches with frayed stem ends, and rabbit browse was identified by clipping at an angle near the base up to about 20 cm from the ground. Pre-fertilization and overall mortality were analyzed with ANOVA as a randomized complete block design (RCBD) using shade as the predictor variable.

For the number of FOLR, a log transformation was used to improve normality. Due to values of zero, 1 was added to the number of laterals. In the rest of the article, the number of FOLR refers to this log transformed variable. Linear regression was used with each growth measure as the response variable and the initial seedling attributes (height, RCD, and number of FOLR) as the predictor variables.

We used mixed models to test for differences among the groups of fertilizer supplement treatment and shade levels.

When we use the treatment name “fertilizer” in the rest of the article, we refer to treatment with fertilizer supplement. The dependent variables were absolute and relative growth in RCD and height. The relative growth is the growth expressed as a proportion of the initial RCD and height, respectively. The relative growth variables are more appropriate for measuring the effects of fertilizer and shade than absolute growth. Furthermore, these variables are more normal and have fewer outliers. Each seedling is treated as an observation in the mixed model. The seedlings are biologically independent due to the planting spacing, application of fertilizer supplement on individual trees, and interspersion of fertilizer treatment (subplot factor) within each shade block (whole plot factor).

The fixed effects are fertilizer, shade, and the fertilizer by shade interaction. The random effects are replication and the replication by shade interaction. Restricted estimate of maximum likelihood methods were used to decrease bias in the mixed model. Type III sum of squares was used due to missing data from mortality. Tukey-Kramer method was used for comparing means. We considered results to be significant if $p<0.1$, but we also provide the actual $p$-values. Statistical analyses were performed in SAS software version 9.1.3 (PROC MIXED procedure; SAS Institute, Cary, NC, 2006).

## Results

Seventy of the 396 chestnut seedlings died by the time of first fertilizer treatment in August (four months after planting). This mortality did not differ among the three shade levels tested as a RCBD ($p=0.29$). By the end of the first growing season the average mortality of seedlings in the open was 57 %, while in the light and heavy shade it was 9 % and 17 %, respectively. However, the differences were still not significant ($p=0.13$; Figure 1).

Seedlings were not significantly different in size at planting or at the time of treatment assignment. Fertilizer, shade, and their interaction all had a significant effect on the relative RCD growth (Table 1).

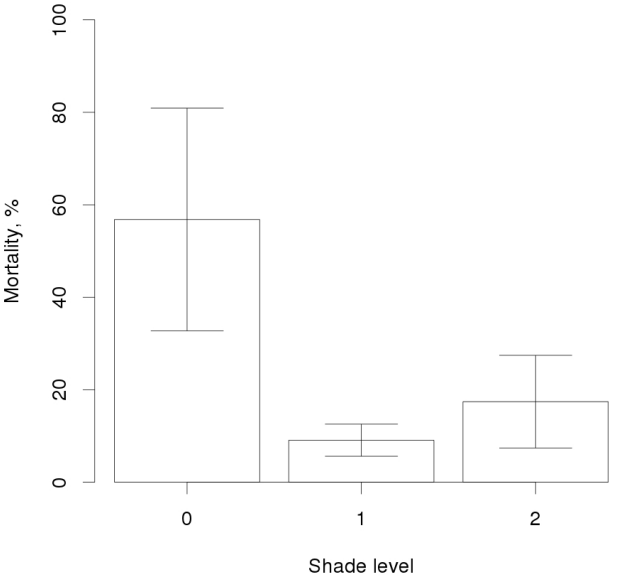


**Fig. 1. Percent mortality with SE bars at the end of the growing season in each shade level.**

Note: 0=open, 1=light shade, 2=heavy shade.

**Table 1. Effect of the treatments on the four measures of chestnut seedling growth.**

| Dependent and predictor variables | *F* (*df*) | *P* |
|---|---|---|
| Absolute root collar diameter growth | | |
| Shade | 6.15 (2.40) | 0.060 |
| Fertilizer | 2.17 (2.24) | 0.116 |
| Shade*fertilizer | 1.66 (4.24) | 0.159 |
| Relative root collar diameter growth | | |
| Shade | 6.40 (2.40) | 0.057 |
| Fertilizer | 2.72 (2.24) | 0.068 |
| Shade*fertilizer | 2.45 (4.24) | 0.047 |
| Absolute height growth | | |
| Shade | 0.09 (2.40) | 0.918 |
| Fertilizer | 1.71 (2.24) | 0.183 |
| Shade*fertilizer | 1.09 (4.24) | 0.364 |
| Relative height growth | | |
| Shade | 0.19 (2.40) | 0.831 |
| Fertilizer | 1.94 (2.24) | 0.147 |
| Shade*fertilizer | 1.15 (4.24) | 0.336 |

Note: Relative growth is the growth as a proportion of the original size. An "*" indicates the interaction term. Shade levels are no overstory, 34 % residual overstory, and 50 % residual overstory. Fertilizer levels are no application, single application, and two applications.

**Table 2. Relative seedling growth as a proportion of the initial size.**

| Predictor | | Root collar diameter | | Height | |
|---|---|---|---|---|---|
| **Hade** | **Fertilizer** | **Mean** | ***SE*** | **Mean** | ***SE*** |
| 0† | All | 0.26[a] | 0.03 | 0.069 | 0.020 |
| 1 | All | 0.23[ab] | 0.02 | 0.061 | 0.017 |
| 2 | All | 0.17[b] | 0.02 | 0.057 | 0.018 |
| All | 0‡ | 0.20[a] | 0.02 | 0.059 | 0.017 |
| All | 1 | 0.20[a] | 0.02 | 0.053 | 0.017 |
| All | 2 | 0.25[a] | 0.02 | 0.075 | 0.016 |

‡Fertilizer: 0=no fertilizer supplement application, 1=single application, 2=two applications, All=compared across all shade levels.
Note: Tukey-Kramer method was used to identify significant differences ($\alpha$=0.1) between means of the relative growth of each main effect group found significant in the ANOVA tests. Means on the first three lines for the same response variable and with the same letter are not significantly different. It is analogous for the last three lines in the table.

However, none of the factors affected relative height growth (Figure 2; Table 1). Additionally, increased shade consistently decreased absolute RCD growth (Figure 2). Linear regression with each dependent variable used in the mixed model showed that number of FOLR was not a significant predictor for any of the growth measurements (all $p$>0.16).

Seedlings in the open grew 26 % in relative RCD, which was significantly more than the 17 % growth of the seedlings in the heavy shade (Table 2). The seedlings in the light shade had intermediate growth, which was statistically the same as the growth in the open and in heavy shade. The pairwise comparisons of relative RCD and height among seedlings treated with fertilizer twice, once, or not sprayed were not different from each other (Table 2).

Fertilizer significantly affected only relative RCD growth (Table 1). However, due to the existence of interaction between fertilizer and shade, none of the pairwise comparisons were

different (Table 2). Fertilization did not have an effect on any of the other measures of seedling growth during the period.

Although there were no significant differences, there was a consistent trend that the single fertilizer application resulted in less growth in the open and heavy shade than in the light shade for absolute and relative RCD (Figure 2).

Browsing and mortality between the time of fertilizer application and the end of the first growing season were unaffected by treatment. Mortality after fertilizer application did not differ among the

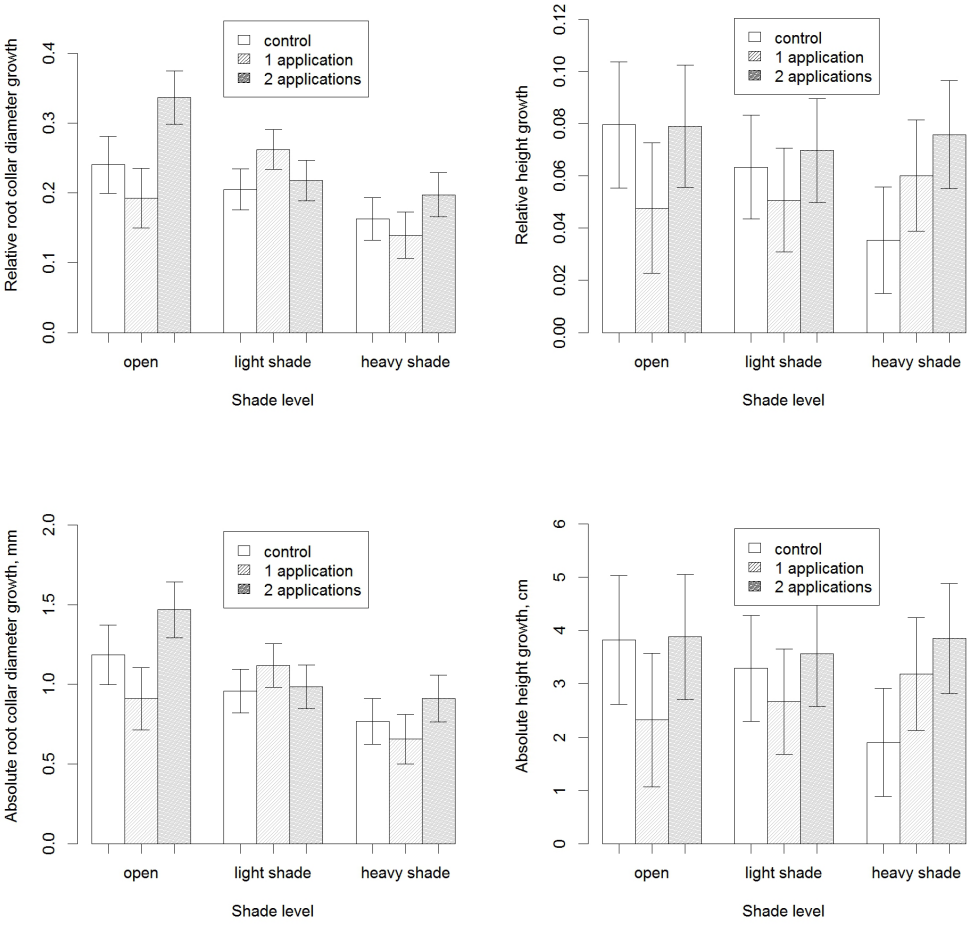


**Fig. 2. Mean ± *SE* for each fertilizer supplement treatment level within each shade level for each dependent variable.**

Note: Relative growth is the growth as a proportion of the original size. Letters indicate significant difference with Tukey-Kramer adjustment for multiple comparisons ($\alpha$=0.1).

three shade levels tested as a RCBD ($p$=0.32).

Rabbit browse was 9.1 % in the heavy shade, 3.8 % in the light shade, and absent in the open. There was no significant main or interaction effect of the nine treatments on percent browsing in the split plot analysis. Hence, we used RCBD to test if the percentage of browsed seedlings was different in the three shade levels before and after treatment. Deer, rabbit, and cumulative browsing at the end of the growing season were all unaffected by shade. Only 4 seedlings were browsed before treatment assignment, all by rabbit, so pre-treatment browsing was not analyzed separately.

## Discussion

The greatest growth in absolute RCD was achieved in the open for seedlings treated twice. However, it was significantly greater than the absolute RCD growth of only two out of the eight other treatments (Figure 2). Greater growth in partial shade when compared to full shade has been found consistently, e.g., *C. dentata* seedlings in 30 % shelterwood treatment can have approximately three times greater annual height growth and twice as large a diameter growth than seedlings in an intact forest (McCament and McCarthy 2005). However, germination, vigor, and survival do not improve significantly in shelterwood (McCament and McCarthy 2005). Rhoades et al. (2009) found that seedling mortality does not differ significantly between 30 % shelterwood and midstory removal treatments. Seedling annual height growth was over 3 times and diameter growth about 4 times as great in shelterwood versus in midstory removal treatments. Visible root disease was noted but not identified in some seedlings. This was twice as common in shelterwood treatments as in midstory removal. Survival of seedlings may be greater under moderate shade (Anagnostakis 2007, Griffin 1989).

Due to the great variation in survival, it cannot be claimed that any of the shade levels resulted in a better survival than any of the other two (the test for the difference among them had a $p$=0.13), even though the value for average mortality of seedlings in the open was approximately three to six times greater than that of the seedlings in the heavy and light shade, respectively. Nevertheless, considering this result and the results from other studies, it can be recommended that at least some care be taken when considering planting in fully open conditions. American chestnut has consistently survived well when planted under some shade (Jacobs 2007). Afforestation of chestnut on agricultural fields has been studied little, but unpublished work in Ohio has found very high mortality (Brian McCarthy, pers. comm., Ohio University, August 4, 2010). However, a study in Indiana found negligible American chestnut mortality two years after planting on a former agricultural field (Selig et al. 2005). Unless there is a delayed growth response that may be seen in subsequent growing seasons, fertilization of the kind used in this study is not recommended. In addition to a longer term response, future studies in the region should also consider the potential impact of *Phytophthora cinamomi* on the American Chestnut Foundation's blight-resistant hybrids. There were indications that this water mold caused some of the seedling mortality in our study.

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# INCREASING THE DOLOMITE STONE CONTENT IN THE GROWING MEDIA REDUCES THE GROWTH OF TWO-YEAR BAREROOT SEEDLINGS OF EUROPEAN HOP-HORNBEAM (*OSTRYA CARPINIFOLIA* SCOP.)

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## Abstract

European hop-hornbeam is a broadleaf deciduous tree, found from Southern France to Anatolia and Transcaucasia, mainly on limestone and dolomite bedrock. The seedlings produced in a mixture of quartz sand and peat in the ratio of 2:1 were transplanted after the first growing season in raised beds filled with growing media of 3 different mixture ratios of peat and dolomite stone. Detailed morphological development and nutrient concentration and content after a second growing season were compared. An increase of the dolomite stone in the growing media has a negative impact on the growth of European hop-hornbeam seedlings. The seedlings from growing media with the highest content of peat in relation to the dolomite stone (70:30) had highest values of all measured morphological attributes. Unlike morphological parameters, influence of the dolomite stone content on studied macronutrients concentration is not conclusive.

**Key words:** growing media, European hop-hornbeam, nutrient ratio, *Ostrya carpinifolia***,** seedling quality.

## Introduction

Areal of European hop-hornbeam covers the Western Balkans from Greece, Albania, Bosnia and Herzegovina, Croatia, Serbia and southern Hungary and extends to the Southern Alps. It occurs in Provence and in Italy including Sardinia and Corsica. In the southeast, European hop-hornbeam areal extends across Asia Minor to the Caucasus and Lebanon (Jovanović 2007). European hop-hornbeam is a semi-shade species with narrow tolerance limit to soil moisture (Popović et al. 1997).

