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NECESSITY, OPPORTUNITIES AND PRIORITIES FOR DEVELOPMENT OF AGROFORESTRY IN BULGARIA

Lubcho Trichkov[1] and Vania Kachova[2*]

[1]Projects and International Activities Directorate, Executive Forest Agency –Bulgarian Ministry of Agriculture and Food, 55 Hristo Botev Blvd., 1040 Sofia, Bulgaria. E-mail: lptrichkov@abv.bg
[2]Forest Genetics, Physiology and Plantations Department, Forest Research Institute – Bulgarian Academy of Sciences, 132 St. Kliment Ohridski Blvd., 1756 Sofia, Bulgaria.
[*]E-mail: vania_kachova@abv.bg

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Abstract

By its nature agroforestry is a form of multifunctional, environmentally friendly and sustainable land management and could provide good income, employment, economic and social stability in many less favoured rural and mountainous areas in Bulgaria. Achievements, policies, legislation, financial instruments and prospects for the development of agroforestry in Bulgaria are reviewed. The review underlines the priorities and opportunities, such as development of agroforestry in mountainous areas of the country; renovation and establishment of new forest protective belts and coastal buffer agroforestry systems; introducing of new agroforestry practices in production of fruit productive trees with high value timber, and in the forest farms. We are pointing out the need for a comprehensive strategy and policy in this field, for facilitating financial access, and for introducing of an assessment system for “good practices” and new agroforestry technologies. Implementation of agroforestry systems in the practice would have important economic, environmental and social impact on the development of local communities and the society as a whole.

Key words: financial instruments, legislations, perspectives, politics.

Introduction

Forests are a key element of the environment and have important economic, ecological and social functions. The reasonable use of forests for providing economic and social benefits is a challenge to the society, landowners, politics and forestry management in Bulgaria. Reconciliation of production functions and environmental protection-related functions concerning forests are the main objectives that are posed in strategic and program documents in sectors “Agriculture” and “Forests”. In terms of economic crisis, climatic changes and natural disasters, new approaches and mechanisms should be found in order to reach a sustainable growth of the sectors and the society as whole. Agroforestry is such approach of land use management allowing diversification of production and reduction of economic risk, together with an improvement and conservation of biodiversity; better carbon retention in ecosystem in comparison with monoculture farming; better combat against desertification and loss of soil fertility (Ramachandran Nair et al. 2009, Ramachandran Nair 2014, Stancheva et al. 2015). As specified in the definitions, agroforestry is a combined system for land management, which includes all systems of land use and technologies of production by which tree and shrub vegetation is incorporated with crop and grass and/or animals on the same land area. These systems are obtained by planting trees on agricultural land or introducing agriculture in existing woodland or orchards. Agroforestry is uniquely suited systems of sustainable land management for improvement nowadays critically reducing ecosystem services. Such artificially created ecosystems should be constructed to be not only more resilient, but also both highly productive and sustainable. Namely, the establishment of vital and functioning forest-agricultural combined ecosystems capable to provide high value of ecosystems services, and simultaneously to provide economic benefits for owners, is the aim of such an approach used for natural resources management. Agroforestry is not only multifunctional but also ecological land management that takes advantages, which would be achieved by combined growing of various biological species, and an appropriate use of the current financial mechanisms and which can provide economic and social stability of many disadvantaged regions of Bulgaria (NAPSMCD 2007–2013). This is suitable method for land management by which boosting of producer's incomes could be achieved whilst improving the environmental conditions (Burgess 1999, Gold et al. 2013, McIvor et al. 2014). In this sense, this is a sustainable management of the land use (Mosquera-Losada et al. 2009). By this method, we can obtain a total increase of biomass output per unit area, better preserved and improved soil fertility and a large number of environmental benefits. These benefits are reached due to a better balance of nutrients in the system and carbon sequestration, better use of water in the system, greater biodiversity, providing more and better habitat for animal species, soil quality, protection against erosion, regulation of the water cycle, increase of local water use due to improved micro-climatic conditions and increasing resilience (better prevention against torrential waters among others) (Garret et al. 1994, Rigueiro-Rodróguez et al. 2009, Ramachandran Nair et al. 2009, Ramachandran Nair 2014, Yakimov et al. 2003, Stancheva et al. 2004, 2015).

The aim of the present review was to identify the best practices of agroforestry systems applied in Bulgaria and to outline prospects and priorities for future development of agroforestry, according to the need and opportunities that are provided on the basis of legal, political and financial instruments available in the country, and to consider the need of improvement of these instruments and measures.

Material and Methods

The methodology consisted of the analysis of available strategic and program documents, scientific publications and theoretical summaries on the matter. Acting political, strategic and regulatory documents promoting the agroforestry development in Bulgaria have been analyzed – laws and regulations (rules, regulations, orders, etc.), archival data, surveys, published materials, statistical reports, etc. Recommendations concerning the harmonization with European legislation were also considered, as well as respective recommendations for further development of agronomic politics in this direction as a solution for current economic and environmental crises in agriculture.

Results and Discussion

As a combined system land management, agroforestry is displayed both in traditional and innovative form by which agro-ecological, socio-economic and innovative-technological benefits for society could be reached through a proper application. In Bulgaria, agroforestry has its traditions, distinctive features and peculiarities. Agroforestry systems with high ecological and economic values were established. Particular successes were achieved in the establishment of protective forest shelter belts in Dobrudja plane and in row shelterbelts along rivers, water basins, roads, industrial objects, etc. Another example is the agricultural use of forest area, establishment of forestry-pasture complexes (silvopastoral practices), and cultivation of Walnut plantations for production of high value timber and nuts. Establishment and maintaining of agroforestry systems like specialized forest plantation complexes and forest farming could help the overcoming of energy crisis related to climate change and reducing the poverty and unemployment, particularly in rural areas.

There were some studies in Bulgaria for identifying of proper combinations and compositions of tree, shrub and crop formations, of appropriate silvicultural actions and measures, as well as agrochemical, agricultural and animal husbandry practices, including identifying proper places and lands for cultivation. These studies contributed to the maintaining of agroforestry systems to provide better implementation of their ecological and economic services (Georgiev 1960, Peev 1989, Peev and Hinkov 2000, Bencheva 2004, Gyuleva 2014). From ecological point of view the role of agroforestry systems to reduce carbon and other gas emissions in the atmosphere is much appreciated. Also these systems could have water-protective, water-retention and water-purification functions, they are able to preserve soil fertility and control of erosion, and to protect biodiversity, etc. (Peev 1989, Peev and Hinkov 2002a, Broshtilova and Broshtilov 2004, Gyuleva et al. 2012). Efforts were posed on clarifying of their economic role to boost incomes of people by diversification of production, and realization effective and rational use of available natural resources (Alexandrov et al. 2001, Peev and Hinkov 2002b, Yonovska 2003, Yovkov et al. 2003, Gyuleva et al. 2013, Milev 2013).

The present review address the following important points: necessity, good practices, opportunities, priorities and financial instruments for development of agroforestry in Bulgaria.

Necessity for development of agroforestry in Bulgaria

It is imposed by the economic, ecological and social advantages, which these systems provide. Economic considerations are connected with increasing demands of timber on global markets and its use as raw materials in a number of production processes. Besides, as renewable energy resource timber could be used to produce new ecologically conforming bio-products (fibres, composts, building materials, “green materials” etc.), and for development of “green economy”, and to reach to “smart” and “intelligent” solutions in agricultural and forestry management. Increasing demands of wood, particularly in bio-economy and bio-energy, requires combining the production process with resource-effective use and sustainable management. The application not only of silvicultural but also the agricultural practices, like intercrop planting of grasses and shrubs among the trees and intensive care such as green manure, usage of soil fertilizers and amendments, inoculation of fungi, mushrooms etc. particularly in forest tree plantations, could lead to overall increasing of productivity and sustainability of ecosystems. Agroforestry allows achieving of good territorial balance of the country development, and to be a tool for improving of economics of many poorly developed and mountainous regions. This could be done by using the biggest resources of biomass, which are located in the less populated and remote areas of the country. By production and/or gathering of wild forest and bio-products, by obtaining of forages and using the forest territories for livestock pastures the economy could be diversified and additional value for all kind of forestry domains and ownerships in the country could be achieved. It was shown that the proper implementation of agroforestry systems has led to overall production increase, generation of more biomass and an increase of incomes of people engaged in agroforestry. Furthermore, the sustainable forest management is a pillar in rural development. The lack of forest management leads to the lack of sustainability of forest ecosystems, particularly in the contemporary conditions of climatic changes, to rapid deforestation and forest fires. The application of good practices only could enhance the entire economic effectiveness in forestry holdings. Such practices could include intensive care against diseases and pests, and for improvement of health conditions, optimization of organic matter (OM) decomposition, improving the carbon cycle in the system, usage of agricultural plants for green fertilizing etc. It could be claimed that the concept of agroforestry in Bulgaria is in accordance with the overall goal of the flagship strategy of EU – Europe 2020 for achievement of “smart, sustainable and inclusive growth”. That concern also relates with intelligent management of resources, and achievement of balance in establishment and managing of forest ecosystems. This concept is consistent with the tree objectives of the Common agricultural policy (CAP): food security; competitiveness and sustainable management of natural resources and climate change; balanced territorial development.

Ecological considerations are related to the positive contribution of agroforestry systems to environment protection, adaptation of natural ecosystems to climate changes, conservation and improvement of biodiversity and soils. Forest areas covered with vegetation are the main greenhouse gas sinks and core tools to mitigate the climate change. Wood is the main binding material of CO_2 in the atmosphere, and forests are main CO_2 – retaining ecosystems on the earth during the millions of years (reservoirs of CO_2). Integration of tree vegetation in the agricultural plots results in the enhancement of land quality (improvement of soil quality and decrease of soil erosion); enhancement of the carbon retention; protection of biodiversity; improvement of water purification and its percolation; combat soil desertification; improvement of local climatic conditions and providing of a better ecological services for local society (Palma et al. 2007, Rigueiro-Rodróguez et al. 2009, Mbow et al. 2014, Stancheva et al. 2015).

Good agroforestry practices in Bulgaria

The opportunities concerning agroforestry development in Bulgaria are connected with the achievements and the good practices in this direction, existing national legislation, developed policies, strategies, programs, and the access to financial instruments and resources.

Good practices are achieved in implementation of Agricultural Uses of Forest Areas. Poplar plantations were proven to be particularly appropriate. They were successfully cultivated with some important crops, like sunflowers, cabbage, corn, pepper and eggplant, melon and pumpkins, cauliflower, wheat, beans. Furthermore, plantations of oaks, walnut and alder with corn were grown in practice (Yakimov et al. 2003). There was increase in crops as a result of better extraction of nutrients and water from a deeper tree root system, reduction of expenses for arboricultural measures – hoeing, nourishing, and watering. Final result was realization of additional income due to increased diversity of the products (Yakimov et al. 2003, Stancheva et al. 2015). Agricultural Uses of Forest Areas is combined well with organic production, especially in clean mountain areas, by applying of agronomic, biological and mechanical methods of tillage. The wrong implementation of Agricultural Uses of Forest Areas is connected with improperly combination of trees and crops and releasing of root phytoncides and substances with allelopathic effects by agricultural plants (Stancheva et al. 2015). There are new technological schemes that should be investigated, like the one developed in Italy along the rivers, with poplar and oak for producing wood together with wheat and other crops. Another very important agroforestry system with extremely good results in Bulgaria is represented by Protective Forest Shelter Belts. Their application resulted in achieved improvement of micro-climatic conditions through protection against the strong winds, purification of the air, improvement of water and temperature regimes, increase of the crops yields and of the quality of products obtained (Peev 1989; Totev et al. 1995; Peev and Hinkov 2000, 2002a; Marinov et al. 2003; Yakimov et al. 2003). Currently there are about 9000 ha protective forest belts in Northern Bulgaria, with main purpose protection against strong winds. There is an understanding about the importance of protective forest belts for agriculture, particularly in areas with harsher climatic conditions, and also for protection of urban places, industrial objects, livestock farms, and roads. This requires supplying of financial resources for establishment of new protective forest belts and reconstruction of the already existing ones. Relatively large Silvopastoral Complexes have been established. In this systems tree and shrubs species are reconciled with fodder culture (grass mixture, etc.), and these areas are being used for grazing of animals. By such established forest–pasture complexes an ecological livestock breeding could be developed, and a balance of animal nutrition could be reached. Additional extraction of wood, economic effectiveness, environmental friendliness and social benefits for society have been achieved (Stancheva et al. 2004). It will be good the foreign experience to be investigated, such e.g. in England and Wales (AGFORWARD 2014) for a reconciliation of tree and shrub species with grazing and mowing of grass for realization of additional incomes. There are new practices in Europe, such e.g. in Germany, where trees are grown in alleys for biomass production (AGFORWARD 2014).

Particular interest from the side of forestry holdings should be paid on the establishment of Forest Tree Plantations with High Value Wood by applying intensive methods for husbandry and management. Parts of these intensive methods are connected with applying of agroforestry practices. In this aspect we recommend the usage of the other countries experience, e.g. new agroforestry practices implemented in Spain for grazing and incorporation of vegetation among high value tree species: wild cherry and walnut (AGFORWARD 2014). In our country plantations of forest fruit species have been established: from walnut (*Juglans regia* L.) and to a lesser extent from hazel (*Corylus avellana* L.) and almond (*Prunus dulcis* (Mill.), as well as plantations of Wild cherry and *Sorbus*. There are some positive results, but some difficulties were reported too (Alexandrov et al. 2001). There is a special interest to the establishment of walnut plantations in Bulgaria. Walnut plantations form sustainable agroforestry systems and are preferred because of the high value of wood and sufficient earnings from fruit selling (Glushkova et al. 2008). The development of Forest Farming in Bulgaria, as an agroforestry system in which naturally and artificially created tree and shrub vegetation is reconciled with economic activities, is still at its early stage. Except wood, the other products can be obtained for consumption and manufacture processing – oil cultures, mushrooms, medical plants, forest fruit, ornamental plants, etc. New ways to incorporate these activities in forestry and agricultural holdings and farms should be developed. Examples that could be given include cultivation of herbs, medical plants, mushrooms, and some useful research was done in this respect (Stoyanova 1997, 2006).

Regulations, politics, programs, strategic documents and financial instruments concerning agroforestry in Bulgaria

Legislation is connected with enforcement of the Law on ownership and usage of agricultural land (LOUAL); the Forest Act (FA), the Law on protection of agricultural land (LPAL); the Law for supporting agricultural producers (LSAP), etc. The Ministry of agriculture and food (MAF) is the institution, which authorizes schemes for single payment according to CAP, provides information to owners, authorizes measures on land protection and proper tillage, also activities concerning restoration of ecological functions and enhancement of soil fertilities, etc. LSAP represents the schemes and measures of single payments: the single payment per areas, the green payments and specifies the rules, approvals and implementations of the Rural Development Program (RDP). The agroforestry system “protective forest belts” is authorized in FA. Forest belts established in 1950s and 1960s are identified as public state property. The terms and conditions of the usage of non-wood products, including mushrooms, medical and aromatic plants, Christmas trees, foliage fodder, etc., are clearly laid down. The terms of grazing in forest areas are also set out. Concerning agroforestry systems specialized forest plantation and forest farming, art. 88 (5) of the FA introduces that the following categories are not managed as forests: 1. Christmas trees and greenery plantations; 2. Trees and shrubs designed for rapid production of biomass; and 3. Fruit productive plantations. The agroforestry system forest-pasture complexes is described and the terms and conditions for grazing in forest areas are specified in art. 123 to art. 125 of FA.

Decree №2 on the terms and conditions of the afforestation lands used for establishment of special protective and economic forests with art. 32 postulates that newly established plantations are managed also by use of “agricultural crops planting among intercrops spaces”.

Policies, which are connected with applying of agroforestry systems, are based on the development of strategies and programs targeted to rural areas, to enhancement of the vitality and competitiveness of agricultural and forest farms, maintaining of its sustainable and environmentally-friendly management. The political documents highlight the need of economic growth, with preventing degradation and contamination of the environment. Through wise implementation, agroforestry could have the advantages over other agricultural practices, whose monoculture intensive production results in soil depletion, water deficit, and significant green gas emissions. Particularly great are the opportunities of agroforestry for finding technologic solutions and resource provision for "green economy" to which is faced the modern world.

The policy of Bulgaria concerning agrarian sector, as the state member of EU, should be harmonized with the policies adopted by European Parliament and European Commission. The EU respects the competences and specifics of the state members, especially in devising of their strategic and program documents. Regulation No1698/2005, article 39 clearly and precisely stated that "Agroforestry systems can acquire a high ecological and social value by combining extensive agriculture and forestry systems, aimed at the production of high quality wood and other forest products. The establishment of such systems should be supported." Unfortunately, the measure 222 of the old RDP (2007–2013) for supporting the development of agroforestry in Bulgaria did not started at all. Currently, realization of the CAP of EU, a number of regulations were adopted: Regulation (EC) №1305/2013 on support for rural development by the European Agricultural Fund; Regulation (EC) №1303/2013 concerning the improvement of coordination and harmonization of CAP from EU funds; Regulation (EC) №1306/2013 for financing, management and monitoring of the CAP. Agroforestry also finds its place in the regulatory documents of the CAP. There is a certain art. 23 supporting the construction of agroforestry systems in the new Regulation (EC) №1305/2013 on rural development. The member states should set out the minimum and maximum number of trees per hectare, taking into account the local soil and climatic conditions, native forest species and need of ensuring of sustainable land use. Further, art. 20 postulates that measures to support forestry as an integral part of rural development should cover the increase and improvement of forest resources through afforestation of land and establishment of agroforestry systems combining extensive agriculture and forestry. These systems are advisable also for recovering of forests affected by fires or other natural disasters.

At the national level, the development of agroforestry is directly linked to the development of the forestry. The National Strategy for Forestry Sector Development in Bulgaria (NSFSD 2013–2020) highlighted that in accordance to the vision 2020 Bulgaria should have a vital, productive and multifunctional forests; conserved biodiversity and quality and quantity of water resources. These are three strategic objectives related to ensuring sustainable development of forestry sector. They have been set out for strengthening the role of forests of ensuring economic growth of the country and for increase of their contribution to "green economy". Of four priorities for achieving the strategic objectives, particularly close and important in terms of opportunities of agroforestry is the fourth one, about the potential of forest sector for green economy development. There are concrete measures and actions for implementing the priorities that are directly or indirectly related to agroforestry systems. For example, priority 1, measure 1.4 recommends elaboration of "modern program for recovering of existing and establishment of new shelterbelts". Priority 4, measure 4.1 recommends the necessary actions concerning research, analysis and evaluation of the potential of forest areas in Bulgaria for the production of energy from wood biomass; development of a National scheme for sustainable production and consumption of woody biomass, which should contain relevant sustainability criteria; developing best practices for creating and managing of intensive plantations for biomass production, including the fostering of energy crops production – planting of tree and shrub species, created for rapid production of biomass. In the analytic part of NSFSD there is a conclusion that during the period 2006–2011 a reduction of the amount of revenues from non-timber products and benefits was established. The incomes from livestock and crop production can be evaluated as a minimum. In the forest sector there are services and activities that could bring significant additional incomes for forest holdings, like gathering of charge for grazing in forest areas; usage of the territory for temporary storage of wood and other building materials; temporary stay of livestock; usage of ski slopes; for placement of temporary facilities and equipment for conducting business activities; setting up of camps, tents and others. Such activities are related to the presence of agroforestry systems, some of which are with a significant value for tourism.

Another important strategic document in the forestry sector is the Strategic Plan for Forestry Sector Development (SPFSD 2014–2020). The document is a brief analysis on the development of the forestry sector, with the major challenges identified. The relevant operational objectives; the activities for achieving of expected results; responsible and related institutions; indicators for performance; necessary financial resources for their implementation; time and more, are specified. In operational objective 1. "Increasing forest areas, timber stock and carbon stock of in forest areas" (that relay to the measure 1.1. of NSFSD) in activity 1.2. the expected results connected with recovering and building of new shelterbelts and reducing the negative influence of wind erosion on agricultural farmland are specified. The operational objective 2 "Improving the management and use of forests" (that relay to the measure 1.2. of NSFSD) in activity 2.7. "Research, analysis and evaluation of the potential of forest areas in Bulgaria on available opportunities to provide services, produce and realization of non-timber forest products and their environmentally friendly use", defines the expected results regarding the improvement of forest services, increase the production of non-timber products and organization of fees for their use. Expected results are also connected with increasing the revenues from these business activities.

The "Program of measures to adapt forests in the Republic of Bulgaria and mitigate the negative impact of climate change on them" includes measures for the establishment, maintenance and improvement of agroforestry systems such as "protective forest belts" and "forest-pasture complexes". Relatively insufficient funds are set in the Programme for realization of the measures, mainly targeted at conduction of arboriculture activities in ecosystems.

In the "Third national action plan on climate change (2013–2020)" is necessary to seek financial resources for the implementation of measures aimed at: increasing the land areas from categories – absorbents of greenhouse gases: forests, pastures and meadows; increasing the potential of forests for carbon sequestration; long-term retention of carbon in wood products.

Main financial instruments for development and maintaining of agroforestry systems in Bulgaria are the European Funds, which are accessible via the following national and European programs:

1. Operational programs: “Environment”, “Science and education for smart growth”, “Innovations and competitiveness”;
2. Operational programmes for cross-border, transnational and international cooperation;
3. Both pillars of CAP – single payments and RDP;
4. Horizon 2020;
5. Other EU and international mechanisms such as: Trading of greenhouse gas emissions and etc.;
6. Additional funding from the Bulgarian government is also possible: e.g. “Green investments” ensuring by Ministry of agriculture and food.

Practically, there is no funding for agroforestry in the draft of the new RDP (2014–2020). However, there are some measures supporting the forests and agriculture, and therefore there are subsidies for certain agroforestry activities or projects. Such activities are: recovery and establishment of new protective forest belts; bio-agriculture; erosion control; increase soil productivity. Subsidised funding is possible for maintenance and recovery of grass fields with high natural value, erosion control, and traditional practice for seasonal grazing also should be financing. Investments are planned for the development and usage of new technologies for recycling of the wood waste.

Programme LIFE+ (Program for environment and climate action) from 2014 has been created with Regulation 1293/12.11.2013 of the European Parliament and the European Council. Funds could be used foreseeing for priority areas “Environment and resource efficiency” and “Adaptation to climate change”.

Horizon 2020 Programme launched in 2014, as well as by new OP “Environment”; “Science and education for smart growth”; “Innovation and competitiveness”, may also be used for obtaining substantial financial resources for the development of agroforestry in Bulgaria.

The financial support, which can be received by agricultural and forestry farmers and producers under National schemes for government aid can be considered as a form of support to agroforestry. There are some short and long-term loans granted by Government Fund “Agriculture” through some commercial banks at preferential terms for guarantees. Specifically, funds for development of agroforestry systems in Bulgaria can be obtained in other national schemes authorized by Ministry of Agriculture and Food as a “green investment”; development of animal husbandry, beekeeping, sericulture. Moreover, the six State Forestry Enterprises (SFE) and their forest and hunting territorial divisions are eligible for financial investments from fund “Investments” established through the Forest Act.

Potential and priorities for the development of agroforestry in Bulgaria

There are opportunities and potential for the development of modern agroforestry in Bulgaria, taking into account the specific environmental and climatic conditions, characteristics of the local economy and existing good practices.