Besides its importance as species inhabiting the least fertile soils, particularly on steep slopes where protective function is predominant, European hop-hornbeam has a great practical value. Wood is hard, sound, heavy and lackluster (Vilotić 2000). Korkut and Korkut (2008) present the results of studies that indicate possibility of European hop-hornbeam utilization for different wood products and recommend the Turkish forest enterprise to “give more at-

tention to European hop-hornbeam wood, which is more valuable wood source for forest industry". Despite wide possibilities for its use in afforestation (Jović et al. 1998, Tomić et al. 2011), foresters neglect European hop-hornbeam in choice of species for afforestation programmes.

Our study objective was to quantify and compare seedlings grown in growing media with 3 different ratios of peat and dolomite stone, in order to investigate their response on production in growing media more similar to soil condition on planting site and to promote the use of European hop-hornbeam in production of planting material and afforestation.

## Materials and Methods

Stratified seeds from 3 provenances (Jagnjenica – 720 m elevation, Junaci – 950 m – elevation and Vojmisliće – 1160 m elevation) were sown in a mixture of quartz sand and peat in the ratio of 2:1. Seeds from all 3 provenances were equally and randomly represented. After the first growing season, 945 seedlings were transplanted into three modified beds (315 per bed with growing density of 160 seedlings per $m^2$), 100 cm wide, 200 cm long, 40 cm height, with 30 cm layer of growing media. Each bed was filled with a growing media made of mixture of peat and dolomite stone at different ratios: treatment A – 70:30, treatment B – 50:50 and treatment C – 30:70. A low mineralized peat, with *pH* in *KCl* of 4.9, *pH* in $H_2O$ of 5.8,89 % of organic components, 2.41 % of nitrogen, 0.18 % of potassium, 0,18% of phosphorous and 2,43 % of other minerals; originate from local source at Pešter plateau, Southwestern Serbia. A dolomite faction – buffer 0/30, originated from local dolomite quarry near Belgrade, Serbia. A study was installed in Belgrade, at nursery of Faculty of Forestry, University of Belgrade. Seedlings were grown under standard nursery practices, irrigated on need and weeded on 15 days.

Values of *pH* in mixture of growing media and deionized water were measured at beginning and end of the study from 3 samples from each bed. Temperature of growing media was measured on daily basis during growing season at 10 cm depth, on 3 spots in each bed.

At the end of the second growing season, all seedlings were measured for morphological attributes of quality: height (*H*), root collar diameter (*D*) and dry masses: the total seedling dry mass ($M_{sd}$), shoot dry mass ($M_{sh}$), root dry mass ($M_{ro}$). Dry masses were measured after drying the seedlings in the thermostat cabinet at a temperature of 68 °C for 48 hours.

From the measured morphological parameters after second growing season, the quality index (*QI*) has been calculated by the formula (1) (Dickson et al. 1960):

$$QI = \frac{Msd\ (g)}{\left(\frac{H(cm)}{D(mm)}\right) + \left(\frac{Msh\ (g)}{Mro\ (g)}\right)} \qquad (1)$$

Concentrations of nitrogen, phosphorus and potassium in shoot and root were measured at the end of the second growing season. Sample preparation for the determination of *P* and *K* was performed with wet combustion of plant material in nitric acid and hydrogen peroxide. From the obtained extract, phosphorus was determined colorimetric with the use tin chloride and ammonium molybdate. Potassium was determined by flame photometry. Nitrogen content was determined by the Kjeldahl method.

Normality of data was tested by Kolmogorov-Smirnov test. Data distribution was not normal, but given a large sample (307 valid pairs for comparison), we did not apply nonparametric statistics, and continued with analysis of variance with growing media as factor. The significant differences between the groups, and the homogeneity of groups were examined by post hoc multiple comparison test or Tukey HSD test. All statistical analyzes were performed using the computer program STATISTICA 7, StatSoft Inc.

## Results

The *pH* value of mixture of growing media and deionized water increased at the end of the study, but there are no significant differences between treatments (Table 1).

There is no significant differences in temperature of substrates in with different treatment (Table 2).

Seedlings from growing media with lowest content of dolomite stone (A) had greater mean values of all measured attributes except nitrogen concentration in both, shoot and root and phos-

**Table 1. Average values of *pH* in mixture of growing media and deionized water at beginning and end of the study from 3 samples from each bed.**

| Treatment | *pH* | |
|---|---|---|
| | beginning | end |
| A | 5.8 | 6.3 |
| B | 5.9 | 6.2 |
| C | 5.8 | 6.3 |

**Table 2. Average value, standard deviation (*Sd*), minimum and maximum temperature (°C) from three seedbeds with different substrates in last three month of vegetation period.**

| Month | | July | August | September | All |
|---|---|---|---|---|---|
| Days | | 31 | 31 | 30 | 92 |
| treatment | | | | | |
| Average | A | 18.745 | 18.952 | 17.947 | 18.554 |
| | B | 18.913 | 19.003 | 17.750 | 18.564 |
| | C | 18.790 | 18.823 | 17.500 | 18.380 |
| *Sd* | A | 0.8254 | 1.2902 | 1.2364 | 1.2035 |
| | B | 0.9872 | 1.5105 | 1.4229 | 1.4298 |
| | C | 1.1998 | 1.5935 | 1.5281 | 1.5605 |
| min-max | A | 17.6-21.1 | 17.3-21.8 | 16.0-19.7 | 16.0-21.8 |
| | B | 17.5-21.4 | 17.4-22.5 | 15.8-19.6 | 15.8-22.5 |
| | C | 17.1-22.1 | 16.9-22.6 | 15.3-19.0 | 15.3-22.6 |

**Table 3. Height (*H*), diameter (*D*), seedling dry mass ($M_{sd}$), shoot dry mass ($M_{sh}$), root dry mass ($M_{ro}$) and Quality Index (*QI*) of European hop-hornbeam seedlings from three growing media.**

| Indicators | Growing media | | | Statistics | |
|---|---|---|---|---|---|
| | A | B | C | *F* | *p* |
| ***H*, cm** | $28.99^{c}$ | $22.57^{b}$ | $20.33^{a}$ | 49.09860 | 0.000000 |
| ***D*, mm** | $3.14^{c}$ | $2.84^{b}$ | $2.51^{a}$ | 22.27941 | 0.000000 |
| **$M_{sd}$, g** | $0.99^{b}$ | $0.68^{a}$ | $0.57^{a}$ | 28.46041 | 0.000000 |
| **$M_{sh}$, g** | $0.65^{b}$ | $0.42^{a}$ | $0.34^{a}$ | 35.83220 | 0.000000 |
| **$M_{ro}$, g** | $0.34^{b}$ | $0.26^{a}$ | $0.22^{a}$ | 15.99357 | 0.000000 |
| ***QI*** | $0.09^{b}$ | $0.07^{a}$ | $0.06^{a}$ | 12.95882 | 0.000003 |

Note: For each measured attribute from each growing media, means with different letters are significantly different at the $\alpha$=0.05 level.

**Table 4. Nitrogen concentration in shoot ($N_s$) and root ($N_r$), phosphorus concentration in shoot ($P_s$) and root ($P_r$), potassium concentration in shoot ($K_s$) and root ($K_k$) and nutrient ratios in shoot ($N_s$:$P_s$, $N$s:$K_s$) and root ($N_r$:$P_r$, $N_r$:$K_r$) of European hop-hornbeam seedlings from growing media.**

| Nutrient concentration, % | A | B | C | *F* | *p* |
|---|---|---|---|---|---|
| $N_s$ | 1.65 | 1.79 | 1.80 | 1.689897 | 0.209842 |
| $N_r$ | 1.43 | 1.60 | 1.58 | 2.613844 | 0.098068 |
| $P_s$ | 0.05 | 0.03 | 0.03 | 1.174716 | 0.329333 |
| $P_r$ | 0.03 | 0.04 | 0.02 | 1.962280 | 0.166671 |
| $K_s$ | 0.30 | 0.27 | 0.28 | 0.861018 | 0.437819 |
| $K_r$ | $0.36^{c}$ | $0.26^{ab}$ | $0.21^{a}$ | 4.246619 | 0.029032 |
| **Ratio** | | | | | |
| $N_s$:$P_s$ | 33 | 60 | 60 | | |
| $N_s$:$K_s$ | 5.5 | 7 | 6 | | |
| $N_r$:$P_r$ | 48 | 40 | 79 | | |
| $N_r$:$K_r$ | 4 | 6 | 7.5 | | |

Note: For each measured concentration from each growing media, means with different letters are significantly different at the $\alpha$=0.05 level.

concentration in both, shoot and root and potassium concentration in shoot. Seedlings grown in growing media C had a greater concentration of nitrogen in shoot. Seedlings grown in growing media B had a greater concentration of nitrogen and phosphorous in root.

Mean values of all measured morphological attributes, as well as potassium concentration in root, are significantly different at $\alpha = 0.05$ level.

phorous concentration in root (Table 3 and 4). Quite opposite, seedlings from growing media with highest content of dolomite stone (C) had smallest mean values of all measured attributes except nitrogen

**Table 5. The mean value of content of *N*, *P* and *K* in seedlings (in mg), *N*:*P* and *N*:*K* ratio and root shoot mass ratio (*R*:*S*) from 3 growing media.**

| | Growing media | | |
|---|---|---|---|
| **Content, mg** | **A** | **B** | **C** |
| ***N*** | 15.60 | 11.70 | 9.65 |
| ***P*** | 0.40 | 0.20 | 0.10 |
| ***K*** | 0.31 | 0.18 | 0.14 |
| **Ratios** | | | |
| ***N*:*P*** | 39 | 58 | 96 |
| ***N*:*K*** | 5 | 6 | 7 |
| ***R*:*S*** | 0.52 | 0.62 | 0.65 |

Unlike concentrations, the content of *N*, *P* and *K* in seedlings decrease with increase of dolomite content in growing media. In the same time, all ratios increase with increase of dolomite content in growing media (Table 5).

## Discussion

There are no significant differences in *pH* value between different treatments at end of the study and expected increasment in all three seedbeds is mainly due to irrigation process.

The results of impact of dolomite stone content in growing media on morphological attributes of seedlings are

consistent with results of similar research with different ratio of peat and stone in the production of Austrian pine (Mataruga 2006). Heiskanen and Rikala (2003) find lack of influence of peat content decreasing on height of Scots pine seedlings, but relatively small negative influence on heights of Norway spruce and birch seedlings. Given to lack of standard dimensions prescribed, as well as the results of other research about production of the European hop-hornbeam seedlings, it is difficult to discuss the quality of the plants examined in this study. However, by comparison with seedling of other species inside the same age, it can be concluded that the seedlings of European hop-hornbeam from the three treatments do not meet their quality, in all observed morphological attributes.

Unlike morphological attributes, influence of dolomite content on concentration of observed nutrients is less pronounced. These results are similar to those reported by Valdecantos et al. (2006) for *Quercus ilex* L. and *Pinus halepensis* Mill. foliar *N* and *P* concentrations. On the other hand, nutrient content decrease with increase of dolomite in growing media, but these differences are not significant.

However, nutrient ratios are more useful than nutrient concentrations in defining of critical nutrient limits (Flückiger and Braun 2003). Results of *N*:*P* ratio indicates that growing of seedlings on all three growing media was very limited by *P*, considering a much larger values than 16, as suggested by Koerselman and Meuleman (1996).

Different conclusions can be made by comparison of nutrient concentrations and nutrient content. The increasment of *R*:*S* biomass ratio with *N* and *P* deficiencies based on nutrient content is consistent with Hermans et al. (2006), which is not so conclusive for ratios based on nutrient concentrations. Nutrient content is dependent on seedling size (Landis et al. 2005) and it is more comparable to *R*:*S* biomass ratio.

There are no defined critical nutrient levels for European hop-hornbeam to our knowledge. Compared to nutrient critical levels suggested by Binns et al. (1983) for other broadleaved species, seedlings grown in all 3 growing media was limited by all 3 observed nutrients.

## Conclusions

An increase of the dolomite stone in the growing media has a negative impact on the growth of European hop-hornbeam seedlings. This impact is more noticeable on morphological attributes compared to content of major nutrients in seedlings. European hop-hornbeam seedlings can be produced in substrates with large level of dolomite stone as part of conditioning process, but further research of field success should be performed.

## Acknowledgment

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# *IN VITRO* PROPAGATION OF *SYRINGA VULGARIS* L.

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## Abstract

Actively growing root suckers from 4-year old shrubs of seed origin were collected in the beginning of spring (April 20, 2012) from open field grown shrubs. Softwood, apical and nodal explants (3–4 cm) were used as initial explants. For the induction of axillary shoots formation, the explants were cultured on Murashige and Skoog (1962) basal medium, supplemented with 0, 0.5 mg·l$^{-1}$ or 1.0 mg·l$^{-1}$ BAP. The formed axillary shoots were cultivated on PGRs free MS medium, or supplemented with BAP (0.5, 1.0 or 5.0 mg·l$^{-1}$) or zeatin (0.5 or 1.0 mg·l$^{-1}$ + 0.1 mg·l$^{-1}$ IBA) for multiplication. For evaluation of the effect of subculture on the multiplication coefficient, the formed axillary shoots (≈ 30 mm) were transferred 3 times on same but fresh medium and cultured for 14 or 21 days. The subcultures did not affected the multiplication rate and maximal number of axillary shoots was reached when the MS medium was supplemented with 5.0 mg·l$^{-1}$ BAP (3.8 ± 0.4). The following variants of an inductive, half-strength MS medium were used for the rooting: free of auxin (variant 1), supplemented with 1.0 mg·l$^{-1}$ and 5.0 mg·l$^{-1}$ IBA (variant 2), supplemented with 5.0 mg·l$^{-1}$ IBA for 48, 72, and 168 h (variant 3), and supplemented with 7.5 mg·l$^{-1}$ IBA for 24, 48, and 72 h (variant 4) and then on half-strength MS medium without auxin. For comparison of the results, the shoots from variant 1 and 2 were rooted on the same medium without transfer to an expressive medium. All pulse treatments with high concentration of IBA (7.5 mg·l$^{-1}$) improved significantly the rooting (between 76.6 ± 8.0 % and 82.2 ± 4.4 %) in comparison with all other treatments. These results demonstrated that the type of initial plant material, the balance between the concentration of the used auxin and the duration of inductive phase are critical for the rooting rate and quality of the root system.

**Key words:** auxin pulse treatment, common lilac, rejuvenation, root suckers.

## Introduction

Common lilac (*Syringa vulgaris* L.) is famous for its great diversity of ornamental cultivars and hybrids (Krüssmann 1986, Dirr and Heuser 1987, Dirr 1998, Fiala 2008, Rudolf 2008) making it very suitable for use in urban areas. However, cloning of ornamental genotypes in a generative way is impossible because of the heterozygosity of this species (Fiala 2008). That is why the seed progeny is used for selection and hybridization or for production of rootstocks for grafting (Dirr and Heuser 1987). *Syringa vulgaris* cuttings are generally considered difficult to root (Waldenmaier and Bünemann 1991, Howard and Ridout 1992, Howard 1993, Tyatyushkina 2007, Patience and Alderson 1984) and vary between the cultivars (Schmidt 1978, Bassuk et al. 1984, Tyatyushkina 2007), their age

(Waldenmaier and Bünemann 1991), period of cuttings collection (Schmidt 1978), different part of the shoots (Bojarczuk and Jankiewicz 1975, Schmidt 1978). Also, the rooting has been found to increase considerably only during the phase of full bloom (Schmidt 1978, Bojarczuk 1975, 1978, 1979, Dirr and Heuser 1987, Dirr 1998, Hartman et al. 2002, Cameron et al. 2003). For this reason the trade production of its cultivars is performed by grafting on seedlings. However, the production of large quantities of grafts is limited by the season, period duration for rootstock production, and the success depends on the method of grafting (Krüssmann 1986, Dirr and Heuser 1987, Dirr 1998, Fiala 2008). Also, the grafting is labour-consuming and needs large areas.

The success of *in vitro* cloning of woody plants depends on the age of stock plant, explants used, and culture conditions. However, *in vitro* propagation could allow large-scale production of plants and may provide rejuvenated plants with high rooting capacity (Bonga and von Aderkas 1992, Hackett and Murray 1993, Hartmann et al. 2002). For this reason some researchers have focused on *in vitro* propagation of the species and cultivars of genus *Syringa* (Hilderbrandt and Harney 1983, Pierik et al. 1988, Gabryszewska 1989, Waldenmaier and Bünemann 1991, Pinker et al. 1993, Refouvelet et al. 1998, Liu et al. 2013).

Nesterowicz et al. (2006) and Skrzypczak (1992) considered April as optimal period for cuttings collection. Tomsone et al. (2007) have indicated the beginning of flowering as the best period. It has been reported that *in vitro* culture of common lilac could be established by axillary buds (Hildebrandt and Harney 1983, Waldenmaier and Bünemann 1991), nodal explants (Einest and Alexander III 1985, Pierik and Steegmans 1985, Pierik et al. 1988, Tomsone et al. 2007), lateral buds with a small part of the stem (Gabryszewska 1989), and shoot tips (Einest and Alexander III 1985). It was shown that *in vitro* propagation depends on the cultivar (Pinker at al. 1993, Tomsone et al. 2007) and age of the stock plant (Pierik et al. 1988). However, some of the publications did not report for the age of stock plant (Einest and Alexander III 1985, Tomsone et al. 2007) and the type of induced shoots (Tomsone et al. 2007).

The aim of this work was to study the effect of the type of explants, their disinfection, and the effect of type, concentration, and duration of application of plant growth regulators (PGRs) for *in vitro* multiplication and rooting of common lilac.

## Material and Methods

### Plant material and disinfection

Four-year old shrubs of seeds origin, growing in the nursery “Sequoia” Ltd. in Sofia were used as stock plants. It was earlier demonstrated that the highest percentage of bud development can be induced when the donor plant is in the end of physiological dormancy or begins active growth (Iliev 1996, Nesterowicz et al. 2006). Therefore, actively growing root suckers were collected in the beginning of spring (April 20, 2012) from open field grown shrubs (Fig. 1).

Softwood, apical and nodal explants (3–4 cm) were used as initial explants. All explants were rinsed for 5 min under running tap water with 2–3 drops of detergent. Then they were disinfected for 2 min

with 0.1 % solution of $HgCl_2$. After being rinsed 3 times in sterile distilled water, the ends (≈ 2 mm) of explants were aseptically removed.

### Culture establishment

For the induction of axillary shoots formation, the explants were cultured on Murashige and Skoog (1962) basal medium, supplemented with 0.5 mg·l$^{-1}$ or 1.0 mg·l$^{-1}$ 6-benzylaminopurine (BAP). The results were compared with control (plant growth regulators (PGRs) free medium). Each treatment contained 20 explants and was repeated 3 times. The percentage of developed explants was calculated from the aseptic explants, after 14 days.

### Multiplication of the axillary shoots

The formed axillary shoots were cultivated on MS medium, supplemented with BAP (0.5 or 1.0 mg·l$^{-1}$) or (6-[4-Hydrooxy-3-methil-but-2-enylamino]purine (zeatin, 0.5 or 1.0 mg·l$^{-1}$ + 0.1 mg·l$^{-1}$ IBA) for multiplication. The results were compared with control (PGRs free medium). For evaluation of the effect of subculture on the multiplication coefficient, the induced axillary shoots (≈ 30 mm) were transferred 3 times on same but fresh medium and cultured for 14 or 21 days. Each treatment contained three replicates and for each of them 10 explants were used. The number and length (mm) of the formed new axillary shoots were calculated after each subculture.

### Rooting of the axillary shoots

After the multiplication stage, shoots that were longer than 20 mm were transferred to

**Fig. 1. Actively growing root suckers.**

the following variants of an inductive, half-strength MS rooting medium: free of auxin (variant 1), supplemented with 1.0 mg·l$^{-1}$ and 5.0 mg·l$^{-1}$ IBA (variant 2), supplemented with 5.0 mg·l$^{-1}$ IBA for 48, 72, and 168 h (variant 3), and supplemented with 7.5 mg·l$^{-1}$ IBA for 24, 48, and 72 h (variant 4) and then on an expressive rooting medium (half-strength MS medium without auxins). For comparison of the results, the shoots from variant 1 and 2 were rooted on the same medium without transfer to an expressive medium. Three replications, each containing 15 explants, were cultured in each variant. The percentage of rooted shoots was determined weekly in duration of 5 weeks and the number and length of induced roots of first and second order were determined after 5 weeks. The length of second order roots was determined as a mean of the rooted shoots.

### Culture conditions and statistical analysis

All variants of the medium contained 50 mg·l$^{-1}$ NaFeEDTA and 8.0 g·1$^{-1}$ agar. The pH was adjusted to 5.5 before autoclaving at 118 kPa and temperature 120 ºC for 20 min. The cultures were

grown in cultivation chamber at 23 ± 1 ºC with 14 h cool white fluorescent light at photon flux density 40 μmol·m$^{-2}$·s$^{-1}$, daily.

The results were analysed by One-Way ANOVA followed by a post hoc LSD test at $p < 0.05$, using SPSS 20.0 for Windows. Percentage values were transformed using arcsine square root $(\sqrt{P})$ (Compton 1994) to normalize error distribution prior variance analysis.

## Results

### Disinfection of the initial explants

The results from disinfection demonstrated that nodal explants contained more contaminations than apical explants. However, they are more resistant to the action of $HgCl_2$ i.e. less of them were damaged from the treatment and significantly more aseptic and viable explants were obtained (Table 1).

### Culture establishment

Callus induction was not noticed on the explants, cultured on cytokinin free medium (control). The first signs of callus formation were detected on the base of the explants 7 days after culture establishment on the variants of the medium, containing BAP and in the end of the experiment its amount was small. Simultaneously, axillary shoot formation was observed on the explants. The level of elongated shoots was higher in the control and low concentration of BAP and after 14 days reached 95.8 ± 4.2 % and 96.7 ± 3.3 %, resp., but there was not found any statistical difference in comparison with the other treatments. It was found that the concentration of BAP does not affect the percentage of explants with bud dormancy breaking and number of the formed shoots. However, the lower concentration of BAP (0.5 mg·l$^{-1}$) had statistically significant effect on the rate of growing buds (55.0 ± 5.0), but the shoots were statistically longer on cytokinin free medium (10.0 ± 1.5 mm) (Table 2).

### Multiplication of the axillary shoots

After 14 days of cultivation and the first subculture there was no significant difference in the rate of formed axillary shoots between all treatments. After the second subculture the rate of axillary shoots increased when the explants were cultured on highest concentrations of BAP (1.0 and 5.0 mg·l$^{-1}$) but remained statistically identical after the third subculture. The serial number of subculture did not affect significantly the number of formed axillary shoots when the explants were cultured on medium supplemented with zeatin. Also, the equal concentrations

**Table 1. Disinfection of the initial explants.**

| Type of the explants | Contaminated explants, % | Damaged explants, % | Aseptic explants, % |
|---|---|---|---|
| Softwood apical explants | 32.3 ± 4.8 a | 35.9 ± 5.0 b | 31.0 ± 7.1 a |
| Softwood nodal explants | 48.6 ± 5.8 b | 8.0 ± 4.5 a | 43.4 ± 8.7 b |

Note: The means (M) ± standard error (SE) within a column followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$.

of BAP and zeatin induced statistically identical effect (Table 3).

After 21 days of cultivation and the first subculture statistical difference between the control and lowest concentration of BAP (0.5 mg·l$^{-1}$) was not observed. The multiplication rate increased with the increasing of the concentrations of BAP. Maximal number of shoots was reached when the medium was supplemented with 5.0 mg·l$^{-1}$ BAP (3.8 ± 0.4). Similar tendency was observed after the second and third subculture (Fig. 2). The zeatin was more effective after first and second subculture. However, zeatin was more effective than BAP when these cytokinins were used in concentration of 1.0 mg·l$^{-1}$ (Table 3).

**Table 2. Effect of BAP concentration on the formation of axillary shoots.**

| Concentration of BAP, mg·l$^{-1}$ | Explants with bud dormancy breaking, % | Growing buds, % | Number of the formed shoots | Length of the shoots, mm |
|---|---|---|---|---|
| Control | 95.8 ± 4.2 a | 45.4 ± 4.4 a | 2.1 ± 0.3 a | 10.0 ± 1.5 b |
| 0.5 | 96.7 ± 3.3 a | 55.0 ± 5.0 b | 2.5 ± 0.3 a | 6.3 ± 1.0 a |
| 1.0 | 80.6 ± 10.0 a | 42.5 ± 3.2 a | 1.9 ± 0.2 a | 6.3 ± 1.0 a |

Note: The means (M) ± standard error (SE) within a column followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$

However, it should be indicated that cytokinin treatments resulted in curling of some leaves in all shoots (Fig. 3).

The number of the formed shoots depended on the type of cytokinin ($F$ = 8.629, $p < 0.05$), its concentration ($F$ = 90.660, $p < 0.05$), and duration of cultivation ($F$ = 69.886, $p < 0.05$). However, the results indicate that prolonged subculturing of the axillary shoots could not lead to increasing of their multiplication rate ($F$ = 2.185, $p < 0.05$) (Table 4).

**Table 3. Factors affecting the number of formed axillary shoots.**

| Duration of the inductive phase (days) | Type and concentration of the PGRs (mg·l$^{-1}$) | Subculture I | Subculture II | Subculture III |
|---|---|---|---|---|
| | Control (PGRs-free) | 1.1 ± 0.1 a A | 1.3 ± 0.1 abc A | 1.2 ± 0.1 a A |
| | 0.5 BAP | 1.2 ± 0.1 abc A | 1.1 ± 0.1 ac A | 1.2 ± 0.1 ac A |
| 14 days | 1.0 BAP | 1.6 ± 0.1 bc A | 2.1 ± 0.2 de B | 1.9 ± 0.1 b–f AB |
| | 5.0 BAP | 1.5 ± 0.2 abc A | 2.6 ± 0.4 ef B | 2.1 ± 0.2 def AB |
| | 0.5 zeatin + 0.1 IBA | 1.5 ± 0.2 a–d A | 2.0 ± 0.3 c–f A | 2.0 ± 0.3 c–f A |
| | 1.0 zeatin + 0.1 IBA | 1.4 ± 0.3 abc A | 1.6 ± 0.2 a–d A | 1.6 ± 0.2 a–f A |
| | Control (PGRs-free) | 1.3 ± 0.1 ab A | 1.2 ± 0.1 ac A | 1.4 ± 0.1 abc A |
| | 0.5 BAP | 1.4 ± 0.1 abc A | 1.7 ± 0.1 bcd A | 2.6 ± 0.1 ef B |
| 21 days | 1.0 BAP | 1.8 ± 0.1 c A | 2.0 ± 0.2 de A | 1.6 ± 0.1 a–d A |
| | 5.0 BAP | 3.8 ± 0.4 f A | 3.5 ± 0.2 g A | 3.5 ± 0.2 g A |
| | 0.5 zeatin + 0.1 IBA | 2.7 ± 0.4 e B | 2.1 ± 0.2 de AB | 2.0 ± 0.2 df A |
| | 1.0 zeatin + 0.1 IBA | 2.4 ± 0.2 de AB | 2.8 ± 0.2 f B | 2.1 ± 0.1 f A |

Note: The means (M) ± standard error (SE) within a column followed by the same small letter and in the rows followed by the same capital letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$.

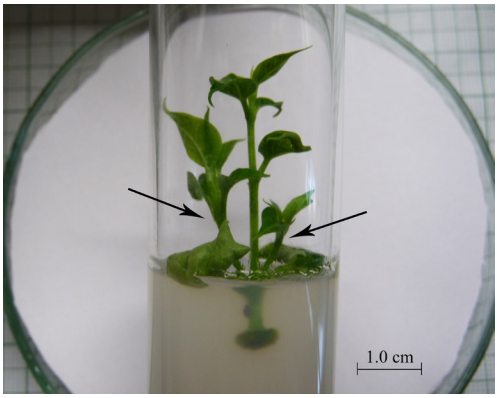


**Fig. 2. Axillary shoot formation during the multiplication stage.**

After 14 days of cultivation and the first subculture the length of shoots was higher in shoots on PGRs free medium (control) but after 21 days of cultivation there were no differences in the shoot length between the used treatments. However, after the second and third subculture, independently of the duration of cultivation, highest length of the shoots was observed on medium supplemented with 1.0 mg·l$^{-1}$ BAP (12.0 ± 1.0 mm and 14.6 ± 1.1 mm; 17.5 ± 1.3 mm and 24.8 ± 2.6 mm, resp.) and the increased concentration of BAP induced depressive effect. The comparison of the equal concentrations of used cytokinins demonstrated that zeatin stimulated statistically significantly the elongation of the shoots after the third subculture, only. In comparison with the lower concentration of zeatin, the higher one (1.0 mg·l$^{-1}$) had stimulating effect after the third subculture, only (Table 5). Key factor for the elongation of the shoots was duration of cultivation ($F$ = 138.862, $p$ < 0.05). The length of the shoots depended on all other investigated factors ($F$ = 5.953, 24.536, 20.999 resp.; $p$ < 0.05) and the interaction between concentration of cytokinin and number of

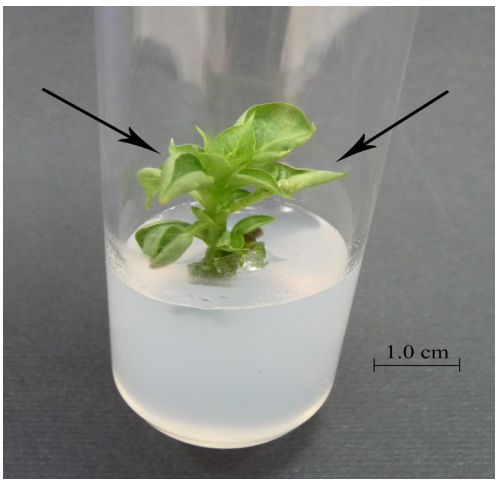


**Fig. 3. Shoots with curling leaves.**

**Table 4. Significance of the studied factors and their combinations on the mean number of the axillary shoots estimated by a post hoc LSD test.**

| Factors | *F* | Level of significance |
|---|---|---|
| TC | 8.629 | 0.003 |
| C | 90.660 | 0.000 |
| D | 69.886 | 0.000 |
| S | 2.185 | 0.113 |
| TC × C | 3.782 | 0.023 |
| TC × D | 2.036 | 0.154 |
| TC × S | 1.137 | 0.321 |
| C × D | 19.152 | 0.000 |
| C × S | 1.336 | 0.238 |
| D × S | 3.117 | 0.045 |
| TC × C × D | 6.230 | 0.002 |
| TC × C × S | 0.646 | 0.629 |
| TC × D × S | 2.504 | 0.082 |
| C × D × S | 2.025 | 0.059 |
| TC × C × D × S | 1.815 | 0.123 |

Note: $R$ Squared = 0.345 (Adjusted $R$ Squared = 0.331), $p$ < 0.05.
Legend: TC = Cytokinin, C = Concentration of the cytokinin, D = Duration of cultivation, S = Number of the subcultures.