A promising activity could be the application of agroforestry technologies for production of tree species with high quality wood (high value trees), and at the same time, forest fruits, nuts and seeds – walnut, wild cherry, chestnut. Good practices in this regard can be developed considering the experience in some European countries. There are some practices in Bulgaria during the last 5–7 years to establish plantations of oak and hazel, whose roots are “inoculated” with fungi of truffles, and there are some recommendations for inoculation with other fungi and mushrooms to stimulate the growth of forest plantations. This is a way to improve the yield in specialized plantations for production of high quality trees and biomass and for realization of good extra income. The introducing of agroforestry systems, especially in forest nurseries and plantations is connected with using of proper herbaceous and crop species for plant nutrition, with biological pest control, intensive organic and ‘green’ fertilisers, using of appropriate soil improvers, soil amendments and physiological regulators. Good practices in this area are particularly needed.

Agroforestry systems can be combined well with organic production of agro- and forest-products as a form of ecological agriculture, and in this direction introducing of new practices and innovative technologies are expected too.

Establishment of effective agroforestry systems for flood protection in the light of the current realities is also urgent and necessary (Petkova 2004).

There is a need in mountainous areas and there are no well-established silvicultural complexes with well-maintained grazing woodland and semi-opened pastures. The good semi-natural grasslands in mountain areas, which were established in the past, were abandoned, but there are expectations for funding in the new RDP (2014–2020) to be restored. There is a possibility for the implementation of best practices of agroforestry connected with grazing of animals and extraction of plant biomass and forage. The country has an understanding of environmental value of the forestry-pasture complexes and they are in the category of lands with recognized high nature value (HNV).

Natural and climatic conditions of the country are particularly suitable for the development of forest farming, and in this direction a number of expectations are connected by land and forest owners, especially in the mountainous areas of the country where the livelihood is connected mainly with tobacco production. In this way the income of people could be significantly increased by cultivation of berries, medicinal plants and herbs, ornamental plants, mushrooms, raw materials for processing and craft activities, and technical plant. Construction of forest farms, however, still in early stage of development. Thinning of forests due to the illegal logging and negative natural phenomena – drought, disease, fires, etc. allows the farmers to build forest farms on their affected lands with little investment. The presence of many small forest owners with limited opportunity for timber harvesting and limited income, provides good opportunities for the development of this form of agroforestry in the country. Particularly valuable system of agroforestry in relation to the improvement of environmental conditions at the local level is forest shelter belts. It is necessary to ensure sufficient funds for their good management, both from the state and from the land owners, roads, industrial sites, farms. For this purpose dissemination and exchange of information and mobilization of expert potential is highly desirable.

Conclusions

Agroforestry, as a social-economic and ecological system for land management targeted at development of rural areas, and in the same time, a system that gives opportunities to introduce "know-how" in the field of "green technologies" takes its place in the European strategically and program documents. The requirements for increase of the total production of the ecosystems, and for their ecological and sustainable management, for reducing the costs from one side, and the observed delegation of the soil, the deficit of water, climate change and the insecurity of the stock markets on the other side are the reasons for introduction of agroforestry, as an intelligent and modern system for land use.

In the State forestry enterprises (SFE) the efforts should be directed towards the construction of plantations for the production of high quality wood and biomass by applying intensive production methods. Suitable are agroforestry systems with proper mixture of tree, shrub and grass species in afforestation of forest areas affected by forest fires.

The implementation of agroforestry systems in practice will have important economic, environmental and social impact on local communities and society as a whole. But, it is necessary to create an overall concept and to be devised National Strategy for development of agroforestry in Bulgaria. It should be clearly pointed out, not only "the principle for sustainability", but also "the principle of subsidiary", which to be introduced as a base of the Program and Conception. In the future National Strategy, besides the analysis of achievements should be indicated also the framework for development of agroforestry in the agricultural and forestry holdings, and to be also set out the access to financial instruments for implementation. Modern National long-term strategic development program of agroforestry in Bulgaria (2016–2025) should be devised and adopted by the Council of Ministers. Other opportunities could be given under National schemes for government aid, and their activities are controlled by Ministry of agriculture and forestry. Moreover, is necessary to change the legislation in the forest sector in order to ensure the possibility of State Forest holdings and their territorial divisions – State forestry and Hunting holdings to have access to funds from the Fund "Investments" and to be able to diversify its revenue. It is also necessary to develop criteria and a comprehensive evaluation system for "best practices". An important point is to ensure a better link between science and practice, and a better interaction and greater use of expertise.

The introduction of agroforestry systems in forestry and agricultural practices will have important economic, social and positive impact on the environment and will boost economy of disadvantaged areas and society as a whole.

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NEW DATA ON ALIEN INSECT PESTS OF ORNAMENTAL PLANTS IN BULGARIA

Aneliya Pencheva[1*] and Mariya Yovkova[2]

[1]Department of Plant Pathology and Chemistry, Faculty of Ecology and Landscape Architecture. University of Forestry. 10 St. Kliment Ohridski Blvd., 1756 Sofia, Bulgaria.
[*]E-mail: ajp84@abv.bg
[2]Institute of Ornamental Plants – 1222 Sofia, Negovan, Bulgaria.
E-mail: mariya_yovkova@abv.bg

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Abstract

In this study, the results of recent surveys (during the period between 2012 and 2015) on alien insects infesting ornamental plants in Bulgaria are reported. Fourteen species, associated with urban landscape areas and indoor plants, are discussed. Two of them, *Ceroplastes sinensis* Del Guercio and *Lepidosaphes flava* (Signoret), are reported for the first time in Bulgaria. Furthermore, *Acizzia jamatonica* (Kuwayama), *Ceroplastes ceriferus* (Fabricius), *Pseudaulacaspis pentagona* (Targioni Tozzetti) and *Cydalima perspectalis* (Walker) have been found in new localities. *Metcalfa pruinosa* Say has widened its host range in Bulgaria. Additional distribution data are also provided about *Aulacaspis yasumatsui* Takagi and *Cacoecimorpha pronubana* (Hübner). Details on current status, host plants, zoogeographical origin and probable pathways of introduction into Bulgaria are reported for each species. Morphological and biological remarks are given for *C. perspectalis* and *C. sinensis*.

Key words: box tree moth, Chinese wax scale, De Stefan scale, new pests, scale insects.

Introduction

In recent years, due to the active marketing of ornamental plants and plant material, a large number of non-native insects have penetrated in different continents and countries. After introduction into a new area, they may become acclimatized, surviving outdoors. In some cases these are very harmful species. They not only cause significant damage to ornamental plants, reduce their aesthetic and market values but also may threaten the native flora. Thus, their invasion becomes a global environmental problem regarded as one of the main factors that lead to a decline in regional biodiversity (FAO 2003, Chornesky et al. 2005).

As far as phytophagous insects are concerned, more than 25 alien species have been recorded as new pests in the last decade in Bulgaria (Beshkov et al. 2015, Doychev 2015, Pencheva et al. 2014, Tomov and Trencheva 2013, Yovkova et al. 2013, Yovkova et al. 2015). About two thirds of them are ornamental plant pests and may cause significant damage to cultivated plants both in Bulgaria and across Europe. Their unexpected massive appearance in urban habitats is attributed mainly to the introduction and distribution of infested plant materials.

The aim of the present study was to investigate the current status and distribution of alien insect recently reported as pests on ornamentals in Bulgaria, as well as old invaders whose populations are increased in the last years. The survey results will contribute to get acquainted with alien insect's distribution and have a practical application, benefiting producers of ornamental plants.

Materials and Methods

The survey was conducted over a period of four years, between 2012 and 2015. Several localities with ornamental plants (city/urban parks, private gardens, ornamental nurseries, greenhouses, garden centres and collection in botanical gardens) were surveyed. They were located in various regions of Bulgaria (Fig. 1). Part of the biological materials was provided by different specialists working in urban parks or in nurseries.

During the visual inspection of ornamental plants, infested plant parts (twigs or leaves) and insects on them were collected and placed separately into plastic bags or in 70 % alcohol for further examination in the laboratory. For each insect's sample, origin of infested host plants or pathways of their introduction were checked. The surveys were carried out during the active phase of the insects' life, namely from May to October. Caterpillars of the lepidopteran pest species were reared to adults under laboratory conditions. Scale insect specimens were mounted for light microscopy according to the procedure detailed by Kosztarab and Kozár (1988). The species found in Bulgaria for the first time were identified using the keys of Danzig (1993), Gimpel et al. (1974) and Fetyko and Kozár (2012). The

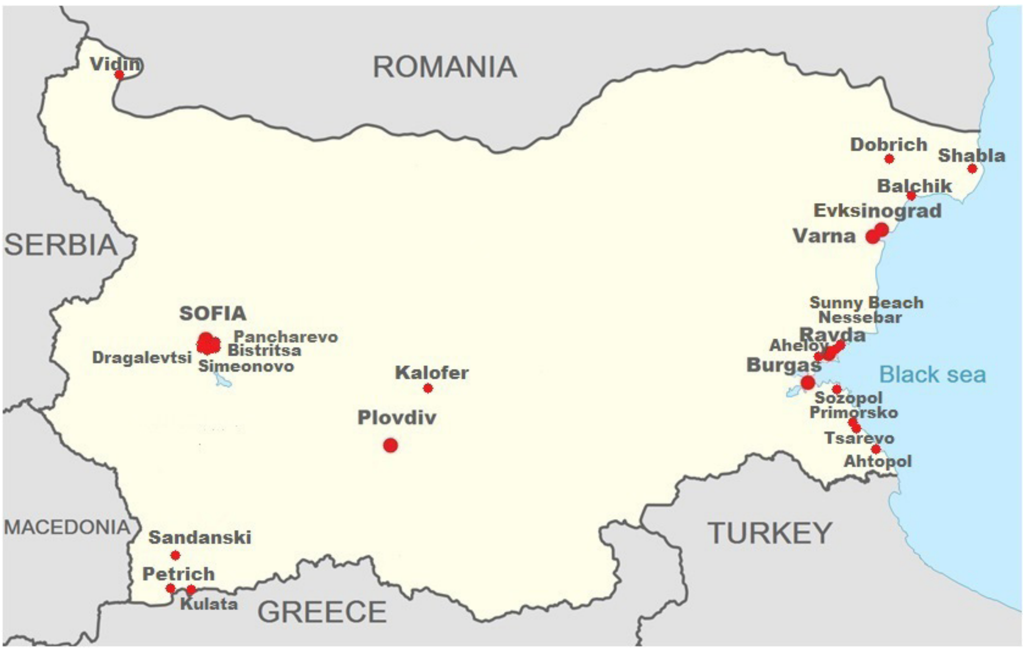


Fig. 1. Regions and locations in Bulgaria where alien insect pests included in this study were established.

identification of *Ceroplastes ceriferus* was confirmed by J. F. Germain, Plant Health Laboratory, Montpellier, France. The collected specimens are preserved in the department of Plant Pathology and Chemistry at the University of Forestry, Sofia.

Results and Discussion

Fourteen alien insects associated with urban green areas were identified in different regions of Bulgaria during the present study (Table 1). They belong to two

Table 1. List of surveyed alien pests.

No	Alien insect pest	First record in Bulgaria	Established localities	Type of localities	Origin
1	*Metcalfa pruinosa* Say	2007– Plovdiv	Petrich, Balchik, Shabla – 2014; Nessebar, Balchik and Varna – 2015	Outdoors (private gardens and city parks)	North America
2	*Acizzia jamatonica* (Kuwayama)	2009 – Nessebar	Sofia – 2014; Nessebar – 2015	Outdoors (private gardens)	East Asia
3	*Trioza alacris* Flor.	2009 – Ahtopol	Aheloy – 2012–2015	Outdoors (private gardens)	Mediterranean origin
4	*Elatobium abietinum* (Walker)	2014 – nearby Sofia	Not found in its previous localities	Outdoors in private gardens	Central and Northern Europe
5	*Icerya purchasi* Maskell	1968 – Sofia	Ravda – 2012–2015	Indoors in garden centres	Australia
6	*Phenacoccus peruvianus* Granara De Willink	2010 –Ravda	Ravda – 2013–2014	Indoors in garden centres and greenhouses	South America (Argentina, Peru)
7	*Ceroplastes ceriferus* (Fabricius)	2011 – Vidin	Nessebar – 2012 and 2013; Sozopol – 2015	Outdoors (private gardens)	Neotropical
8	*Ceroplastes japonicus* Green	2007 – Sofia	Ravda – 2014	Outdoors (private gardens and garden centres)	Eastern Asia
9	*Ceroplastes sinensis* Del Guercio	**New record**	Nessebar – 2013	Indoors (garden centres)	Central or South America
10	*Aulacaspis yasumatsui* Takagi	2009 – Tsarevo	Sofia and Sunny Beach – 2013; Ravda – 2014	Indoors (greenhouses and garden centres)	Southeast Asia
11	*Lepidosaphes flava* (Signoret)	**New record**	Sofia, 2012 Burgas, 2013	Indoors and garden centres	Near East and Mediterranean Palaearctic
12	*Pseudaulacaspis pentagona* (Targioni Tozzetti)	1968 – Petrich, Sandanski, Kulata	Plovdiv, 2013; Sofia, 2012–2015; Balchik – 2014; Sunny Beach – 2013	Outdoors (private gardens, city parks and botanical gardens)	Eastern Asia
13	*Cacoecimorpha pronubana* (Hübner)	2009 – Aheloy	Ravda, Burgas, Aheloy – 2012–2015	Outdoors and indoor (garden centres)	Mediterranean region
14	*Cydalima perspectalis* Walker	2014 – Balchik	Dragalevtsi, Dobrich – 2014; Burgas, Plovdiv, Primorsko, Sofia, Kalofer – 2015	Outdoors (private gardens and city parks)	Asia

orders (Hemiptera and Lepidoptera) and 8 families. Half of them are scale insects (Coccoidea), which are able to spread easily with infested plant materials, because of their small size and cryptic behaviour. Details on detected species are given further down.

Metcalfa pruinosa (Say, 1830) [Hemiptera, Flatidae] – citrus flatid planthopper.

Collected material: 2014: 20 July – Petrich; 26 August – Balchik and Shabla; 2015: 15 July – Ravda and Nessebar; 20 August – Balchik and Varna.

In August 2014, in Balchik Botanical garden *M. pruinosa* had infested more than 45 herbaceous, ornamental and fruit plant species (Fig. 2). The most infested trees and shrubs were: *Acer platanoides* L., *A. campestre* L., *Broussonetia papyrifera* (L.) Vent., *Catalpa bignonioides* Prints, *C. speciosa* Prints, *Cornus mas* L., *Diospyros kaki* Thunb., *Fraxinus excelsior* L., *Ficus carica* L., *Hibiscus syriacus* L., *Parthenocissus tricuspidata* (Siebold & Zucc.) Planch., *Philadelphus coronarius* L. and *Vitis vinifera* L. The population density of citrus flatid planthopper was considerably lower in 2015, but number of infested host plants increased. At other sites *M. pruinosa* mainly infested weeds, including *Marsdenia erecta* (L.) R. Br. and *Clematis vitalba* L. (around Nessebar), and only in Petrich the insect was found on a single *Catalpa bignonioides* tree.

The North-American planthopper *M. pruinosa* was accidentally introduced into Europe (first in Italy), and subsequently caused economic damage to orchards and vineyards in some South-European countries (Strauss 2010). In Bulgaria it was first detected in 2004 on *Thuja occidentalis* L. at a locality near Plovdiv (Trenchev et al. 2007). The planthopper is gregarious and extremely polyphagous species. According to some authors it may feed on 200 to 300 plant species (Souliotis et al. 2008, Grozea et al. 2011). Three of infested plants species determined in Balchik – *Lonicera maacki* (Rupr.) Herder, *Securinega suffruticosa* J. F. Gmel. and *Cephalotaxus harringtonia* (Knight ex J. Forbes) K. Koch – have probably not been recorded earlier as hosts of *M. pruinosa*.

This species overwinters as eggs inserted in woody tissue or under tree bark. The first nymphs were found on the leaves and stems in the beginning of May. The total development period of the larval stages takes an average of 42 days (Lucchi and

a b

Fig. 2. *Metcalfa pruinosa*: adults (a) and signs of pest infestation to *Cornus mas* (b).

a b

Fig. 3. Psyllids: a – white wax on a leaf of *Albizia julibrissin* excreted by nymphs of *Acizzia jamatonica*; b – nymphs and gall of *Trioza alacris* on a leaf of *Laurus nobilis*.

Santini 1993). Nymphs surround themselves with long, waxy filaments, which protect them from their copious honeydew (Fig. 2b). In 2015 adults were present in all localities from the beginning of July to the end of September. Besides infested plants, *M. pruinosa* could be transported over long distances on vehicles, which often park along the roadsides near food plants of the pest. Local invasion of the surrounding area follows and is facilitated by the presence of uninterrupted belts of host trees and shrubs (Pantaleoni 1989). The protected waxy cover of the citrus flatid planthopper, the way it spreads and its adaptability, are factors that lead to the development of the species in large population density during the last 4 years in Bulgaria.

Acizzia jamatonica (Kuwayama, 1908) [Hemiptera, Psyllidae] – albizia psyllid.

Collected material: Simeonovo (nearby Sofia) – 25 September 2014; Nessebar – 16 July 2015.

In September 2014, psyllid nymphs and adults were collected on a young (about 6–7-year-old) solitary silk tree (*Albizia julibrissin* Durazz.) located on a private property nearby Sofia. Psyllid nymphs had excreted honeydew and spirals of white wax on the leaves (Fig. 3a). The damage caused by *A. jamatonica* was substantial and associated with leaf discoloration and premature leaf drop. The following year (2015) the infested tree died, probably due to bark necrosis. In the middle of July 2015, psyllid nymphs were also found around Nessebar.

A. jamatonica is native to East Asia, but has rapidly increased its geographical distribution during the last decade, becoming established in Europe and North America (Mifsud et al. 2010). It develops on *Albizia* spp. and is most commonly recorded on *A. julibrissin*. The first occurrence of *A. jamatonica* in Bulgaria was in July 2009 in Nessebar (Vétek and Rédei 2009). Since then, the insect has rapidly spread in South-eastern Bulgaria and particularly in the coastal areas (Harizanova et al. 2012).

Trioza alacris Flor, 1861 [Hemiptera, Triozidae] – laurel psyllid.

Collected material: Aheloy, during May – August in 2012 – 2015.

The abundant leaf galls on *Laurus nobilis* L. (used as a hedge), laurel psyllid adults and nymphs have been detected from April to October since its first appearance in 2009 (Pencheva et al. 2009). At present *T. alacris* is limited to a locality situated at a private property in Aheloy and since 2009 it has hibernated successfully for about 6 years. It is suspected that the laurel psyllid has been introduced in Bulgaria on imported plants originating from the Netherlands.

The species is most likely of Mediterranean origin but was introduced to Central and Northern Europe on cultivated bay laurel (Mifsud et al. 2010). Generally it develops on *L. nobilis*, but is also reported on *L. azoricus* (Seub.), producing characteristic large leaf galls by rolling down leaf margins (Fig. 3b). *T. alacris* has two to five broods in the year according to the weather (Ossiannilsson 1996), the last one maturing in the end of October. Its chemical control is difficult because of the lavishly waxy secretion covering the nymphs.

Elatobium abietinum (Walker, 1849) [Hemiptera, Aphididae] – green spruce aphid.

Collected material: 20 April 2014 – nearby Sofia.

The green spruce aphid was detected for the first time in April 2014 on three private properties nearby Sofia – Bistritsa, Pancharevo and Dragalevtsi. Dense colonies of larvae and apterae females have been registered on the branches of *Picea abies* (L.) Karst. and *P. pungens* Engelm. The damage to infested plants was significant and has led to needle necrosis and premature needle drop (Yovkova et al. 2015). Further observations conducted during growing season in 2015, the aphid was not found in either the previous or in any of the other aforementioned detected locations.

According to Carter and Halldórsson (1998) and CABI (2015a), *E. abietinum* originates from Central and Northern Europe. It is distributed throughout Europe (Blackman and Eastop 2015, CABI 2015a). The question why this species still has not been found in Bulgaria and why it disappeared so quickly remains open, especially taking into account that the *Picea* species are often used in landscaping and in forest wood production and are periodically investigated. Due to the lack of data on the presence of *Elatobium abietinum* in neighbouring countries (Turkey, Greece, FYR of Macedonia and Serbia) where, like in Bulgaria, the aphid's fauna is comparatively well investigated, and due to its very local appearance only near Sofia, it can be concluded that the green spruce aphid was probably introduced into Bulgaria via imported infested Picea plants.

Icerya purchasi Maskell, 1878 [Hemiptera, Monophlebidae] – cottony cushion scale.

Collected material: Ravda: 20 July 2011 and 25 October 2015; Burgas – 20 May 2015.

During the present study, dense colonies of cottony cushion scale nymphs and adult females have been registered on *Pittosporum tobira* (Thunb.) W. T. Aiton, *Citrus lemon* (L.) Burm, *Laurus nobilis, Parrotia persica* (DC.) C. A. Mey and *Liquidambar styraciflua* L. (Fig. 4a) in two garden centres in Ravda and in greenhouses in Burgas. In both locations the host plants were just imported from Italy.

I. purchasi was found for the first time in Bulgaria in 1968, when it was recorded as an important pest in the Botanical garden in Sofia, affecting *Acacia* spp., *Magnolia* sp. and other 4 ornamentals (Tsalev 1968). Although this species was not detected in the following investigations (carried out from 1990 to 1995 in ornamental greenhouses) (Pencheva 1995), it has appeared periodically in garden centres in the last 10 years because of the intensive trade of ornamental plants (Pencheva 2007).

The cottony cushion scale is an Australian species but now is widespread

a b

Fig. 4. Scale insects: Adult females of *Icerya purchasi* on *Parrotia persica* (a) and *Phenacoccus peruvianus* (b).

throughout the world. It has a wide climatic tolerance and has become established in Southern Europe and in greenhouses of temperate regions. *I. purchasi* is extremely polyphagous pest and infested more than 180 plants belonging to 66 families (García et al. 2015). Once introduced into glasshouses, it may become a significant risk for production of ornamentals in Bulgaria, because of its ability to rapid distribution. The cottony cushion scale spreads by crawling from plant to plant, via wind, on machinery, and with labour crews. Accidental introduction to new territories is possible through infested live plants, particularly in shipments of whole ornamentals and fruit trees (CABI 2015b).

Phenacoccus peruvianus Granara de Willink, 2007 [Hemiptera, Pseudococcidae] **–** Bougainvillea mealybug.

Collected material: Ravda – May 2012 and July 2014; Burgas – May 2012.

Specimens of Bougainvillea mealybug were detected two times in garden centre in Ravda and in greenhouses in Burgas on several *Bougainvillea glabra* Choisy plants (Fig. 4b), imported from Italy. Although *P. peruvianus* has been recorded in Bulgaria in 2014 (Pencheva et al. 2014), this species was first detected in May 2010 on the same hosts.

The mealybug is of Neotropical origin (Argentina, Peru) and was recorded to Europe (Spain) in 1999, prior being described by Granara de Willink. Since then *P. peruvianus* has been found in different European countries (Sicily, Italy, Corsica, Croatia, Portugal, Monaco, France and Greece) (Beltrà et al. 2010, Gkounti and Milonas 2013, Masten et al. 2015).

Bougainvillea mealybug feeds mainly on Bougainvillea plants, which are widely cultivated in gardens of the Mediterranean region and frequently traded as a potted plant (Mazzeo et al. 2014). It is also recorded on *Alternanthera* spp., *Araujia sericifera* Brot., *Aucuba japonica* Thunb, *Buddleja* spp., *Solanum vespertilio* Ait., *Plectranthus scutellarioides* (L.) R. Br., chilly peppers and others (Beltra et al. 2010). Among its hosts *P. peruvianus* shows a preference for members of Solanaceae and can successfully complete its life cycle on *Solanum lycopersicum* L. and *Nicotiana tabacum* L. (Beltrà et al. 2013). Large mealybug populations cause necrosis of the foliage, leaf loss, dieback and sooty mould development on the excreted

a b

Fig. 5. Adult females of *Ceroplastes* spp.: *C. ceriferus* (a) and *C. japonicus* (b).

honeydew (Malumphy and Eyre 2011).