**Table 5. Factors affecting the length (mm) of formed axillary shoots.**

| Duration of the inductive phase, days | Type and concentration of the PGRs, mg·l$^{-1}$ | Subculture I | Subculture II | Subculture III |
|---|---|---|---|---|
| 14 days | Control (PGRs-free) | 10.2 ± 0.9 c A | 10.0 ± 0.9 bcd A | 9.1 ± 0.7 a A |
| | 0.5 BAP | 4.9 ± 0.5 ab A | 5.2 ± 0.5 ab A | 5.0 ± 0.5 a A |
| | 1.0 BAP | 8.7 ± 0.6 abc A | 12.0 ± 1.0 d–g B | 14.6 ± 1.1 d B |
| | 5.0 BAP | 4.3 ± 0.4 a A | 6.4 ± 0.7 a A | 6.3 ± 0.6 a A |
| | 0.5 zeatin + 0.1 IBA | 6.1 ± 2.2 abc A | 6.6 ± 1.1 abc A | 6.0 ± 1.1 a A |
| | 1.0 zeatin + 0.1 IBA | 6.1 ± 1.2 abc A | 9.3 ± 1.2 a–g A | 9.2 ± 1.5 ac A |
| 21 days | Control (PGRs-free) | 10.6 ± 1.1 c A | 13.9 ± 1.1 g A | 18.5 ± 1.7 e B |
| | 0.5 BAP | 11.0 ± 1.0 c A | 13.7 ± 1.1 efg A | 13.3 ± 1.0 cd A |
| | 1.0 BAP | 10.5 ± 0.8 c A | 17.5 ± 1.3 h B | 24.8 ± 2.6 f C |
| | 5.0 BAP | 10.1 ± 0.6 c A | 13.7 ± 0.6 fg B | 13.1 ± 0.5 bcd B |
| | 0.5 zeatin + 0.1 IBA | 9.7 ± 0.7 bc A | 9.2 ± 0.7 a–d A | 13.1 ± 0.9 bcd B |
| | 1.0 zeatin + 0.1 IBA | 11.1 ± 1.4 c A | 10.9 ± 0.9 c–g A | 20.9 ± 1.3 e B |

Note: The means (M) ± standard error (SE) within a column followed by the same small letter and in the rows followed by the same capital letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$.

subcultures, and duration of cultivation and number of subculture ($F = 4.605$ and 12.412, resp; $p < 0.05$) (Table 3 and 6).

## Rooting of the axillary shoots

In the absence of IBA (control) the rooting rate was low and the emergence of new roots progressed slowly (Table 7). Very small amount of callus was observed on the base of the shoots in the end of the rooting period cultured on all variants of the medium, supplemented with IBA (Fig. 4).

On medium without transfer of the shoots, the beginning of adventitious root formation was observed after 21 days. Rooting of the shoots was observed after 28 days in all rooting variants of the medium. Statistical difference in the rooting rate after longer period of cultivation (35 days) was found on PGRs free medium, only. The addition of IBA increased rooting response up to 55.6 ± 12.4 %, but statistical significant differences were not observed between investigated concentrations of IBA (Table 7).

The beginning of rooting process for all pulse treatments was observed earlier in comparison with experiments without pulse treatment i.e. after 7 days when the shoots were pulse treated with 7.5 mg·l$^{-1}$ IBA for 72 h and after 14 days in all other durations of inductive phase. The same tendency was found about the finishing of rooting process in comparison with the experiments without transfer of the shoots. All pulse treatments with high concentra-

**Table 6. Significance of the studied factors and their combinations on the mean length of axillary shoots estimated by a post hoc LSD test.**

| Factors | *F* | Level of significance |
|---|---|---|
| TC | 5.953 | 0.015 |
| C | 24.536 | 0.000 |
| D | 138.862 | 0.000 |
| S | 20.999 | 0.000 |
| TC × C | 5.305 | 0.005 |
| TC × D | 0.891 | 0.345 |
| TC × S | 0.855 | 0.425 |
| C × D | 0.766 | 0.513 |
| C × S | 4.829 | 0.000 |
| D × S | 12.412 | 0.000 |
| TC × C × D | 0.912 | 0.402 |
| TC × C × S | 0.746 | 0.560 |
| TC × D × S | 1.271 | 0.281 |
| C × D × S | 1.804 | 0.094 |
| TC × C × D × S | 0.515 | 0.725 |

Note: *R* Squared = 0.135 (Adjusted *R* Squared = 0.124), $p < 0.05$.
Legend: TC = Cytokinin, C = Concentration of the cytokinin, D = Duration of cultivation, S = Number of the subculture.

tion of IBA (7.5 mg·l$^{-1}$) improved significantly the rooting (between 76.6 ± 8.0 % and 82.2 ± 4.4 %) in comparison with all pulse treatments with lower concentration of IBA (Table 7). However, our results showed that there is not statistical difference in the rooting rate between pulse treatments with 5.0 mg·l$^{-1}$ IBA or between pulse treatments with 7.5 mg·l$^{-1}$ IBA. Also, the roots in some shoots appeared above the callus formed (Fig. 5) and in others shoots through the callus (Fig. 6).

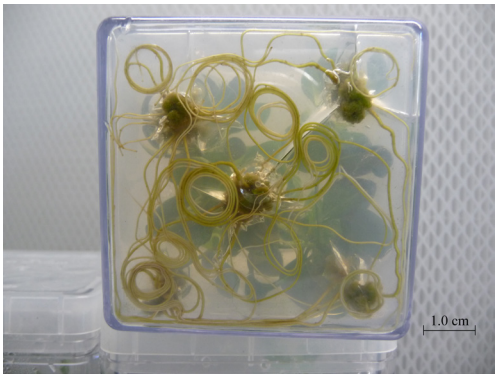


**Fig. 4. Callus formation on the basal part of the shoots in the end of rooting experiments.**

Our results demonstrated that the percentage of rooting depended on all investigated factors (Table 8).

The number of first order roots was higher after the cultivation of the shoots for 48 and 72 h on inductive medium, supplemented with 7.5 mg·l$^{-1}$ IBA, and after cultivation without transfer on medium, containing 5.0 mg·l$^{-1}$ IBA (2.8 ± 0.2, 3.9 ± 0.5 and 3.9 ± 0.5, resp.). Statistical difference between them was not found. The percentage of shoots, originated from PGRs free medium formed significantly more roots of second order (40.8 ± 12.3 %). However, their number was not strongly affected by the different treatments. The first order roots reached highest length after the cultivation of shoots for 48 and 72 h on inductive medium, supplemented with 7.5 mg·l$^{-1}$ IBA (48.4 ± 2.0 mm and 45.4 ± 1.4 mm, resp.). Similar tendency was observed for the second order roots. However, their length was significantly lower (Table 9).

## Discussion

It is well known that *in vitro* propagation of woody plants is strongly affected by the age of stock plant (Bonga 1982, Bonga and von Aderkas 1992, Hartmann et al. 2002). One of the most juvenile plant material are the root suckers (Bonga 1982, Hackett 1985, Waldenmaier and Bünemann 1991, Bonga and von Aderkas 1992, Hackett and Murray 1993,

**Table 7. Factors affecting the dynamics of rooting process, %.**

| Concentration of IBA, mg·l$^{-1}$ | Duration of the inductive phase, h | 7$^{th}$ day | 14$^{th}$ day | 21$^{st}$ day | 28$^{th}$ day | 35$^{th}$ day |
|---|---|---|---|---|---|---|
| Control (PGRs-free) | Without transfer | 0.0 ± 0.0 a A | 0.0 ± 0.0 a A | 0.0 ± 0.0 a A | 2.1 ± 2.1 a A | 22.2 ± 11.8 a B |
| 1.0 | | 0.0 ± 0.0 a A | 0.0 ± 0.0 a A | 8.9 ± 4.4 ab AB | 20.0 ± 3.9 ab BC | 35.6 ± 11.1 ab C |
| 5.0 | | 0.0 ± 0.0 a A | 0.0 ± 0.0 a A | 22.2 ± 5.9 bcd B | 42.2 ± 5.9 b C | 55.6 ± 12.4 bcd C |
| 5.0 (from control) | | 0.0 ± 0.0 a A | 0.0 ± 0.0 a A | 15.6 ± 2.2 bc A | 44.4 ± 5.9 b B | 51.1 ± 9.7 abc B |
| 5.0 | 48 | 0.0 ± 0.0 ab A | 11.1 ± 4.4 ab AB | 13.3 ± 6.7 ab AB | 20.0 ± 6.7 ab B | 24.4 ± 8.0 a B |
| 5.0 | 72 | 0.0 ± 0.0 a A | 24.4 ± 2.2 b B | 28.9 ± 2.2 cd B | 31.1 ± 2.2 ab B | 37.8 ± 5.9 ab B |
| 5.0 | 168 | 0.0 ± 0.0 a A | 8.9 ± 2.2 a A | 33.3 ± 6.7d B | 42.2 ± 5.9 b BC | 51.1 ± 5.9 abc C |
| 5.0 (from control) | 72 | 0.0 ± 0.0 a A | 8.9 ± 2.2 a AB | 20.0 ± 7.7 bcd BC | 35.6 ± 14.6 b C | 57.8 ± 12.4 b–e D |
| 7.5 | 24 | 0.0 ± 0.0 a A | 40.0 ± 7.7 c B | 57.8 ± 2.2 e C | 73.3 ± 7.7 c CD | 76.6 ± 8.0 cde D |
| 7.5 | 48 | 0.0 ± 0.0 a A | 51.1 ± 9.7 cd B | 75.6 ± 2.2 f C | 80.0 ± 3.9 c C | 82.2 ± 4.4 e C |
| 7.5 | 72 | 4.4 ± 4.4 b A | 55.6 ± 5.9 d B | 68.9 ± 8.0 fe BC | 75.6 ± 11.8 c C | 77.8 ± 9.7 de C |

Note: The means (M) ± standard error (SE) within a column followed by the same small letter and in the rows followed by the same capital letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$.

Hartmann et al. 2002). To date, *in vitro* propagation of common lilac has been reported by using explants from mature or young stock plants (Pierik et al. 1988). It was found that rejuvenation by frequently subculturing of the shoots would be necessary for induction of high rate of multiplication and rooting of the axillary shoots. The number of subcultures depends on the age and genotype of stock plant (Pierik et al. 1988). It is known that one of the limiting factors for the autovegetative propagation of woody plants is physiological status of the stock plants i.e. the season of explants collection. However, we found that root suckers, collected in the beginning of spring in active growth are suitable initial plant material for the establishment of *in vitro* culture. Our results indicated that subculture is not obligatory for the rejuvenation and obtaining of high multiplication rate *in vitro*. Also, the results demonstrated that the subcultures did not decrease the multiplication rate. It was found that BAP in concentration of 1.0 mg·l$^{-1}$ is the best for the induction and elongation of axillary shoots (Jankiewicz and Orlikowska 1990, Nesterowicz et al. 2006). However, our results are in agreement with the findings of Pierik et al. (1988), Gabryszewska (1989), and Refouvelt et al. (1998) that the formation and elongation of axillary shoots requires higher level of BAP.

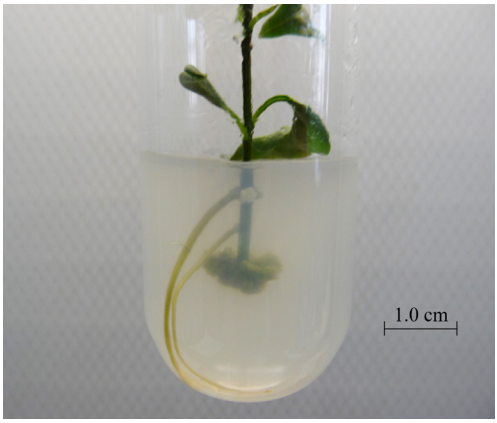


**Fig. 5. Roots appeared above the callus formed.**

**Fig. 6. Roots appeared through the callus.**

Common lilac was considered as difficult-to-root species (Waldenmaier and Bünemann 1991, Marks and Simpson 2000, Ford et al. 2002). It was found that rooting ability of common lilac *in vitro* decreased with the increasing of stock plant age (Waldenmaier and Bünemann 1991). The rooting of the shoots depends on their rejuvenation by subculturing (Pierik 1990), their length, and culture origin (Hildebrandt and Harney 1983, Marks and Myers 1994, Marks and Simpson 2000). Our results demonstrated that subculturing of the shoots during the multiplication stage is not necessary if root suckers are used as initial plant material.

**Table 8. Significance of the studied factors and their combinations on the dynamic of rooting estimated by a post hoc LSD test.**

| Factors | *F* | Level of significance |
|---|---|---|
| C | 103.521 | 0.000 |
| DIP | 4.426 | 0.001 |
| DR | 93.786 | 0.000 |
| C × DIP | 6.780 | 0.009 |
| C × DR | 8.046 | 0.000 |
| DIP × DR | 2.107 | 0.006 |
| C × DIP × DR | 1.659 | 0.157 |

Note: *R* Squared = 0.347 (Adjusted *R* Squared = 0.335), $p < 0.05$.
Legend: DR = Duration of rooting, C = Concentration of IBA, DIP = Duration of inductive phase.