Ceroplastes ceriferus (Fabricius, 1798) [Hemiptera, Coccidae] – Indian wax scale.

Collected material: 2013: 3 June and 25 July – Nessebar; 2015: 30 October – Sozopol.

A few *C. ceriferus* females (Fig. 5a) were detected in two private properties nearby Nessebar in 2013, on single *Pyracantha* sp. and *Ilex aquifolium* shrubs. This scale was found again in October 2015 on twigs of *Euonymus alatus* (Thunb.) Siebold in a private garden in Sozopol. In both localities *C. ceriferus* had been distributed along with plant material imported from Italy two years ago.

Indian wax scale was detected for the first time in Bulgaria in June 2009 on a private property in Vidin on a solitary *Acer palmatum* 'Atropurpureum' tree (Pencheva and Yovkova 2011).

C. ceriferus is most likely to be native to Asia, but it has already been widely distributed all over the world (García et al. 2015). In Europe, it has been intercepted several times at the imports of ornamental plants (Pellizzari et al. 2004). *C. ceriferus* has been found also in Netherlands, UK and Slovenia (Fetyko and Kozár 2012, Seljak 2012).

The Indian wax scale may use a wide variety of hosts comprising more than 120 plant species belonging to 51 families and 71 genera (García et al. 2015). Besides fruit crops, it attacks also lots of ornamentals (*Acer, Berberis, Buxus, Cornus, Deutzia, Euonymus, Ficus, Ilex, Lagerstroemia, Laurus, Magnolia, Platanus, Pyracantha, Rhododendron, Salix* and *Viburnum*) (EPPO 2015). Like all the others, wax scales *C. ceriferus* secretes large amount of honeydew which provides a medium for sooty mould. The sooty mould may become so dense that it interferes with photosynthesis (Hamon and Williams 1984, Seljak 2012).

The observations over the last three years indicated that *C. ceriferus* can successfully overwinter in locations around the Black Sea coast.

Ceroplastes japonicus Green, 1921 [Hemiptera, Coccidae] – Japanese wax scale.

Collected material: 2013: 25 July – Ravda and Aheloy; 2015: 20 July – Aheloy.

Adult females, first and second instar nymphs of *C. japonicus* were detected on leaves and twigs of *Ilex aqufolium*, *Citrus* sp., *Laurus nobilis, Viburnum thinus* L. and *Pyracantha coccinea* M. Roem (Fig. 5b) in garden centre in Ravda and in private property in Aheloy. The country origin of infested ornamentals was Italy. Japanese wax scale was found for the first time in Bulgaria in 2006 on plant material collected in greenhouses (Pencheva 2007).

C. japonicus is native to Eastern Asia and is currently a pest of significant economic impact for citrus and other fruit crops in Asia. In Europe it represents a serious phytosanitary threat mainly to laurel, holly and ivy (Mazzeo et al. 2014). *C. japonicus* is widespread on ornamentals in several European countries, both outdoors (i.e. Armenia, Croatia, Georgia, Italy, France, Netherlands, Russia and Slovenia) and indoors (i.e. Hungary, Unitved Kingdom) (Fetykò and Kozár 2012). The present investigation confirms that the species is established in the southern Black Sea coast where it overwinters outdoors (Pencheva 2009).

Ceroplastes sinensis Del Guercio, 1900 [Hemiptera, Coccidae] – Chinese wax scale.

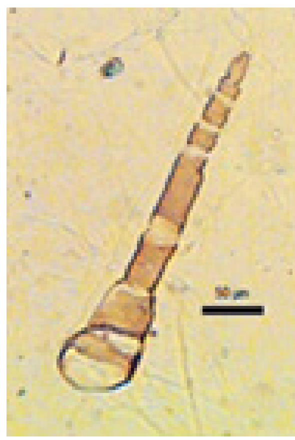
Collected material: 2013: 25 July – Ravda.

Specimens of *C. sinensis* were identified on *Ilex aquifolium* imported from Italy to a garden centre in Ravda (Fig. 6). This is the first record of this scale species in Bulgaria. The adult females are convex, reach up to 3–7 mm in length and 2.0–6.0 mm in width. The dorsal wax cover is formed by 7 not very distinctly divided plates – 1 dorsal and 6 lateral. The wax cover is white in young females and reddish brown in old specimens (Gimpel et al. 1974).

Based on cladistics analysis of wax scale insects, Qin et al. (1994) predicted that *C. sinensis* originates from Central or South America. This supposition was supported later by the discovery of heavily parasitized individuals of Chinese wax scale in Argentina (Hodgson and Peronti 2012). It is a highly polyphagous pest and can damage more than 130 plant species belonging to 54 families (García et al. 2015). Currently *C. sinensisis* is distributed in several European countries (Croatia, France, Greece, Italy, Portugal, Spain, Turkey and subtropics of the former USSR) (Fetyko and Kozár 2012).

Chinese wax scale completed a single annual generation. The optimal conditions for its development are: temperature 22,4–26,0 ºC and air humidity 70–80 %. The pest is not as cold resistant as *C. japonicus*. The mean fecundity of adult females was 3260 ± 770 eggs per female (García et al. 2015). The heavy infestation leads to the death of branches and, some-

a



b

Fig. 6. *Ceroplastes sinensis*: a – adult females; b – the antenna of Chinese wax scale is 7-segmented.

times, plants. Its introduction in Bulgaria may become a problem in ornamental plant production.

Aulacaspis yasumatsui Takagi 1977 [Hemiptera, Diaspididae] – Cycads aulacaspis scale.

Collected material: 2013: 3 April – Ravda and 28 July – Sofia.

A. yasumatsui was detected on a few imported plants of sago cycas (*Cycas revoluta* Thunb.) in a garden centre in Ravda in April 2013. Although the infestation had not been detected prior to carrying the plants to the greenhouse, within two months all leaves were covered with a white crust, comprising scales of live and dead insects. Damage initially appears as chlorotic spots, but most of the fronds eventually become brown and withered. Later *A. yasumatsui* was collected in a greenhouse in Sofia, also on imported sago cycas plants. Although this infestation was not so drastic, in both cases the plants died. Cycads aulacaspis scale was first detected in Bulgaria by Trencheva et al. (2010a) in 2009 in a garden centre in Tsarevo, at the southern Black Sea coast.

A. yasumatsui is native to Southeast Asia. Its host plants are from the gymnosperm order Cycadales, which consists of three families – Cycadaceae, Stangeriaceae and Zamiaceae (CABI 2015c). It is highly damaging to cycads, which include horticulturally important and endangered plant species. It is difficult to control, as it forms dense populations and spreads quickly to new areas via infested plant trade (Howard et al. 1999).

Lepidosaphes flava (Signoret, 1870) [Hemiptera, Diaspididae] – De Stefan scale.

Collected material: 2012: 13 April – Sofia and 2013: 26 April – Burgas.

Only sporadically infestations of *L. flava* were detected on olive trees (*Olea europaea* L.) grown indoors in two garden centres. This is the first record of the De Stefan scale in Bulgaria. Apparently, all infested plants had been imported from Greece and Italy.

De Stefan scale is native to Near East and Mediterranean Palaearctic. In Europe it is distributed in Cyprus, Greece, Italy, Montenegro, France, Spain, Turkey, Ukraine and Russia (Central and Southern) (García et al. 2015). Their females are brown, elongated and 2.4–3.0 mm long. The male puparium is clear brown, 1.8 mm long (Danzig 1993). In Italy *L. flava* has one generation per year and it overwinters as a mature fertilized female. Each female produces 25–30 eggs (García et al. 2015). This pest prefers *Olea europaea* (Pellizzari.et al. 2011) and *Ligustrum* spp. (Gill 1997) as hosts, and should not be a major risk for Bulgaria since olive trees are grown predominantly in greenhouses and interior.

Pseudaulacaspis pentagona (Targioni Tozzetti, 1886) [Hemiptera, Diaspididae] – white peach scale.

Collected material: Balchik (13 May 2014), Sofia (from May to October in the period 2012–2015) and Plovdiv (6 June 2013).

P. pentagona was detected in Balchik (on *Broussonetia papyrifera*), Plovdiv (*Platanus* × *acerifolia* (Aiton) Willd.) and in different private properties on Sofia (on *Cornus alba* L. ‘Elegantissima’, *Syringa* x *persica* L. ‘Alba’, *Rosa hybrida* L. and 12 other arboreal hosts) mainly on *Catalpa bignonioides* and *Morus alba* f. *pendula* (Fig. 7). The last two hosts were imported from Italy and had been planted in private gardens approximately in 2007 or 2008. Heavily infested trees exhibited dieback and died after a few years.

The white peach scale is native to Eastern Asia, where it is a pest of *Prunus* and *Morus.* It was accidentally introduced to Italy in the nineteenth century, and has nowadays spread to all major continents (CABI 2015d). The scale was first recorded in Bulgaria in 1968 (Tsalev 1968), but its distribution was limited predominantly in south-western regions (Trencheva et al. 2010b) as an important economic pest of peach trees.

A batch of infested ornamental shrubs from a single nursery can easily spread the pest widely. In temperate regions, dense populations can form thick crusts of scales on tree trunks and older branches. In Central Europe it has colonized both cultivated and natural habitats. Heavy outbreaks of the scale insect have occurred on ornamental plants in Hungary (Kosztarab and Kozár 1988). *P. pentagona* can be mentioned as a significant invasive scale present in the highway margin zone (Bayoumy et al. 2011).

Fig. 7. Dense colonies of *Pseudaulacaspis pentagona* on branches of *Morus alba*.

Cacoecimorpha pronubana (Hübner, 1799) [Lepidoptera, Tortricidae] – carnation tortrix moth.

Collected material: Burgas – 26 May 2012; Aheloy – from May to September in the period during 2012–2015; Ravda – 2013: May and July.

Last four years *C. pronubana* was detected in Aheloy (in its previous locality) (Pencheva et al. 2009), with a low population density, because of continuous control, including pruning the branches with hibernating caterpillars and pheromone traps. In Burgas and Ravda the species was found in garden centres on *Viburnum tinus* L. and *Aucuba japonica* Thunb. (Fig. 8), imported from the Netherlands and Italy.

This Mediterranean species is widespread in Europe and North Africa. At present its range is expanding across Europe, having been able to invade even northern countries such as Lithuania, which is a confirmation of the great adaptability of this insect (Signorile 2012). Over 100 plant species have been noted as hosts, however, *Dianthus* is the most seriously affected, although other species have suffered infestations regarded as serious. In international trade, *C. pronubana* may be carried on plants for planting or cut flowers of carnations, chrysanthemums, pelargoniums, roses and other host plants (CABI 2015e).

Fig. 8. Adult of *Cacoecimorpha pronubana.*

Cydalima perspectalis (Walker, 1859) [Lepidoptera, Crambidae] – box tree moth.

Collected material: 2014: 25 August – Balchik, Dragalevtsi (near Sofia); 28 August – Dobrich; 2015: 20 May – Balchik and Evksinograd (near Varna); from 15 July to 15 October – Balchik, Burgas, Kalofer, Primorsko, Sozopol, Plovdiv and Sofia.

During the present observations in the end of August 2014, damage to *Buxus sempervirens*, as well as larvae, pupae and moths (Fig. 9a) were found in new locations: private gardens in Dobrich and Sofia. In summer 2015 the pest was detected both in private gardens and in city parks located in Burgas, Primorsko, Sozopol, Sofia, Plovdiv and Kalofer. Plants of different ages – from 5 to 80 and more years old (in Euxinograd) – were attacked in both old plantings and newly created sites. The infested box trees were defoliated, particularly in lower parts of the crowns. This damage reduced completely the aesthetic value of plants. Odher's were webbing of the branches with frass and residues of moulting. Heavy damage or repeated attacks lead to total defoliation and may cause the death of the shrubs. The young larvae of the box tree moth feed on the lower surfaces of the leaves, leaving the upper epidermis intact (Fig. 9b). Older caterpillars gnaw roughly on leaves, leaving thick veins only. They can also eat the green bark of the young twigs.