It is well known that the type and concentration of auxin has a central role in the induction of adventitious roots (De Klerk 2001, De Klerk et al. 1997, 1999, Kurepin et al. 2011). These factors could lead to variation in the percent of rooting and asynchrony of root expression in Common lilac (Wainwright 1987, Marks and Simpson 2000). Also, it was demonstrated that auxin pulse treatment is critical for the rooting of plants *in vitro* and depends on the duration of inductive phase (De Klerk 1996, 2001, 2002, De Klerk et al. 1999, Mitras et al. 2009, Dancheva 2012). After the inductive phase, once the cells have been determined to root formation, auxin is no longer required for the rooting of *Syringa vulgaris* shoots (Marks and Simpson 2000). The effect of IBA and its concentration is documented in a few studies (Waldenmaier and Bünemann 1991, Nesterowitz et al. 2006), but in some of them the percentage of rooted shoots is not indicated (Nesterowitz et al. 2006). Our results showed that rooting response and number of roots depend on the duration of cultivation on inductive medium. The cultivation of shoots to a root induction medium for shorter period improved the rooting and number of the formed roots. These results demonstrated that the balance between the concentration of the used auxin and the duration of inductive phase are critical for the rooting rate and quality of the root system.

**Table 9. Factors affecting the quality of the root system.**

| Concentration of IBA, mg·l$^{-1}$ | Duration of the nductive phase, h | First order roots | | Second order roots | | |
|---|---|---|---|---|---|---|
| | | Number | Length, mm | Shoots with second order roots, % | Number | Length, mm |
| Control | Without transfer | 1.6 ± 0.3 a | 23.4 ± 4.4 abc | 0.0 ± 0.0 a | 0.0 ± 0.0 ab | 0.0 ± 0.0 a |
| 1.0 | | 2.3 ± 0.4 a | 26.8 ± 3.2 abc | 4.8 ± 4.8 a | 0.3 ± 0.3 ab | 0.8 ± 0.4 a |
| 5.0 | | 3.9 ± 0.5 bc | 20.0 ± 1.3 a | 8.5 ± 4.3 a | 0.1 ± 0.1 a | 0.2 ± 0.24 a |
| 5.0 (from control) | | 2.3 ± 0.5 a | 33.7 ± 2.1 c | 40.8 ± 12.3 c | 1.2 ± 0.4 bc | 5.4 ± 0.9 bc |
| 5.0 | 48 | 1.4 ± 0.2 a | 19.9 ± 5.1 ab | 11.1 ± 11.1 ab | 0.6 ± 0.5 abc | 3.1 ± 1.2 ab |
| 5.0 | 72 | 2.3 ± 0.5 a | 21.7 ± 1.8 ab | 17.9 ± 9.0 ab | 0.8 ± 0.6 abc | 2.4 ± 0.7 ab |
| 5.0 | 168 | 2.3 ± 0.4 a | 43.6 ± 3.2 de | 9.7 ± 5.0 a | 0.1 ± 0.1 a | 1.3 ± 0.8 a |
| 5.0 (from control) | 72 | 2.0 ± 0.2 a | 30.2 ± 2.5 bc | 32.4 ± 9.0 bc | 1.3 ± 0.4 c | 7.8 ± 1.1 c |
| 7.5 | 24 | 2.7 ± 0.3 ab | 41.4 ± 1.9 d | 14.6 ± 7.3 ab | 0.4 ± 0.2 ab | 3.6 ± 1.0 ab |
| 7.5 | 48 | 2.8 ± 0.2 abc | 48.4 ± 2.0 e | 19.3 ± 4.0 abc | 0.7 ± 0.3 abc | 4.9 ± 0.9 b |
| 7.5 | 72 | 3.9 ± 0.5 c | 45.4 ± 1.4 de | 18.8 ± 5.1 ab | 0.8 ± 0.3 abc | 5.3 ± 0.9 bc |

Note: The means (M) ± standard error (SE) within a column followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$.

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# EVALUATION OF THE PROBLEMS OF LAKE PAMVOTIDA ASSOCIATED WITH THE RESIDENTS OF THE CITY OF IOANNINA (GREECE)

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## Abstract

Lake Pamvotida offers an array of significant advantages to the inhabitants of the city of Ioannina that are related both to the enhancement and recreation of the landscape, and the traditional usages of water. However, the ongoing intensification of water use, in combination with the pollution and faulty interventions that were undertaken in the past, have created problems in the lake as well as in the wider area. According to the inhabitants who live within the extent of the lake, the overuse of agricultural pesticides, lack of cleansing, increase of waste, as well as the inappropriate use of biological cleaning material are among the most serious problems that the surrounding lakeside region encounters. Lack of signposting and a museum of natural history also constitute part of the problem in the area. More particularly, inhabitants consider that the causes of their inability to solve these problems are to be found in the insufficient environmental education, scarcity of financial resources and lack of coordinated actions on the part of public stakeholders, such as the local administration, the prefecture administration and the central power, as well as private individuals and groups in the area, the union of hotel owners and/or freelance etc, environmental organizations and the local press, for instance. Based on the inhabitants' evaluations with respect to the significance of the problems facing the lake display little satisfaction concerning the actions that they have undertaken with regard to the protection of the lake.

**Key words:** stakeholders activities, protection, management of the lake.

## Introduction

Lakes and rivers that are within easy reach of cities offer a variety of significant advantages both to the urban centers and, more particularly, to the people who live close to the water and, thus, can improve their quality of life (Tampakis et al. 2013). These advantages are closely related both to traditional quantitative usages of water such as domestic use, for agricultural purposes, fishing, hunting, as well as protection against natural disasters (Pacione 2003, Tsiadikoudis et al. 2012) and benefits that are associated with the enhancement and recreation of the landscape's aesthetics (Lansford and Jones 1995, Klessig 2001, Vesterinen et al. 2010).

Generally, the fundamental role of water for the human life is recognized by water

legislative frameworks in developed societies (Scott and Coustalin 1995, Cullet 2011, Grafton and Hussey 2011), which set rights and obligations on its use, as a result of demographic expansion, use-oriented conflicts and perceived problems of scarcity (Kuks 2004, Pettella et al. 2012). However, the ongoing intensification of water use of lakes and rivers threatens the balance of their sensitive ecosystems in a variety of areas of our planet and more evidently in underdeveloped countries (Eiswerth et al. 2011). Lake Pamvotida in the prefecture of Ioannina constitutes a case study in Greece. The harmonic coexistence of the city and the lake has been disturbed over the last years due to the growth of the city of Ioannina and its surrounding area (Albanis et al. 1986, Panou 2011). The industrial and especially the agricultural production have increased rapidly, improving the standards of living of the inhabitants in the area but at the same time they cause ecological problems both in the lake as and the wider region (Lambrou 1998, Vrigka 2003). Nowadays, the lake is confronting significant problems of eutrophication that are related to the elimination of water transparency, the characteristically unpleasant odor, death of fish, reduction of fishing, excessive increase of aquatic vegetation, etc. The increased figures of phosphorus, nitrogen and chlorophyll-a are indicative of the eutrophication in the lake (Albanis et al. 1986).

In the past, many interventions were undertaken and, although being resolved with initially good intentions, they have had a negative impact on the ecosystem of the lake on a long-term basis (Barba 2008, Panou 2011). Such interventions as the drainage of the Lapsista (a small lake that united with Lake Pamvotida), the construction of lakefront roads and low walls surrounding the lake acted as dams that closed some water sources up and obstructed the incoming of clean water into the lake. The polders used for the configuration of places of public utility (squares, etc.) have contributed, in the long-term, to a change in the natural coast, a reduction of the lake surface and its total water volume, as well as the limitation of its cleansing potential. Most importantly they have changed the society's perception and image of the lake.

The aim of the present research was to study how inhabitants in Ioannina perceive the problems that have been caused due to the faulty interventions and improper management of the lake. The evaluation of these problems is very important for their resolution, as it is believed that only when problems have ultimately become a source of constant disturbance in the life of the greatest part of local population it is possible that unanimous decisions for their solution will be taken and the inhabitants themselves will be even more willing to aid both ethically and financially.

## Materials and Methods

The research has been conducted with the use of individual interviews in the municipality of Ioannina and random sampling has been applied for the sake of their simplicity in procedure (Damianos 1999, Matis 2001, Kalamatianou 2000).

The population ratio that is also impartial evaluation of the real ratio of the population p and the assessment of the standard error of the population ratio of the $S_p$ without correction of the finite population as the sampling fraction is small, has been calculated using formulae of simple random sampling.

To calculate the size of the sample we thought it would be necessary to conduct pre-sampling with a sample size of 50 individuals. The size of this sample was calculated based on the formulae of simple random sampling (where $t$ = 1.96 and $e$ = 0.048) (Kalamatianou 2000, Matis 2001). Even though simple random sampling without off reset was used, the correction of the finite population can be omitted, as the sample size n is small in relation to the population size $N$ (Pagano and Gauvreau 2000). More specifically, the sample size was set to 417 individuals.

The data collection was carried out during July to December of 2009. The individuals of the sample were then located and personal interviews were conducted. In some cases, in which this was not possible to be done, we followed the same procedure in the selection of new units of sampling.

The total number of questions which were reported to be possible problems of the lake constitutes a multi-theme variable on which reliability analysis was applied. In particular, in order to find out the internal reliability of a questionnaire (Frangos 2004), i.e. if our data have the tendency to measure the same thing we used the alpha coefficient (or reliability coefficient a-Cronbach (Howitt and Cramer 2003). If the alpha coefficient is 0.70 or higher it is regarded satisfactory (Howitt and Cramer 2003), and if it is higher than 0.80 it is regarded very satisfactory. In real practice, it is frequent that lower reliability coefficients, that are with values no higher than 0.60, are also accepted (Siardos 1999).

However, the checking must not only be reliable, but credible as well and this is performed through the application of factor analysis (Siardos 1999). Factor analysis is a statistical method which aims to discover the existence of factors, common within a group of variables (Sharma 1996). In particular, we used the method of principal components which is based on the spectral analysis of the variance table (correlation) (Djoufras and Karlis 2001). Regarding the significance of the principal components, the criterion which was used was the one suggested by Guttman and Kaiser (Cattell 1978, Frangos 2004), according to which, the limit for the collection of the appropriate number of the principal components is determined by the values of typical roots, equal or higher to one. Furthermore, we also used the matrix rotation of the main factors applying the Kaiser's method of maximum variance rotation (Harman 1976).

## Area of Study

Lake Ioannina or the Pamvotida or the Pamvotis (the name Pamvotis first appears in the 12$^{th}$ century commentary on the Odyssey of Eustathius of Thessaloniki) is Europe's second-oldest lake and the largest lake of Epirus, located in the central part of the Ioannina regional unit in northern Greece. The city of Ioannina to the west and the town of Perama to the north are urban settlements fringing the lake, while the remaining of its periphery is comprised of farmland. The lake features small fishing ports and a boating port. There is a regular boat service to the Island of Ioannina, where Ali Pasha was hiding during the last days of his reign, and it is situated near the northern shore. The Greek National Road 6 surrounds the northern half of the lake. The magical scenery of Lake Pamvotida is a true gem for the city of Ioannina and a pole of attraction for the region of Epirus in northern Greece. Its calm waters constitute the

ideal setting for the hosting of the water ski tournament.

Lake Ioannina is home to the Tsima, a species of fish endemic to the lake. Two bryozoan species have recently been reported from the lake (Massard and Geimer 2008).

### Demographic characteristics

With regard to the information sought after concerning the demographic characteristics of the population of Ioannina, Table 1 shows that most of them are graduates of secondary education, and their age ranges between 18–30 years old, private employees, married and with no children.

## Results of the Study and Discussion

### The problems of Lake Pamvotida

Since 1900 up to the present day, the ecosystem of Lake Pamvotida has undergone

**Table 1. Citizens' Socio-demographic profile.**

| | | | | | |
|---|---|---|---|---|---|
| 1. **Gender** | | | | | |
| Male | Female | | | | |
| 49.1 % ($S_p$=0.0179) | 50.9 % ($S_p$=0.0179) | | | | |
| 2. **Age** | | | | | |
| 18–30 | 31–40 | 41–50 | >50 | No answer | |
| 20.4 % ($S_p$=0.0176) | 50.4 % ($S_p$=0,0153) | 23.3 % ($S_p$=0,0178) | 5.4 % ($S_p$=0,0069) | 0.5 % ($S_p$=0.0024) | |
| 3. **Marital Status** | | | | | |
| unmarried | married | divorced or widowed | did not answer the question | | |
| 23.2% ($S_p$=0.0181) | 64.4% ($S_p$=0.0208) | 12.3% ($S_p$=0.0196) | 0.2% ($S_p$=0.0015) | | |
| 4. **Childhood** | | | | | |
| Without children | one child | two children | three children | more than three children | |
| 29.1 % ($S_p$=0.0213) | 26 % ($S_p$=0.0171) | 30 % ($S_p$=0.0171) | 10.4 % ($S_p$=0.0098) | 4.6% ($S_p$=0.0062) | |
| 5. **Educational level** | | | | | |
| Primary School | Lower Secondary School | Technical School | Upper Secondary School | Technological education | University |
| 4.9 ($S_p$=0.0053) | 5.4 % ($S_p$=0.0079) | 16.1 % ($S_p$=0.0120) | 22.8 % ($S_p$=0.0196) | 20.5 % ($S_p$=0.0169) | 28.9% ($S_p$=0.0231) |
| **6. Profession** | | | | | |
| private employee | public servants | self-employed | students | | |
| 37 % ($S_p$=0.0144) | 16.5 % ($S_p$=0.0168) | 18.2 % ($S_p$=0.0197) | 6.3 % ($S_p$=0.0114) | | |
| unemployed | housewives | farmers or stock-breeders | pensioners | did not answer the question | |
| 3 % ($S_p$=0.0059) | 11,6 % ($S_p$=0.0083) | 3 % ($S_p$=0.0059) | 4 % ($S_p$=0.0068) | 0.4 % ($S_p$=0.0021) | |
| 7. **Annual income** | | | | | |
| <5,000 euro | 5,000–10,000 euro | 10,001–15,000 euro | 15,001–20,000 euro | >20,000 euro | No answer |
| 5.1 % ($S_p$=0.0101) | 7.5 % ($S_p$=0.0058) | 26.5 % ($S_p$=0.0218) | 21.4 % ($S_p$=0.0189) | 5.4 % ($S_p$=0.0074) | 34 % ($S_p$=0.0199) |

significant changes, although the lake has been integrated within the European Ecological Network of Special Conservation Areas 'Natura 2000' (Environment DG – European Commission 2001).

The surface of the lake has been reduced by 25–30 % and its water volume has also been reduced by more than 32 % while the reduction of its average depth is measured around 57 % (Management Agency of Lake Pamvotida 2012). With respect to the drop of the water level in the lake, 41 % of the inhabitants in the area claimed that this problem is moderate, while 25.7 % stated that it is a big problem (Table 2).

The mound that was raised around the lake of Ioannina during the period of 1969–1974 as well as the respective concrete scaffolding delimited the lake arbitrarily and also detracted vital areas from the lake that are up to 10 % of its surface such as the temporarily flooded wet pastures in the north (Amfithea-Perama) and the currently polded plots in the south extending from Ioannina to the Katsika area. It can be stated that 50 years ago, the lake surface was a lot more expanded than it is nowadays. The arbitrary delimitation of the lake using the artificial scaffolding has led to polders and plotting, mainly upon the south section of the area (Management Agency of Lake Pamvotida 2012). Inhabitants' perception of the problems the area faces due to arbitrary constructions was reported to be as moderate in 27.8 % of the cases and big (Fig. 1), in 22.8 %.

Referring to the problem of lack of policing in the area of the lake, its surveillance seems to be relatively important to the inhabitants of the area, who claim that the lack of policing may have balanced the situation. In 31.4 % of the cases, inhabitants consider this problem to be moderate, another 23.5 % big and 17.7 % very big. However, the lack of surveillance in the lake is the main cause of a series of other problems that have appeared in the

**Table 2. Evaluation of problems in the Pamvotida lake by the citizens.**

| Problem | Very little | | little | | Moderate | | Big | | Very big | | No answer | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | $S_p$ | % | $S_p$ | % | $S_p$ | % | $S_p$ | % | $S_p$ | % | $S_p$ |
| Drop of the water level | 7.4 | 0.0128 | 17.7 | 0.0187 | 41.0 | 0.0241 | 25.7 | 0.0214 | 7.7 | 0.0130 | 0.5 | 0.0034 |
| Arbitrary Constructions | 13.7 | 0.0168 | 20.9 | 0.0199 | 27.8 | 0.0219 | 22.8 | 0.0205 | 13.7 | 0.0168 | 1.2 | 0.0053 |
| Lack of Surveillance/ Policing | 9.8 | 0.0146 | 16.3 | 0.0181 | 31.4 | 0.0227 | 23.5 | 0.0208 | 17.7 | 0.0187 | 1.2 | 0.0053 |
| Presence of Rubbish and lack of cleansing | 4.3 | 0.0100 | 7.7 | 0.0130 | 22.1 | 0.0203 | 33.1 | 0.0230 | 32.6 | 0.0230 | 0.2 | 0.0024 |
| Inappropriate use of biological cleansing | 6.7 | 0.0123 | 9.4 | 0.0143 | 21.1 | 0.0200 | 32.6 | 0.0230 | 28.1 | 0.0220 | 2.2 | 0.0071 |
| Use of agricultural pesticides | 4.3 | 0.0100 | 6.5 | 0.0121 | 16.1 | 0.0180 | 31.4 | 0.0227 | 39.8 | 0.0240 | 1.9 | 0.0067 |
| Lack of an Exhibition Venue – Natural History Museum | 5.0 | 0.0107 | 11.3 | 0.0155 | 33.1 | 0.0230 | 29.7 | 0.0224 | 20.1 | 0.0196 | 0.7 | 0.0041 |
| Lack of signposting | 4.6 | 0.0102 | 12.2 | 0.0160 | 27.8 | 0.0219 | 35.3 | 0.0234 | 19.9 | 0.0196 | 0.2 | 0.0024 |
| Illegal Hunting | 8.2 | 0.0134 | 19.4 | 0.0194 | 28.3 | 0.0221 | 25.7 | 0.0214 | 17.7 | 0.0187 | 0.7 | 0.0041 |
| Overfishing | 7.2 | 0.0127 | 19.2 | 0.0193 | 36.7 | 0.0236 | 26.6 | 0.0216 | 10.3 | 0.0149 | | |

**Fig. 1. Polders in Lake Pamvotida (http:/www.lakepamvotis.gr/articles/draseis).**

lake in the last years and are related to the lack of cleansing and the increase in the quantity of waste in and around the area of the lake.

The creation of the mound and the scaffolding has led to a dramatic restriction of the potential of natural self-cleansing of the lake. The coast that once existed in front of the gangway or pier/dock, where the lake washed up waste and deposited organic material which was oxygenated by the waves, and was rising every time north winds were blowing, no more exists. Nowadays, the whole area has been polded with concrete and a municipality park has been constructed in this site. The cleansing of Lake Pamvotida with the aid of mechanical means is scarce for the lack of organized cleansing planning. There are only two pieces of machinery that clean the water and this is done only in cases of water pollution reaching the highest level. As for the question concerning the lack of cleansing and presence of waste in the lake, 33.1 % of the participants in the study believes that it is a big problem, and 32.6 % of the inhabitants answering that it is a very big problem. It was only in 1992 when biological cleansing started in Ioannina. However, all urban sewage from the island, as well as from other lakefront settlements pours out in the Pamvotida, since these areas have never been connected with the system of biological cleansing and most sewers are located below the surface of the lake. Moreover, inhabitants' allegations abound concerning the sewage leakage from the residences in the city, but the authorities state that they are unaware of the accurate source of the sewage. The municipality and the Municipality Agency of Water Supply and Sewage (MAWSS) insist that the problem is attributed to the illegal connections to the system of biological cleansing, while the prefecture claims that the central tube has waterproof problems that have never been dealt with (Management Agency of Lake Pamvotida 2012). In regard to the question of the inappropriate use of biological cleansing in the lake, results show that the existence of this problem is a big concern to the inhabitants. Therefore, 32.6 % of the participants answered that it is a big problem and 28.1 % said that that the problem is very big (Fig. 2).

The lake is largely burdened with increased quantities of nitrogenous and phosphorus-based pesticides that are flushed from agricultural crops. The quantities of pesticides and insecticides are considered to have been quadrupled in the average use. For this reason, inhabitants consider pesticides to be the most

important problem of all. More specifically, 39.8 % of the participants notes that this problem is very big and 31.4 % ($S_p$ = 0.0227) of the inhabitants thinks that this problem is big as it leads to the eutrophication of the lake that is directly related to the extinction of fauna and the reduction of water quality.

**Fig. 2. Illegal sewage leakage (http:/www.lakepamvotis.gr/limne-kai-antr).**

About a year ago, in April 2012, the information centre of Lake Pamvotida was founded. Its contribution is deemed to be important. However, the foundation of the Centre for the Protection of Natural Environment – Museum of Natural History dedicated to the study, conservation and protection of natural environment can establish a new line of educational policy for the preservation of natural environment and educate tens of visitors on a daily basis, mostly children of school age. The primary purpose of such a place should be the collection and preservation of every species of fauna, flora and mineral wealth. Their exposition offers an opportunity for study, research, education and recreation. Visitors have the possibility to come in contact with the kingdom of plants and animals, in order to observe the interaction of plant and animal world as well as the interdependence and balance of the ecosystems. The lack of an exhibition venue and of a Natural History Museum of the lake is considered to be a disadvantage for the area according to the inhabitants. Consequently, 33.1 % of the inhabitants classify this problem as moderate, 29.7 % of them stated that this problem is big and 20.1 % of the individuals asked, stated that this problem is very big. Similarly, these percentages are relative to those who believe that lack of signposting for the flora and fauna of the area is a grave omission. According to their responses, 35.3 % of the inhabitants believe that lack of signposting is a big problem, 27.8 % of them report it as a moderate problem.

The Lake is also a place where many species of birds, such as ducks, geese, and coots spend their winter there, while ferruginous (*Aythya nyroca* Güldenstädt, 1770) has additionally been observed, which is a globally endangered species. Their populations are smaller compared to those of the past. Hunting is altogether prohibited in the lake. Nevertheless, according to many allegations, made on behalf of people who are inhabitants of the area, there are references to systematic hunting carried out every afternoon in the little island, in the Katsikas area and in the Perama-Amfithea area even with the use of a boat inside the lake. With respect to the question concerning illegal hunting, the responses we elicited reinforce their allegations making reference to a visible danger. More specifically, 28.3 % of the participants said that this problem is moderate while 25.7 % of the inhabitants stated that it is big.

Excessive exploitation of natural wealth and overfishing also cause problems to the ecosystem of the lake. Nevertheless, the responses concerning this specific problem showed that 36.7 % ($S_p$ = 0.0236) considers overfishing a problem of moderate significance, 26.6 % of the inhabitants stated that it is big. In an open-ended question regarding other kinds of problems that they can spot, only 10 % of them provided an answer. These problems are related with the allocation of responsibilities for the management of the lake, inhabitants' indifference and lack of governmental willingness to take action. Furthemore, problems are found in regard to the lack of delimitation of the lake, the encroachment of coastal areas, the excessive number of nighclubs, that lead to degradation of the area, the lack of advertising of the lake, its consequent tourist decline and the concentration of sediment in the seabed. Problems were also reported, on the subject of the reduction of clean water from sources so that the lake water can be renewed and the lack of enriching of the lake with fish, the intense movement of boats within the lake, the traffic jams in the vicinity of the lake as well as the presence of noise pollution in the area, the lack of a cycle lane and of a sporting centre.

For this multi-form variable we applied a realiability analysis after we have done the necessary checks. Reliability coefficient alfa is 0.8510 and this result constitutes strong evidence that the grades of the scale are logically consistent, i.e. our data affirm this to measurement. Besides, this is also confirmed by the significantly high individual correlation coeffiecients alfa, after the deletion of any infrastucture, an increase in correlation coefficient can not be achieved.

Moreover, before we proceed on with the application of factor analysis, the necessary checks were done. The index Keiser-Meyer-Olkin (KMO) has a figure of 0.823. It is suggested that the index KMO should be bigger than 0.80, however figures bigger than 0.60 are also accepted (Sharma 1996). Furthermore, Bartlett's sphericity checking rejects the null hypothesis so that the correlation table is unitary and that the coefficients of partial correlation are low. Also the suitability measures taken for the sampling procedure (MSA) have range from high to very high figures, which supports the view that the paradigm of factor analysis is acceptable.

In Table 3 we can see that the factors that were extracted were three and they all have a characteristic root bigger than 1. Additionally, the second column shows the percentage of variation that is attributed to every factor while the third column shows the percentage of variation that is attributed to every factor succeeding rotation. The bigger the loading of a variable the more this factor is responsible for the total variation of the grades taking the variable into consideration. The variables that 'belong' to every factor, are those for which the loading (columns 1, 2, 3) is bigger (than the figure of 0.5) in this factor.

Factor 1 includes the variables 'arbitrary constructions', 'lack of surveillance – policing' 'presence of rubbish and lack of cleansing', 'inappropriate use of biological cleansing' and 'use of agricultural pesticides' and we can name it as 'Pollution problems that are related to the supervision and management of the lake'.

Factor 2 can be named as 'Problems related to the protection of fauna' and includes the variables 'illegal hunting' and

'overfishing'. However, it would not be wrong if problems that are associated with 'arbitrary constructions' and the 'reduction of water level' could also be included in this factor even if their figure is below 0.5. The second variable with the same figure is also included in Factor 1. Therefore the two variables can be considered as act as bridges between Factor 1 and Factor 2.

Factor 3 includes the variables 'lack of an Exhibition Venue – Natural History Museum' and 'lack of signposting' and we can name it as 'Advertising and Prominence Problems of the lake'.

**Table 3. Table of Factor Loadings, before and after rotation concerning the problems of the lake.**

| Variable | Factor loadings | | | | | |
|---|---|---|---|---|---|---|
| | Before rotation | | | After rotation | | |
| | 1 | 2 | 3 | 1 | 2 | 3 |
| Reduction of the water level | 0.533 | –0.222 | –0.133 | 0.421 | 0.417 | 0.016 |
| Arbitrary Constructions | 0.699 | –0.346 | –0.014 | **0.658** | 0.418 | 0.004 |
| Lack of Surveillance/ Policing | 0.664 | –0.004 | 0.168 | **0.564** | 0.216 | 0.323 |
| Presence of Rubbish and lack of cleansing | 0.708 | –0.347 | 0.349 | **0.850** | 0.118 | 0.077 |
| Inappropriate use of biological cleansing | 0.666 | –0.235 | 0.332 | **0.759** | 0.100 | 0.153 |
| Use of agricultural pesticides | 0.758 | –0.003 | 0.195 | **0.645** | 0.244 | 0.371 |
| Lack of an Exhibition Venue – Natural History Museum | 0.555 | 0.668 | 0.144 | 0.154 | 0.120 | **0.858** |
| Lack of Signposting | 0.565 | 0.677 | 0.117 | 0.144 | 0.146 | **0.865** |
| Illegal Hunting | 0.702 | 0.100 | –0.581 | 0.160 | **0.856** | 0.286 |
| Overfishing | 0.671 | –0.085 | –0.574 | 0.229 | **0.850** | 0.112 |

## Actions taken on the part of Local Stakeholders for the protection of the lake

It is known that one of the most fundamental elements in the formulation of environmental policy is the approach adopted and the level of participation exhibited by all interested parties – stakeholders and this is because they act differently and according to their values, knowledge and interest (Duerksen et al. 1997). The improvements that are required so that the quality of nature can be protected pertain to the political and local financial interests even for protected areas (Bachert 1991). As a consequence, one of the first priorities in the environmental policy should be the effective management and improvement of the already existing network of the protected area. Sufficient funding is basically the same thing as political support (Eidsvik 1977). In our case here, funding for this purpose is insufficient as it is shown by the dissatisfaction expressed by most of the inhabitants in Ioannina in their responses when asked to evaluate various stakeholders – groups that participate in actions for the protection of the lake (Table 4).

More specifically, in Table 4, it seems that citizens of Ioannina evaluate the actions taken on the part of local administration rather negatively as the 24 % of the cases are not satisfied at all and the 53.5 % of the cases seem to be very little

**Table 4. Satisfaction degree for the stakeholders activities for the protection of the lake.**

| Stakeholders (Institution or group) | Absolutely | | Very much | | A Little Bit | | Not al all | | No answer | |
|---|---|---|---|---|---|---|---|---|---|---|
| | % | $S_p$ | % | $S_p$ | % | $S_p$ | % | $S_p$ | % | $S_p$ |
| Local Administration | 3.4 | 0.0080 | 18.9 | 0.0192 | 53.5 | 0.0244 | 24.0 | 0.0209 | 0.2 | 0.0024 |
| Prefecture Administration | 7.9 | 0.0132 | 24.9 | 0.0212 | 48.7 | 0.0245 | 18.2 | 0.0189 | 0.2 | 0.0024 |
| Central Government (State) | 2.4 | 0.0075 | 7.7 | 0.0130 | 49.2 | 0.0245 | 40.3 | 0.0240 | 0.5 | 0.0034 |
| Groups of citizens (e.g. inkeepers' union, freelance union) | 1.9 | 0.0067 | 13.7 | 0.0168 | 52.8 | 0.0244 | 30.5 | 0.0225 | 1.2 | 0.0053 |
| Non-governmental ecological organizations | 2.6 | 0.0078 | 17.7 | 0.0187 | 57.3 | 0.0242 | 20.9 | 0.0199 | 1.4 | 0.0053 |
| Local Press | 4.6 | 0.0102 | 24.2 | 0.0210 | 57.3 | 0.0242 | 13.2 | 0.0166 | 0.7 | 0.0041 |

satisfied. However, they evaluate the actions taken by administration of the prefecture a bit more positively as most of the inhabitants (48.7 %) state that they are a little bit satisfied and 24.9 % of them are very satisfied.