The box tree moth was first detected in Bulgaria in July 2014 in Balchik Botanical garden and in Varna by Beshkov et al. (2015). It is a very destructive insect, injurious to *Buxus* spp. (*B. sempervirens* L., *B. microphylla* Siebold & Zucc. and *B. colchica* Pojark.). In its native range (China and Japan) it feeds on *B. sinica* (Leuthardt 2013). In Southern Russia *C. perspectalis* also attacks *Ruscus aculeatus* L. and *Prunus laurocerasus* L. (Skvortsov 2013).

The species is a well-known pest within its original range in Asia. It has spread widely throughout Europe in recent years, probably introduced with box tree seedlings. Due to a lack of natural enemies

a b

Fig. 9. ***Cydalima perspectalis*****: a – adults; b – damage by a young larva.**

in its invaded range, *C. perspectalis* can reach large population sizes and densities (Leuthardt 2013, Hizal et al. 2012, Ramel and Ross 2013*).* Observation of the dispersal of European populations of *C. perspectalis* in northwest Switzerland allowed an estimate of the natural dispersal velocity of adults to be made at 7–10 km per year (Leuthardt et al. 2010).

The box tree moth has two to three generations per year in Europe, while in the native range up to 5 generations per year are possible (Perny 2010). It overwinters as a larva, spinning a cocoon between box leaves in autumn and completes its development the following spring. In Balchik, after overwintering, the larvae continued feeding until the middle of May 2015 and pupated in the crown of plants. The first generation moths appeared at the beginning of June**.** A new flight of moths was also detected in the middle of August. In Sofia defoliation of box trees was detected in the middle of July and flight of moths – in the end of August. The new generation larvae were in diapause in the middle of October. The results from the observations carried out in 2015 demonstrate that the box tree moth is spreading quickly throughout the country. Its introduction could represent a threat to nurseries, historical parks and gardens, as well as to plant hosts growing in the wild.

The above presented alien insects are mainly polyphagous except for five monophagous ones – *A. jamatonica, T. alacris,* the coccids *A. yasumatsui* and *L. flava*, and the moth *C. perspectalis.* Polyphagous insects are more likely to become major pests when introduced to new areas because the existing plant hosts may allow them to successful development and reproduction.

Five of the discussed insects are restricted to greenhouses or are merely interceptions, with the slight possibility of outdoors surviving. The species, which are able to adapt to outdoor conditions, are mostly detected as a small sample or in limited localities (with exception of *Metcalfa pruinosa* and *Pseudaulacaspis pentagona*). Probably within quite a short period of time they may release their potential to be more widely spread and established on the territory of Bulgaria, especially in its south-western part. Global warming and mild winters also indirectly affect acclimatization frequencies by increasing the abundance of suitable host plants for alien insect species (Mazzeo et al. 2014). Within Europe, the Mediterranean basin is especially susceptible to insect invasions, due to its climatic conditions being favourable for the establishment of tropical and subtropical non-native species (Roques et al. 2009). Currently the greatest part of ornamental plants in Bulgaria is produced in Italy, the Netherlands and Hungary. The surveying insects in these countries are common pests and their accidental introduction in Bulgaria is possible via imported plants because of reduced border control between EU-countries.

Conclusion

Fourteen insect species associated with urban landscape areas have been surveyed. Two of them have been reported for the first time in Bulgaria – *Ceroplastes sinensis* and *Lepidosaphes flava*. Three species (*Cacoecimorpha pronubana*, *Ceroplastes ceriferus* and *Metcalfa pruinosa*) have widened their host range in Bulgaria. *Aulacaspis yasumatsui, Acizzia jamatonica*, *Pseudaulacaspis pentagona* and *Cydalima perspectalis* have been found in new locations. The quick spread of the box tree moth indicates that the pest will be a serious risk for *B. sempervirens* throughout the country.

The results of investigation indicate that exotic pests and especially scale insects use mainly ornamental plants as a pathway of introduction in new areas, so that urban landscapes and nurseries are the first habitats in which they become established.

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ECOPHYSIOLOGICAL METHOD FOR ASSESSMENT OF *ORCHESTES FAGI* L. INFESTATION ON COMMON BEECH TREES

Petya Dimitrova-Mateva[1*], Svetoslav Anev[1], Simona Georgieva[1], Ganka Chaneva[2], and Nikolina Tzvetkova[1]

[1]University of Forestry, 10 St. Kliment Ohridski Blvd. 1797, Sofia, Bulgaria.
[*]E-mail: petiape@abv.bg
[2]Faculty of Biology, Sofia University 'St. Kliment Ohridski',
8 Dragan Tzankov Blvd. 1164 Sofia, Bulgaria

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Abstract

The paper presents an applicable method for investigation and assessment of the damage caused by beech weevil (*Orchestes fagi* L.) on the leaves of Common beech (*Fagus sylvatica* L.). Selecting of appropriate ecophysiological approaches is a workable tool for an assessment of leafminers' effects as these methods can be applied for leaves with a gradient of damaged area. Thus, it can be established a tendency in the attack effect and determined the limits of the plant protective system. This methodology was applied successfully and can provide reliable set of data for clarifying the relationship between the mining larvae, developing in the infested leaves, and the host plant. The application of this methodology in other environmental conditions and woody species could contribute to a development of biological means for a control on the leafminers in the forests.

Key words: ecophysiology, leafminer, plant defense system, process disruption.

Introduction

Leafminers are a serious problem for the most of tree species in Bulgaria. The complex of leafminers may cause up to 78 % of the total damaged area of beech leaves (Dimitrova-Mateva 2008). Our previous investigations have established that *Orchestes fagi* L. is the first in importance in the group of leafminers on Common beech leaves whose feeding induces alterations in the activity of some antioxidant systems (Miteva et al. 2007, Dimitrova-Mateva et al. 2014). The growth of beech weevil in our country is connected with the Common beech (*Fagus sylvatica* L.) and Oriental beech (*Fagus orientalis* Lipsky) trees. Damages are observed both by the adults and also by the larvae of *O. fagi*. During its feeding the imago makes holes on the beech leaves. This allows an estimation to be made for a degree of infestation by beech weevil. The imago destroys an average of about 20 % (in the particular cases up to 93.2 %) of the photosynthetic leaf area (Dimitrova-Mateva 2008).

Feeding of the larvae is tightly connected with the parenchyma layers of the leaves. Larvae make a large rounded mine which is visible from both leaf sides. It initiates from the midrib and goes to the top of leaf blade, whereas two epidermal layers remained unaffected. The accurate assessment of the defoliation is difficult to be made as an absence of a visible loss of leaf area.

In the mined leaves arises physiological stress, provoked by the interaction between insect and host plant. On one side, the insect damages leaf tissue and induces plant's response. On the other, the physiological activity of larvae affects the physiological and biochemical processes of the host plant (Dimitrova-Mateva 2014). This imposes the necessity a new approach to be applied for an assessment of infestation effect by leafminers.

In Bulgaria so far has been applied mainly a morphological analysis of damaged beech leaves. Examination of artificial defoliation of *F. orientalis* seedlings established that the reduction of the photosynthetic area leads to decrease in the annual growth (Stalev 1990, 1991). The author has tried to estimate the defoliation by leaf chewing insects, considerably different from that, caused by leafminers.

The first steps in the biochemical analysis of infested beech leaves have showed that the feeding and developing of the beech weevil larvae causes remarkable changes in the enzymatic and non-enzymatic components of plant defence system as endogenous phenols, low molecular thiol compounds and products of lipid peroxidation (Miteva et al. 2007).

In Europe, where is the natural region of *Fagus sylvatica* L., such kind of researches have not been made. There are no systematic investigations on the changes in the physiological activity of any forest tree species caused by infestation of leaf-mining insects. Estimation has been made on the germination of *Aesculus hippocastanum* L. at a strong mining of leaves of *Cameraria ohridella* Deschka & Dimic (Takos et al. 2008). Isolated analyses of photosynthesis are made on mined leaves of some grasses, fruit trees and agriculture species (Hespenheide 1991).

Separate studies established, that as a result of damages, caused by *Orchestes fagi* L., the vitality of *Fagus grandifolia* Ehrh in North America weakened and trees become more susceptible to the root fungi from genus *Armillaria* sp. (Sweeney et al. 2012). It was observed a preliminary fall of beech leaves (Pritchard and James 1984), which may be regarded as a mechanical defence (Dimitrova-Mateva 2014).

The larvae start to develop still in the beech buds and cause reduction in the infested leaf area. At a weak level of infestation the physiological processes in the unaffected leaf area can be stimulated and do not show a negative effects on the plant (Retuerto et al. 2004). Due to the faster flow of assimilates in some kind of damages the rate of photosynthesis increases and thus compensates the reduction in the leaf area (Meyer and Whitlow 1992). Stimulating effect of the attack by other insects on the physiological activity was observed even in the lightly infested leaves. In very strong infestation, when most of the leaf area is affected, the physiological indicators decreased sharply (Anev et al. 2013). The preliminary drying and falling of the leaves causes not only a lack of assimilates during the growth period but also decreases plants' ability to accumulate nutrients for the unfavourable winter period (Eyles et al. 2011). This serves as a predisposition for the weakening of plant and its defensive system in the winter as well as for a delayed and weakened start in the next growth period and finally leads to gradually exhausting of the whole plant.

This paper presents a set of ecophysiological methods for an investigation of beech weevil *Orchestes fagi* L. on the leaves of Common beech. The complex methodology consisted of four groups of methods and was applied successfully in young beech forests at two altitudes in the region of Western Balkan mountain (Anev et al. 2015, Chaneva et al. 2015a, Chaneva et al. 2015b, Dimitrova-Mateva et al. 2015).

Morphological Analysis of Beech Leaves

Leaf material was collected during the entire growth period in samples plots at two different altitudes (Anev et al. 2015). In order to be determined the level of infestation by beech weevil randomly chosen leaves from the lower crown parts of stock trees were analyzed (Stalev 1990, 1991; Nielsen and Ejlersen 1977; Nielsen 1968, 1978). Biometric parameters of the leaves (total leaf area, damaged and undamaged area, total leaf length and width as well as a length of petiole) were determined with open source software ImageJ (Rasband 2016).

The established leaf damages were classified in the following groups: number of mines and mined area by *O. fagi*, chewed area by the adults of beech weevil, mined area and number of mines, caused by leaf mining moth *Ph. maestingella*, damages by gall-makers and leaf-chewing insects.

The data collected allow to be calculated:

1. Frequency of the every one of the insect species or group of pests as a percentage of the infested leaves to the total number of the analyzed leaves (Connor et al. 1983, Vorontzov et al. 1991).

2. Participation of each type of damage as a percentage of the total leaf area and the total area of damage.

3. Coefficient of the quantitative density (*K*) – the number of the individuals per leaf area (Nielsen and Ejlersen 1977, Nielsen 1978):

$$K = \frac{Z}{N}.p.m.t$$ (according to Segebade and Schaefer 1979)

where:

N – number of observed trees;

Z – number of infested trees;

p – ratio between the number of infested leaves and the total number of leaves;

m – an average number of mines to the number of the holes on a leaf;

t – percentage of damaged area to the total leaf area.

4. Index of the degree of damage (*R*) (Rosnev et al. 2007)

$$R = \frac{\sum n.k.100}{N.K}, \%$$

where:

n – number of the model trees with a definite degree of infestation;

k – degree of infestation;

N – total number of model trees;

K – maximal degree of infestation.

This formula is applied in the field of forest protection for defoliation assessment of whole trees (Rossnev et al. 2007), using five degrees of infestation (*k*) by the following percentages of defoliation: 0 (0–10 %), 1 (11–25 %), 2 (26–59 %), 3 (60–99 %) и 4 (100 %). Previous studies have shown that the damage from leaf insects rarely exceeds 30 % (Dimitrova 2005, 2008). For this reason different type of grouping was determined in dependence on the highest recorded level of attack. Such exemplary grouping (Table 1) was used in our field experiments in order to determine the relationship between the level of infestation and the physiological and biochemical parameters (Anev et al. 2015).