With respect to the actions taken on the part of central government concerning the protection of the lake the biggest percentage of the citizens (49.2 %) says that they are a little bit satisfied while 40.3 % states that they are totally dissatisfied. With respect to the actions taken on the part of different local stakeholders such as the innkeeper's union, freelance union, etc the inhabitants display some satisfaction in a percent of 52.8 % while the 30.5 % of the cases are not at all satisfied with them.

With respect to the actions taken on the part of ecological organizations concerning the protection of the lake, it seems that the inhabitants are not satisfied at all (20.9 %) and around 57.3 % seem to be a little bit satisfied. Oftentimes, the non-governmental organizations (NGO) try to get support in order to organize actions that are not directly related to the preservation of the lake. Their rationale is that if they offer to the inhabitants programs that can fulfill their needs then they are even more willing to cooperate with NGOs and take part in actions adopted for the protection and upgrade of the lake. The inhabitants' in Ioannina hold a positive view with respect to the actions taken on the part of local press concerning issues of the lake. Their views with regard to these actions show that 57.3 % of the inhabitants are a little bit satisfied and 24.2 % of them are very satisfied.

Afterwards, the reliability analysis was applied for these questions after having done all necessary checks. The figure of correlation coefficient alfa is 0.796 and this constitutes strong evidence that the grades of the scale are logically consistent, i.e. the data have the tendency to measure the same thing. This is also supported by the significantly high individual reliability coefficients alfa when after the deletion of any variable, a reliability coefficient increase cannot be achieved.

Moreover, before we move to the application of factor analysis all necessary checks were done. The index Keiser-

Meyer-Olkin has a figure of 0.768. Furthermore, Bartlett's sphericity checking rejects the null hypothesis in that the correlation table is unitary and that the coefficients of partial correlation are low. Also the suitability measures taken for the sampling procedure (MSA) vary from high to very high figures, which support the view that the paradigm of factor analysis is acceptable.

The factors that were extracted were two and they have a characteristic root bigger than 2. Table 5 shows the loadings which are the partial correlation coefficients of the six variables with each of the two variables that have come out of the analysis.

Factor 1 includes the variables 'local administration', 'prefecture administration' and 'central government (state)' and we can name it as 'stakeholders of state administration'. Factor 2 can be named as 'stakeholders that affect decisions taken by the administration' and includes the variables 'groups of citizens (e.g. inkeepers' union, freelance union, etc),' 'non-governmental ecological organizations' and 'local press'.

More specifically, citizens regard insufficient environmental education (50.6 %) as one of the most important obstacles for the solution of the problems in the lake (Fig. 3), followed by lack of financial funding (45.1 %) and lack of coordinated actions for the solution of the issues that accrue in relation to the area of the lake (41.7 %). On the other hand, citizens consider insufficient updating (20.1 %) and non-participation of citizens in the making of decisions concerning the lake (25.2 %) to be obstacles of minor importance.

## Conclusions

Over the last few decades, huge changes have been brought about in agricultural and intensive farming, which have had an impact on the environment and the quality of life of people who have been living near lakes (Seihas et al. 2012, Tampakis et al. 2013). An example of this is Lake Pamvotida which is an integral part of the city of Ioannina affecting its inhabitants' lives. The most important problems that inhabitants of the lake are facing are the excessive use of agricultural pesticides, lack of cleansing and the increase of waste in and around the lakefront area as well as the inappropriate use of biological cleansing. Moreover, lack of signposting and a Natural History Museum are also big problems for the inhabitants of this

**Table 5. Table of factor loadings, before and after rotation related to the stakeholders' actions.**

| Variable | Factor loadings | | | |
|---|---|---|---|---|
| | Before rotation | | After rotation | |
| | 1 | 2 | 1 | 2 |
| Local Administration | 0.801 | –0.400 | **0.878** | 0.176 |
| Prefecture Administration | 0.784 | –0.327 | **0.819** | 0.224 |
| Central Government (State) | 0.730 | –0.311 | **0.767** | 0.204 |
| Groups of citizens (e.g. inkeepers' union, freelance union) | 0.668 | 0.290 | 0.349 | **0.639** |
| Non-governmental ecological organizations | 0.540 | 0.650 | 0.027 | **0.844** |
| Local Press | 0.681 | 0.380 | 0.303 | **0.719** |

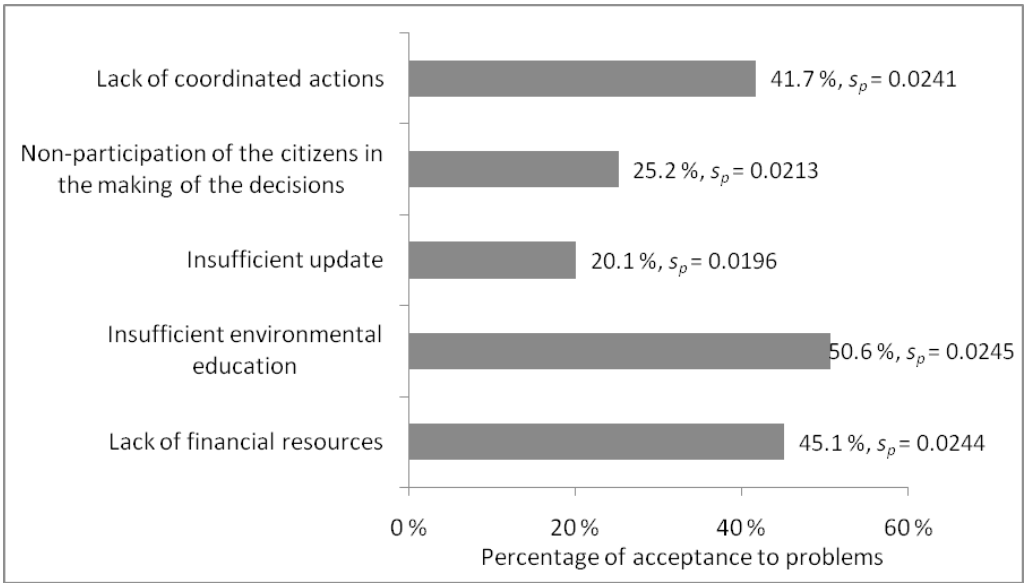


**Fig. 3. Citizens' acceptance of the obstacles for the solution of problems in the lake.**

area. The reduction of water level, overfishing, illegal hunting and arbitrary constructions along the lake were evaluated as problems of moderate importance.

It is only until recently that necessary attention has been given to these problems and great efforts have been employed for their solution. Ignorance on the part of the inhabitants of Ioannina about the adverse consequences that these problems will have on their quality of life and their lust for more arable land have led to the drainage of Lapsista lake. The consequences of the ecological disaster are obvious up to the present day. The flora and fauna of the ecosystem have been greatly affected and the quality of the water in the lake has been downgraded.

What is more, the lake has been encroached by the local stakeholders as well as by individuals who have been dealing with a variety of activities in the surrounding area that result in profile distortions of the area as the time goes by. The human factor is responsible for the increase of filth that inserts into the lake and, consequently causes a significant increase of the phenomenon eutrophication. The reduction of water level and water volume of the lake as well as the fact that its water is never renewed necessitate its enrichments with water from other areas. Nevertheless, no actions have ever been taken in order this problem to be solved. However, it is important that the actions that are to be assumed in the future will not only be related to the improvement of the environment and to the inhabitants' quality of life but they also have to be based on the implementation of financially viable activities and on the actual participation of the local population in the decision-making process (Seihas et al. 2012). Their participation in this process of making ecological decisions is mostly reported in these decisions whose results can affect in one way

or another local community in its entirety (Giannoulas et al. 2004). Citizens' involvement is essential in broadening the conceptualization of the lake values and the implementing management plans (Klessig 2001).

The citizens state that they are very little satisfied considering the actions adopted for the protection of the lake by state stakeholders such as the local administration, the prefecture administration, the central government and by other local stakeholders such as innkeepers' union, freelance union, etc, ecological organizations and the local press. What is more, among the most important obstacles for the solutions of these problems are the insufficient ecological education, the lack of financial resources and the lack of coordinated activities.

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# NEW DATA ON THE VERTICAL DISTRIBUTION OF SOME ARBOREAL SPECIES OF THE FLORA IN BULGARIA

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## Abstract

During floristic studies in different floristic regions of Bulgaria in the period 2006-2012, we found habitats of *Juniperus deltoides*, *J. sibirica*, *J. pygmaea*, *Pinus nigra* ssp. *pallasiana, Quercus coccifera, Q. dalechampii, Alnus incana, Rhamnus saxatilis, R. rhodopaeus, Astracantha thracica, Ilex aquifolium* that expand our knowledge of the vertical distribution of these species in Bulgaria, and hence their ecological niche in the country. The work also specifies the vertical distribution of *Pseudotsuga menziesii*, an adventitious species of the flora in Bulgaria, whose vertical distribution has not been noted in any literary source.

**Key words:** arboreal plant species, vertical distribution, new chorological information, Bulgaria.

## Introduction

The vertical distribution of each plant species provides valuable information about its ecological requirements and it is cited in all "Handbooks for identification", "Synopses of the flora", "Checklists" and "Floras". Until 1967 vertical distribution of species of the flora of Bulgaria was marked with approximate digits from 1 to 3 (Stoyanov and Stefanov 1948, Stoyanov et al. 1966–1967). Digit 1 means that the species is spread in the lower or oak belt, which lies approximately between 0 and 1000 m altitude. The digit 2 indicates that the species is spread in the mountain belt, roughly between 1000 and 2000 m altitude, and digit 3 indicates species distribution in highland (alpine) zone, i.e. higher than 2000 m above sea level. When the species is found in more than one zone, combinations of digits are used. A dash before the digits 2 (–2) and 3 (–3) means that this species can be seen down and below 1000, respectively below 2000 m altitude, and a dash after the digits 1 (1–) and 2 (2–) indicates that the species is up and above 1000, or 2000 m altitude, respectively. These markings give very ap-

proximate notion about the species' vertical distribution and for many species it had never been mentioned. In the multivolume edition of the Flora of Bulgaria (Yordanov ed. 1963–1979, Velchev ed. 1982–1989, Kozhuharov ed. 1995, Kozhuharov and Anchev eds. 2012), the vertical distribution was indicated with real altitudes, but most often only the upper limit of species distribution was specified. "Identification Guide to higher plants in Bulgaria" (Kozhuharov ed. 1992) and "Identification Guide to the plants in Bulgaria" (Delipavlov and Cheshmedzhiev eds. 2003, 2011) indicated altitude distribution of the species represented by specific numbers, as a result of dividing the altitude by 1000. Dashes were also used before or after the altitudes, like in the older literature sources; for some species the range of altitudes was listed as well.

Since 2000, a major and most accurate reference source for the vertical distribution of plant species has become the "Conspectus of the Bulgarian vascular flora", compiled by a group of Bulgarian botanists. The Conspectus had already four editions, which is due to the rapid accumulation of information about the floristic richness and distribution of plant species in Bulgaria (Dimitrov ed. 2001, 2002; Assyov anf Petrova eds. 2006, 2012). It is evident, for example, that the first edition included 3807 species of higher plants, while the fourth edition has 4102 species, i.e. 295 plant species have been added to the flora of Bulgaria just for 11 year period. With a few exceptions, the "Conspectus" provides information about the range of altitudes (m) of distribution of each species. There are species, such as *Pseudotsuga menziesii* with no information about the vertical distribution, because this species has only recently been recognized as part of the adventitious flora of Bulgaria (Tashev et al. 2012).

The purpose of the present work is to provide new data on the vertical distribution of some arboreal plants in Bulgaria, which could be considered in the next edition of the "Conspectus of the Bulgarian vascular flora" and in the publications elsewhere.

## Materials and Method

The data presented have been obtained during various floristic studies in the period 2006–2012. During our work we paid special attention to the altitude where plant species have been found, and if different from the well-known one, herbarium specimens were collected. The exact coordinates of localities and altitudes were determined by means of GPS. The plant names follow The Euro + Med PlantBase – the information resource for Euro-Mediterranean plant diversity (2011) and Assyov and Petrova (2012).

## Results

Below we present descriptions of the new localities of arboreal species, found at altitudes not reported previously for the habitats of these species, thus representing new ecological niches. The following information is provided for each locality: floristic region and sub-region; geographic distribution, habitat, orography, exposition, slope, altitude, coordinates, date of collection and numbers of herbarium specimens in the Herbarium of the Institute of Biodiversity and ecosystem research – BAS (SOM), in the Herbarium of the Sofia University (SO) and in the Herbarium of the Agricultural University

in Plovdiv (SOA). In some cases, floristic characteristic of the localities is provided. At the end we cite the references with altitudinal ranges of distribution different from these reported by us.

**Cupressaceae**

***Juniperus communis* L.**

Rhodopi Mts (Central): Mursalski Rid, Chaeva chuka locality, within a grass community dominated by *Nardus stricta*. Above the treeline, on a northwest facing roof and inclination 2 º, 1900 m a.s.l. (+200 m a.s.l.), N 41º38'48.0", EO24º29'56.8", 9.09.2006, coll. Al. Tashev, A. Vitkova (SOM 163615).

Rhodopi Mts (Central): Mursalski Rid, Mt Karmilyata. Above the treeline, on a roof, 1900 m a.s.l. (+200 m a.s.l.), 18.11.2006, coll. S. Bechev (SOM 163603).

This species was reported to date within the range 200–1700 m a.s.l. (Delipavlov 2011: 37, Assyov and Petrova 2012: 243).

***Juniperus pygmaea* C. Koch**

Balkan Range (Central): in the area of Kalofer, locality called "Kamenlivitsa", near the trail to the hut "Ray", in the only known habitat of *Alchemilla mollis* (Buser) Rothm. in Bulgaria, on a rocky glade, SW exposition, slope of 25 º, 1150 m a.s.l. (–350 m a.s.l.), N 42º41'33.1", EO 24º57'13.6", 21.07.2007, coll. Al. Tashev, A. Vitkova (SOM 164045, SO 104853).