Table 1. Degree of infestation (k) of the leaves depending on their damage.

Degree of infestation	Group
0 – 20 %	1
20 – 25 %	2
25 – 30 %	3
> 30 %	4

Gas Exchange and Chlorophyll Content of Beech Leaves

Measurements of the rate of basic physiological indicators (photosynthesis, transpiration, and stomatal conductance) during the growth period were performed by using Portable Photosynthetic System Li-6400 for an infrared gas analysis (Li-Cor 2012) on attached beech leaves with different degree of infestation. The total content of chlorophyll was determined on living leaves with a portable chlorophyll meter AT LEAF by the method of non-invasive measurements on the basis of difference in the optical density between 660 nm и 940 nm.

Biochemical Analyses of Beech Leaves

After a determination of the level of damage, rate of gas exchange and total content of chlorophyll the sampled leaves were fixed in liquid nitrogen for subsequent biochemical analyses.

The concentrations of chlorophyll *a*, chlorophyll *b* and carotenoids were measured spectrophotometrically after an acetone extraction (Arnon 1949).

Malonedialdehyde (MDA) content was measured according to Dhindsa et al. (1981). Endogenous hydrogen peroxide (H_2O_2) was determined spectrophotometrically at 390 nm (Jessup et al. 1994). The determination of free proline levels was done according to Bates et al. (1973). The content of soluble proteins was measured by method with Kumasi Brilliant Blue G250 (Bradford 1976). The activity of enzymatic defence system was determined by methods as followed: superoxide dismutase (EC 1.15.1.1) – after Beauchamp and Fridovich (1971); guacol peroxidase (EC 1.11.1.7) – after Hart et al. (1971); catalase (EC 1.11.1.6) – after Aebi (1984).

The histochemical detection of active oxygen ions was made with the method of Sakamoto et al. (2005). The content of hydrogen peroxide and superoxide anions – with the method of Shinogi et al. (2003). The content of phenols was determined by the method of Singleton et al. (1999).

Statistical Analysis

All measurements were processed statistically in the following order: tests for a type of data distribution, descriptive statistics and ANOVA-test for estimation of the significance of the differences among the groups of infestation in relation to the investigated physiological and biochemical parameters on the respective altitude.

Conclusions

The presented methodology is an original complex of classic and up-to-date ecological observations, physiological measurements and biochemical analyses of the infested by the beech weevil Common beech leaves. This methodology was applied successfully and can provide reliable set of data for clarifying the relationship between the mining larvae, developing in the infested leaves, and the host plant. Its application in other environmental conditions, and other woody species, will be a contribution to the development of biological tools for leafmining pests control in the forests.

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GAS-EXCHANGE DYNAMICS WITHIN THE CROWN OF NORWAY SPRUCE

Svetoslav Anev

Department of Dendrology, Faculty of Forestry, University of Forestry, 10 Kliment Ohridski blvd., 1797 Sofia, Bulgaria. E-mail: svetoslav.anev@gmail.com

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Abstract

The balance between CO_2 income during photosynthesis and H_2O release during transpiration is strongly influenced by differentiation of leaves within the canopy. Gas-exchange and water use efficiency (WUE) were studied in respect to branch position in the crown of 37-year-old Norway spruce [*Picea abies* (L.) Karst.] tree, growing in the Western Stara Planina. The needles of the middle-crown branches had significantly highest rate of net photosynthesis compared to the upper and lower crown. The rate of transpiration and vapour pressure deficit gradually decreased to the upper-crown branches. The highest water use efficiency was established for the needles in the top of the crown. The lowest photosynthesis and WUE, along with the highest transpiration and vapour pressure deficit of the branches in the crown base of the tree are indicative for the diminished adaptive capacity. The study results could contribute to better understanding of the physiological mechanisms of branch self-pruning.

Key words: photosynthesis, self-pruning, stomatal conductance, transpiration, water use efficiency.

Introduction

Recent models of canopy physiology (Cannell and Thornley 1998, Jones 2013) are based on the assumption that leaf physiological characteristics are constant throughout the tree crown. However, Rosell and Sanz (2012) have shown that variation in leaf geometry can result in photosynthetic variation related to changes in the surface area for light interception. The conifer needles are grouped closely together, so that photosynthesis is strongly affected by shading of one needle by another (Leverenz and Jarvis 1979) or of one branch by another (Norman and Jarvis 1974). Leaves that are shaded by other leaves of the same plant experience limitations similar to those experienced by a plant shaded by other plants (Berry 1975). Björkman (1981) explained that separate crown part of tree plants reacts differently to light variation. There are several reviews on the specific anatomical and morphological characteristics of the sun and shade leaves that provoke big variation in the physiological processes (Leverenz and Jarvis 1980, Masarovicová and Štefančík 1990, Thornley 2002, Terashima et al. 2006).

Furthermore, there are large changes in the microclimatic conditions with increasing of depth in the forest canopy (Campbell and Norman 1998). The light direction, light intensity and light spectrum are strongly affected by the crown and canopy architecture (Jones 2013). Bazzaz and Williams (1991) found out that the air concentration of CO_2 in the crown base is higher than that in the top of crown. The failure of conventional flux-gradient relationships in the crown space, which may be explained with the sporadic penetration of transporting eddies into the canopy (Denmead and Bradley 1985).

The data about the differences of the photosynthesis intensity for sun and shade shoots in Norway spruce are controversial. Wierzbicki (1980) has reported that maximal photosynthesis is higher in sun shoots. On the other hand Fuchs et al. (1977) found higher photosynthetic rate in the shade than in the sun crown. Hinckley and Ritchie (1970) showed that stomatal activity increased, but water stress decreased with increasing of branch position in the crown of spruce. Their results suggest that water levels within the crown are in spatial gradient. Sellin and Kupper (2004) define a hypothesis that stomatal conductance at the base of the crown is constrained not only by low light availability but also by plant's inner hydraulic limitations.

The aim of this study is to find out the specificity in the gas-exchange within the vertical crown profile of Norway spruce during the phase of intensive branch self-pruning. This knowledge could be useful for better understanding of the physiological mechanisms of branch self-pruning.

Table 1. Site description and plant material.

Site description		Plant material	
Altitude, m a.s.l.	825	Age, years	37
Latitude, °	43.15	Height of tree, m	20.0
Longitude, °	23.14	Height of crown, m	11.2
Slope, °	8	Diameter at breast height (DBH), cm	21.6
Exposure	East	Trees density, ha^{-1}	1150

Materials and Methods

Site description and plant material

The experiment was conducted in the Western Stara Planina. Site description and plant material characteristics are shown in Table 1.

The study was carried out in even-aged forest monoplantation in phase of branch self-pruning. The measurements were performed on an experimental tree, chosen on the basis of mean diameter at breast height (DBH) and mean crown length. Thirty-three yearling branches with three different geographical expositions were taken from eleven crown levels (at every one meter from the base to the top of the crown). The third leading shoot from the top of the branches were used for gas-exchange measurements.

Gas-exchange measurements

Foliar gas-exchange measurements were acquired using a LI-6400 portable infrared gas analyzer (LI-COR Ltd., Lincoln, Nebraska, 68504 USA) with the conifer chamber (6400-05) on mature needles at ambient conditions: rate of photosynthetically active radiation, CO_2 concentration, air temperature and relative humidity (Table 2).

The rate of photosynthesis (An), transpiration rate (E), stomatal conductance (gs)

Table 2. Microclimatic conditions during the gas-exchange measurements.

Parameter	Value ± SE
Air temperature, °C	23.35 ± 0.09
Air humidity, %	50.82 ± 0.18
PAR, $\mu mol \cdot m^{-2} \cdot s^{-1}$	867.8 ± 9.4
Air CO_2 concentration, ppm	402.9 ± 0.1

and vapour pressure deficit of leaves (VpdL) were measured in mid-October, in a clear and sunny day. The water use efficiency (WUE) as ratio between An and E and the intrinsic water-use efficiency (iWUE), defined as ratio between An and gs, were calculated. Data were taken after a minimum 30-minute LI6400 calibration period. Readings were logged when gas-exchange was become stable as marked by: (a) visually stable intracellular CO_2 concentration and net CO_2 assimilation rates values and (b) a total coefficient of variation less than 0.5 %, according to the Li-6400 manual (2012). For recalculation of the measured elements of gas-exchange, the projected area of the needles of the experimental shoots was determined with open source software ImageJ (Rasband 2016).

Data analysis

Due to a random distribution of the physiological data (Shapiro-Wilk Normality Test), the medians and median absolute deviations were calculated. The quadratic polynomial regression was used for the analysis of the relationships between branch position throughout the crown and the rate of particular physiological parameter. The significance of the regression coefficients (R^2) was tested by F-statistics. Only significant coefficients were discussed.

Results and Discussion

The highest rate of photosynthesis was measured within the range from 35 % to 90 % of the crown length (Fig. 1). The rate of photosynthesis decreased along with light attenuation in the base of crown and also a sharp decrease was found in the top of crown. Such tendency may refer to the specific crown architecture of Norway spruce, which is adapted to efficient light utilization (Schulze 2005). The photosynthetic rate at light limitation is largely determined, apart from the light, and by the air temperature (Kirschbaum and Farquhar 1984), whereas at light saturation is mostly determined by CO_2 concentration and ribulose-1.5-bisphosphate carboxylase/oxygenase (Rubisco) activity (von Caemmerer et al. 2009).

The transpiration rate decreased gradually from the base to the top of crown, showing big variation among the separate crown levels. However, the greatest variation and lack of significant tendency within the crown were found about gs. Present results are in agreement with the findings of Hinckley and Ritchie (1970) that in the top of spruce crown water stress increased, but do not confirm the hypothesis of Sellin and Kupper (2004) for a gradient of stomatal conductance. The highest E in the base of the crown is probably due to favourable air humidity in this canopy level. In the same time the highest E and lowest light intensity in the base of crown probably reflect in a decrease of An. VpdL decreased gradually from the base to the top of the crown. The big VpdL in the lower crown parts provokes an intensive transpiration and limited photosynthesis. The clearest tendency was found out in the vertical dynamics of WUE. The low efficiency of water utilization of the branches in the base of the crown is probably evidence for a suppressed wa-

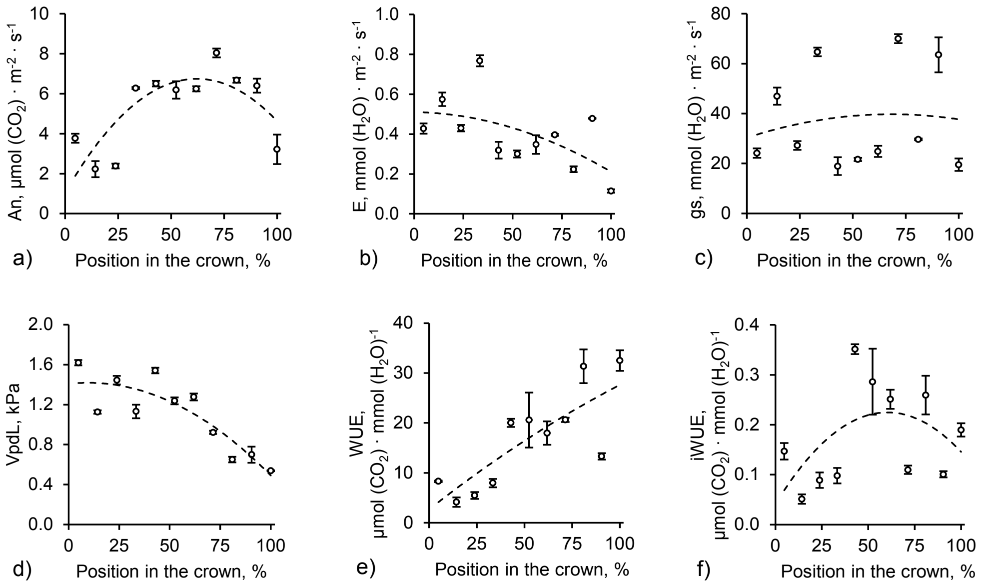

Fig. 1. Vertical gradient of: a) rate of photosynthesis (An); b) rate of transpiration (E); c) stomatal conductance (gs); d) vapour pressure deficit of leaves (VpdL); e) water use efficiency (WUE); f) intrinsic water-use efficiency (iWUE) within Norway spruce crown.