The species was reported till now within the range 1500–1700–1800 m (Delipavlov 2011: 37, Assyov and Petrova 2012: 243).

***Juniperus deltoides* R.P. Adams**

Rhodopi Mts (Central): above the village of Trigrad, locality Kraevo. On a rocky slope, SW exposition, 15 º. On limestone, 1320 m a.s.l., (+220 m a.s.l.) 41°36'24.0"N, 24°23'18.7"E, 26.02.2007, coll. Al. Tashev (SOM 163795).

Rhodopi Mts (Western): close to Devin, Arsaz Tepe locality. In bush community with *Juniperus communis* on a rocky southern slope, inclination 40 º. On rhyolite, 1430 m a.s.l. (+330 m a.s.l.), KG-82, 41°47'33.4"N, 24°23'48.0"E, 08.09.2006, coll. Al. Tashev (SOM 164043, 164044).

The species (sub *J. oxycedrus* L.) has been reported from sea level to 1100 m altitude (Delipavlov 2011: 37, Assyov and Petrova 2012: 243).

***Juniperus sibirica* Burgsd.**

West Frontier Mts: Osogovo, under Mt Choveka, locality Gramadite. In the upper part of a SW slope, inclination 15 º, 1635 m a.s.l. (-265 m a.s.l.), 42°11'17.3"N, 22°37'07.3"E, 18.09.2006, coll. Al. Tashev, A. Vitkova (SOM 163605).

West Frontier Mts: Osogovo, locality Oborite. In the upper part of a SW slope, inclination 12 º, 1560 m a.s.l. (+200 m a.s.l.), 42°10'46.5"N, 22°36'35.5"E, 19.09.2006, coll. Al. Tashev, A. Vitkova (SOM 163606).

The species has been reported from 1900 to 2400 m a.s.l. (Delipavlov 2011: 37, Assyov and Petrova 2012: 243).

**Fagaceae**

***Quercus coccifera* L. var. *coccifera***

Balkan Range (Eastern): "Sinite kamani" Nature park, locality Karakyutyuk, on a highly eroded slope, 25–30 ° inclination, south-eastern exposition, 850 m a.s.l. (+500 m a.s.l.), N 42º44'07.3", EO 26º17'27.9", MH-43, 8.06.2010, coll. Al. Tashev (SOM 165939, 165940, 165941).

The habitat is located in a narrow vertical strip between 849 and 851 m altitude and its geographical coordinates at the center of the field are 42º44'07.3" N and 26º17'27.9" E, at an average altitude 850 m. Eleven individuals of *Quercus coccifera* L. var. *coccifera* with shrubby habitus were found on an area of about

100 m$^2$. Distance between the two most distant individual was 30 m. The height of the specimens found varied from 11 to 77 cm. They had typical globular crowns with diameters between 15 and 90 cm. This shape and sizes are result of livestock grazing in the area. Some parts of the branches and leaves in several individuals were dry. Leaves of the individuals were smaller than normal and had length of 7 to 30 mm and width of 4 to 17 mm. The plant community, where the individuals were found is mostly shrub with single individuals of tree species with height up to 3–4 m. The soil cover undergoes erosion. Tree vegetation was represented by *Carpinus betulus* L., *Quercus pubescens* Willd., *Fraxinus ornus* L., *Acer hyrcanum* Fisch. & C. A. Mey., *Acer platanoides* L., *Prunus avium* L., *Malus sylvestris* Mill., *Pyrus pyraster* Burgsd. and *Pinus sylvestris* L. Shrubs were represented by *Rosa canina* L., *Ligustrum vulgare* L., *Cornus sanguinea* L., *Crataegus monogyna* Jacq., *Chamaecytisus supinus* (L.) Link, *Thymus* spp., *Prunus spinosa* L., *Rubus canescens* DC., *Clematis vitalba* L., etc. In the grassy floor we found wheat grasses: *Dactylis glomerata* L., *Festuca rubra* L., *Festuca valesiaca* Schleich. ex Gaudin, *Phleum phleoides* (L.) Karst., *Koeleria nitidula* Velen., *Brachypodium sylvaticum* (Huds.) P. Beauv. Singly and in larger spots there are *Pteridium aquilinum* (L.) Kuhn. Singly or in small groups there are: *Digitalis lanata* Ehrh., *Pulsatilla montana* (Hoppe) Rchb., *Hieracium* spp., *Campanula lingulata* Waldst. et Kit., *Viscaria vulgaris* Röhl., *Teucrium chamaedrys* L., *Fragaria vesca* L., *Filipendula vulgaris* Moench, *Primula veris* L., *Trifolium montanum* L., *T. ochroleucon* Huds., *Euphorbia cyparissias* L., *Sanguisorba minor* Scop., *Dorycnium herbaceum* Vill., *Leontodon* sp., *Carex* sp., *Rumex acetosella* L. and some others. The total coverage of vegetation cover in the field was about 50 %. There were typical damages resulting from grazing on all trees and shrubs.

The habitat is at altitude more than 400 m above the highest known habitat in Bulgaria. Furthermore, the habitat represents the northernmost locality of the species in Bulgaria, and hence, in Europe – i.e. this is the most northern point of the species' distribution. Till now, the known habitats of this rare for the Bulgarian flora species are in the valley of the Mesta River, Eastern Rhodopes and South Struma Valley, up to 350 m altitude. (Delipavlov ed. 2011: 62, Assyov and Petrova 2012: 338).

***Quercus dalechampii* Ten.**

Rhodopi Mts (Western): in the territory of the State Forestry "Alabak", locality called Kladova, below the hut "Kladova", in the periphery of a forest of *Picea abies*, *Pinus sylvestris* and *Abies alba*. The locality is situated at the summit part of a south-eastern 5 º slope at 1600 m a.s.l. (+100 m a.s.l.), N 42º04'42.7", EO 23º56'47.9", 30.08.2012, coll. Al. Tashev (SOM 168943, SOA 059746).

The vertical distribution reported for this species is from sea level to 1500 m a.s.l. (Delipavlov ed. 2011: 62, Assyov and Petrova 2012: 338).

**Betulaceae**

***Alnus incana* (L.) Moench**

Rhodopi Mts (Central): in the territory of the State Forestry "Smolyan", near the village of Mugla, close to Kasaka, on the banks of the Little River together with *Picea abies* in the natural habitat "White alder woods" (EUNIS: G1.B24 Rhodopide grey alder woods; PAL. CLASS.: 41.S24 Rhodopide grey alder

woods) – a habitat included in the Red Data Book of Bulgaria with the category "Endangered" (Peev ed. 2011). 1463 m a.s.l. (+63 m a.s.l.), 41º36'08.4"N, 24º31'10.9"EO, 2.08.2010, coll. Al. Tashev (SOM 168941).

Rhodopi Mts (Western): in the territory of the Training and experimental forestry "Yundola", locality Mochura, along with undergrowth of *Pinus sylvestris* and *Picea abies* in the natural habitat "Mochurna gora" (91D0) – a priority habitat under Directive 92/43 / EEC, on a northeastern slope and tilt 2 º, 1516 m a.s.l. (+116 m a.s.l.), N 42º03'44.6", EO 23º53'02.1", 29.08.2012, coll. Al. Tashev (SOM 168941, SOA 059748).

The vertical distribution reported for this species is from 600 to 1400 m a.s.l (Delipavlov ed. 2011: 63, Assyov and Petrova 2012: 61).

**Rhamnaceae**

***Rhamnus saxatilis* Jacq. ssp. *tinctorius* (Waldst. et Kit.) Nyman**

Rhodopi Mts (Central): in the territory of the State Forestry "Hvoyna", below the hut "Persenk", near a dirt road to the village Orehovo and old-growth *Picea abies* forest with *Abies alba* and *Pinus sylvestris*. In the upper part of the southern slope with 6 º inclination, 1640 m a.s.l. (+440 m a.s.l.), N 41º50'51.2", EO 24º33'52.6", 8.09.2012, coll. Al. Tashev (SOM 168949).

The vertical distribution reported for this species is from the sea level to 1200 m a.s.l (Delipavlov ed. 2011: 279, Assyov and Petrova 2012: 345).

***Rhamnus rhodopaeus* Velen.**

Rhodopi Mts (Central): within the biosphere reserve "Chervenata stena", on the peak "Popa", 1300 m a.s.l. (+800 m a.s.l.), 30.08.1992, coll. P. Zhelev (SOM 154059).

The vertical distribution reported for this species is from 200 to 500 m a.s.l (Delipavlov ed. 2011: 279, Assyov and Petrova 2012: 345).

**Fabaceae**

***Astracantha thracica* (Griseb.) Podl. ssp. *jankae* (Degen et Bornm.) Greuter**

Balkan Range (Eastern): in the territory of the natural park "Sinite kamani", the locality Natural landmark "Trite peshteri". At a small glade near the road to the locality Karandila near the town of Sliven. Near a forest of *Fagus sylvatica* L. ssp. *moesiaca* with *Carpinus betulus* and *Ostrya carpinifolia* Scop. In the middle part of the slope, southeastern exposition and 3 º inclination, on shallow rocky soil on sandstone, with flowers, 1021 m a.s.l. (+221 m a.s.l.), N 42º44'23.6", EO 26º22'36.4", 20.10.2012, coll. Al. Tashev (SOM 168947, SOA 059744).

The vertical distribution reported for this species is from the sea level to 800 m a.s.l (Assyov and Petrova 2012: 86).

**Aquifoliaceae**

***Ilex aquifolium* L.**

Rhodopi Mts (Western): in the territory of the state forestry "Borino", locality Kremakliev Dol, in the middle part of an eastern slope with inclination 30 º, near a moist, shady vale at 1348 m a.s.l. (+148 m a.s.l.), N 41º42'08.1", EO 24º17'00.2", KG-69 28.11.2009, coll. Al. Tashev (SOM 165562, 165563).

One individual was found in a 70-year old natural forest of *Picea abies*. It is a bush with 14 stems, the highest one reaching 0.85 m, and some of the peripheral stalks are lying on the soil surface. There is an old dead stem with diameter at the base of 32 mm. The projective cover of *Picea abies* in the first floor is up to 90 %, and the undergrowth covered up to 20 %. In the ground floor, representa-

tives of Bryophyta (70–80 %) dominate. The low shrubs found include *Vaccinium myrtillus* L., *V. vitis-idaea* L., *Bruckenthalia spiculifolia* (Salisb.) Rchb., and the herbaceous species occurred singly or in small groups: *Dryopteris filix-mas* (L.) Schott, *Calamagrostis arundinaceae* (L.) Roth, *Melica uniflora* Retz., *Carex sylvatica* Huds., *Aegopodium podagraria* L., *Anthriscus sylvestris* (L.) Hoffm., *Aremonia agrimonoides* (L.) DC, *Asarum europaeum* L., *Cirsium appendiculatum* Griseb., *Cruciata glabra* (L.) Ehrend., *Euphorbia amygdaloides* L., *Fragaria vesca* L., *Geranium robertianum* L., *Hieracium murorum* L., *Hypericum perforatum* L., *Mycelis muralis* (L.) Dumort., *Oxalis acetosella* L., *Potentilla micrantha* Ramond ex DC., *Primula veris* L., *Salvia glutinosa* L., *Sanicula europaea* L.

The vertical distribution reported for this species is from 400 to 1200 m a.s.l (Delipavlov ed. 2011: 277, Assyov and Petrova 2012: 236).

**Pinaceae**

***Pinus nigra* Arnold ssp. *pallasiana* (D. Don) Holmboe**

Rhodopi Mts (Central): Mursalo-Perelik part of the Central Rhodopi, locality Chaeva chuka (Eminvoto). In the upper treeline, in the upper part of a slope, south-facing and angle 30 º, 1850 m a.s.l. (+250 m a.s.l.), N 41º38'24.8", EO 24º29'30.5", 2.08.2010, coll. Al. Tashev (SOM 166162, 166163, 166164).

Single trees of *Pinus nigra* ssp. *pallasiana* in sparse forest together with *Picea abies* (L.) Karst., *Abies alba* Mill,, *Pinus sylvestris* L., *Betula pendula* Roth and *Sorbus aria* (L.) Crantz. Shrub vegetation was represented by *Juniperus communis, J. pygmaeus* C. Koch and *Helianthemum nummularium* (L.) Mill. The plants in herbaceous floor included *Briza media* L., *Festuca rubra* L., *Sesleria latifolia* (Adamović) Degen, *Brachypodium sylvaticum* (Huds.) P. Beauv., *Luzula sylvatica* (Hudson) Gaudin, *Achillea clypeolata* Sm., *Trifolium alpestre* L., *Sideritis scardica* Griseb., *Carlina acanthifolia* All., *Dianthus petraeus* Waldst. & Kit., *Potentilla cinerea* Chaix ex Vill., *Campanula persicifolia* L., *Fragaria vesca* L., *Sedum* spp., *Euphorbia amygdaloides* L., *E. seguerrana* Nick., *Scabiosa ochroleuca* L., *Acinos alpinus* (L.) Moench, *Primula veris* L., *Thymus* sp., *Hieracium alpestre* Jacq., *Nepeta nuda* L., *Leontodon autumnalis* L. etc.

The vertical distribution reported for this species is from sea level to 1600 m a.s.l. (Assyov and Petrova 2012: 316).

***Pseudotsuga menziesii* (Mirb.) Franco ssp. *menziesii***

Rhodopi Mts (Eastern): in the territory of the State Forestry "Kirkovo", in the lands of village Tihomir, around the frontier post "Tihomir", in a glade near a forest road in the locality Proletnika. In the upper part of a western slope, with 6 º inclination, 582 m a.s.l., N 41º17'02.8", EO 25º31'32.5", 08.08.2012, coll. Al. Tashev, N. Tashev (SOM 168935).

Rhodopi Mts (Western): in the territory of the State Forestry "Dospat", in the lands of village Barutin, the locality Lambova livada, mass regeneration under culture of *Pinus sylvestris*, together with undergrowth of *Picea abies* and *Juniperus communis*. On a northwest slope 5 º, 1197 m a.s.l., N 41º36'29.8", EO 24º12'04.9", KG-59, 24.07.2009, coll. Al. Tashev (SOM 165568, 165569).

In the Conspectus of the Bulgarian vascular flora of this species, adventitious for the flora of Bulgaria is not reported. (Assyov and Petrova 2012: 333). Therefore, basing on field data, we offer that

range to be specified – from 500 to 1200 m a.s.l.

## Conclusion

This work presents new data on the distribution of 12 species of the Bulgarian dendroflora expanding our knowledge of their ecological niche. For the first time data are reported about the vertical distribution of the adventitious species *Pseudotsuga menziesii*, which begins to show signs of the invasiveness in the Rhodopes. The data presented can be used in the preparation of new editions concerning the distribution of species in Bulgaria.

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