Note: Median (○) ± median absolute deviation and quadratic polynomial regression (---) is presented.

ter and CO_2 balance. Such tendency may lead to intensive self-pruning of the trees. The iWUE is influenced by the high variation of gs and the well-defined trend of An within the crown profile. The lowest levels of iWUE in the base crown part contributes to restricted ability for survival of these branches. Great variance in measured physiological parameters obtained in the present study definitely do not support the idea for the permanence of leaf physiology throughout the crown, which is the basis of some models of canopy physiology.

Table 3. Coefficients of determination and F-statistics of polynomial regressions between branch position in Norway spruce crown and photosynthetic rate (An), transpiration rate (E), stomatal conductance (gs), vapour pressure deficit of leaves (VpdL), water use efficiency (WUE) and intrinsic water use efficiency (iWUE).

Physiological parameters	R^2	F test	*P* value
An	0.497	14.831	< 0.001
E	0.396	9.823	< 0.001
gs	0.007	0.103	0.902
VpdL	0.737	42.036	< 0.001
WUE	0.551	18.442	< 0.001
iWUE	0.211	4.013	0.029

The regression analysis revealed significant dependence of An, E, VpdL, WUE and iWUE on branch position within the vertical profile of crown (Table 3).

The highest dependence for VpdL (R^2 = 0.737) is probably due to morphological gradient of the needles within the crown. The volume of intercellular space increases with increasing of light and tree height (Niinemets and Kull 1995, Grassi and Bagnaresi 2001). The WUE of needles is depended close to linearly on the branch positions within the crown. The needles in the top of crown had highest WUE, i.e. the most adequate CO_2 and H_2O balance compared to needles in the crown base. Apart from the light deficit in the base of the crown (Sorrensen-Cothern et al. 1993), the lowest WUE along with the highest VpdL probably have a key role for branch self-pruning.

Conclusions

The following tendency within the Norway spruce crown is found:

- The results obtained in the present study definitely do not support the idea of permanence of leaf physiology throughout the crown.
- The needles of the middle-crown branches showed significantly higher rate of net photosynthesis compared to the upper- and lower-crown branches.
- The rate of transpiration and vapour pressure deficit gradually decreased to the upper-crown branches.
- The highest water use efficiency was established for the needles from the top of the crown.
- The gas-exchange dynamics within the vertical crown profile approved the inability of base-crown branches to keep a positive H_2O balance, which is probably the significant reason for self-pruning.

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ENHANCED GROWTH CHARACTERISTICS OF *CASTANEA SATIVA* MILL. SEEDLINGS UNDER LED LIGHTING WITH CONTINUOUS SPECTRUM DURING INDOOR CULTIVATION

Sonia Smirnakou and Kalliopi Radoglou*

Department of Forestry and Management of Environment and Natural Resources, Democritus University of Thrace (DUTH), Pandazidou 193, 68200 Orestiada, Greece. E-mails: sosmirnakou@gmail.com, kradoglo@fmenr.duth.gr*

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Abstract

Previous studies have demonstrated that monochromatic LEDs could be used as an alternative to conventional lighting sources for cultivation of several species in artificial environments, which have led to satisfactory results. However, the application of LED lights of continuous spectrum as an artificial light source in plant growth chambers has not been tested yet. This study examines the influence of five different LED light qualities (L20AP67, AP673L, G2, AP67 & NS1) that emitted a mixture of continuous spectrum based on various percentages of ultraviolet, blue, green, red, far-red and infra-red radiation or Fluorescent light (FL as a control) on growth of sweet chestnut seedlings into mini-plug containers during one month indoor cultivation. Leaf characteristics of chestnut seedlings were better promoted under LEDs by means of faster leaf formation of greater leaf area that showed significantly higher stomata and epidermal cell number compared to the FL light. Therefore among LEDs G2, NS1 and AP67 showed the greatest effect by inducing significantly higher stomatal density (SD), stomatal index (SI%) and cell density (CD) compared to the FL light and L20AP67 LED. Similar shoot development was found irrespective of light spectrum; however, significantly longer roots were formed by the L20AP67 than the FL light that showed the shortest. Further, root system architecture analysis revealed that NS1 and AP673L LEDs produced seedlings with significantly higher root fibrosity index compared to the FL. Dry weight accumulation especially of the shoots and roots of the seedlings treated with the AP673L was the highest by far. In contrast, FL light demonstrated the lowest dry weight mass thus exhibited the lowest R/S ratio compared to LEDs. These results provide new strategies for using LEDs of continuous spectrum for adequate cultivation protocols into growth chambers for *Castanea sativa* Mill. and possibly other forest tree species.

Key words: chestnut, growth chamber, LEDs, light quality, photomorphogenesis.

Introduction

Plant growth and development is the result of the interaction between genotype and environment. This interaction is perceived by specialized photosensory receptor proteins that adapted to signals of the incident light spectrum due to best utilization. In the artificial plant production, light quality, quantity, and duration constitute the information that will ultimately contribute to plant productivity and quality (Singh et al. 2011, Barrero et al. 2012).

Until today, a wide variety of artificial lighting has been used in horticulture including incandescent, fluorescent and high intensity discharge lights (Sager et al. 1982). Incandescent lighting is typically high in the red and infrared wavelengths. Fluorescent lights produce more white light but the fixtures must be located very close to the plants. High intensity discharge (HID) lights, such as high-pressure sodium (HPS) and metal halide, have been used in growth chambers to supplement natural sunlight and increase photosynthetic rates (Seelye and Mullan 2010). Due to the large amount of electrical energy required, adding lights to increase photosynthesis is for most reforestation services and native plant nurseries, economically impractical (Downs 1977, Warrington et al. 1976).

Light emitting diodes (LEDs) are the newest light source being used in controlled environments and greenhouse plant culture, which are solid-state, durable, lightweight, long-lasting, and come in selectable narrow-waveband emissions such as red and blue that can be matched to the absorption spectra of plant pigments by eliminating other wavelengths found within normal white light, thus reducing the amount of energy required for power (Goins et al. 1997, Kim et al. 2005, Landis et al. 2013).

Plant growth under the combination of blue and red light has been studied, for instance, in lettuce, spinach, komatsuna (Japanese mustard spinach) and radish (Yorio et al. 2001, Hanyu and Shoji 2002, Ohasi-Kaneko et al. 2007). The combination of red and blue light was an effective lighting source to produce plant biomass, but also the combination of green, blue and red light was effective (Kim et al. 2004, Pardo et al. 2013). Additionally it has been shown in cucumber plants, which were grown under different combinations of red and blue light supplied by light-emitting diodes (LEDs), that light quality by itself can induce photosynthetic and morphological properties in leaves that normally occur at high light intensities, although the plants were grown under low light intensity (Hogewoning et al. 2010). On the other hand, the number of stomata, rate of photosynthesis and transpiration, and stomatal conductance increased progressively with increasing photosynthetic photon flux density (PPFD) in plantlets of *Withania somnifera* L. (Lee et al. 2007). Also raising the PPFD from 25 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increased the average number of stomata and stomatal length and reduced stomatal frequency in barley (Kubinova 1991). Most of the studies with LED lighting were performed in controlled environment growth chambers, where the main environmental parameters, such as temperature, humidity, CO_2 concentration and photosynthetic daily integral can be controlled independently of external influences. Unfortunately, successful lighting strategies in phytotrons not necessarily produce the same results in greenhouse conditions (Pinho et al. 2007), especially when variable daylight effect is involved. Therefore individual experiments should evaluate the background growth conditions in conjunction with the natural effect lighting.

Sweet chestnut (*Castanea sativa* Mill.), the only native species of *Castanea* genus in Europe, is distributed in majority of the Mediterranean countries, extending from Caucasus to Italy, France, Spain, Portugal and south England. Thus, this wide-range distribution throughout southern Europe highlights the ability of the species to adapt to varying environmental conditions (Lauteri et al. 1997, Martin et al. 2010). Chestnut is one of the multipurpose species of major economic importance in the Mediterranean basin, valued not only for fruit and timber but also for its contribution to the landscape and environment. Because of the multipurpose characteristics of the species, chestnut populations have been affected by clonal propagation and silvicultural practices. This along with the changes in land use and the accelerating dynamics of global and climatic changes have resulted in a fragmentation of habitats, a reduction of population size and probably a loss in biodiversity (Conedera et al. 2004).

The objective of this study was to examine the effects of LEDs with continuous spectrum on seedling growth and quality characteristics of sweet chestnut seedlings and to compare their responses with those grown under fluorescent conventional light.

Materials and Methods

Plant material and culture conditions

Castanea fruits were collected from natural stands located in Petrokerasa, Zagliveri, Thessaloniki, Greece (40°32'40.86" N, 23°15'26.31" E). Prior to 24 h hydration of the nuts the spiny involucres were removed. In order to achieve higher germination, we stratified the nuts in the refrigerator at 2–5 °C in plastic bags filled with moist vermiculite for five months. Only pre-germinated nuts were used, in order to maximize the uniformity of seedlings in trays.

Pre-germinated nuts were sown into each cavity of the mini-plug plastic trays (QP60/7R QuickPot®, Herkuplast-Kubern GmbH, Ering, Germany) of size 310×530 mm; cell size 46×75 mm; volume 95 cc; 363 plants/m^2 and filled with enriched peat soil substrate (Klassmann TS1, Klassmann-Deilmann GmbH, Geeste, Germany) mixed with perlite on the surface. In total 60 seedlings were randomly selected, specifically 10 seedlings of each of two mini-plug container trays per light treatment were used.

Mini-plug trays were transferred for a cultivation period of 21 days to environmentally controlled growth chambers. There were six different light treatments: fluorescent (FL) treatment with white fluorescent lamps, Osram Fluora Philips-TLD (36W/54 daylight) as the reference light, and five light treatments provided by Valoya LED lights spectra (Anonymous 2016): L20AP67, AP673L, G2, AP67 and NS1. The selected light spectrum percentages are shown in Table 1. Both chambers reach 2 m height and consist of three shelves. Each shelf has 1.20 m length, 0.60 m height, and 0.55 m depth. The distance from the lights to the top of the seedlings in the mini-plugs is 0.40 m. In chamber 1, at the first shelve four L20AP67 tubes were placed. In the middle one (of chamber 1 only) four fluorescent lamps were placed as the reference lighting type, with 30 cm space between them. On the bottom shelf AP673L light was used. In Chamber 2 the top shelf had the G2 light, the second – the AP67 light and third shelf – the NS1 light.

The environmental conditions inside the chambers were 17 h photoperiod, 150±10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density (PPFD), 80±10 % relative air humidity (RH), and 20 °C/15 °C day/night temperature. Irradiance and PPFD of light sources were quantified at plant height with a LI-1800 portable spectroradiometer (LI-COR, Lincoln, Nebraska, USA) with the sensor at ≈25 cm from the light tubes in 10 different spots through the growth chamber shelves. Watering was applied twice a day by automatic sprinklers followed by full rotation of the

Table 1. Spectral distribution and red:far-red ratio of the five light treatments.

Light treatments	Continuous Spectrum data including different percentages out of 400–800 nm, %					R:FR ratio
	380–400 nm	400–500 nm	500–600 nm	600–700 nm	700–800 nm	
FL	0	34.8	24.1	36.7	4.4	5.7
L20AP67	0	10.5	26.2	48.9	14.4	2.9
AP673L	0	11.9	19.3	60.5	8.3	5.6
G2	0	7.7	2.4	64.4	25.5	2.5
AP67	0	13.8	15.1	53	18.1	2.7
NS1	1.8	20.2	38.9	35.7	5.2	8.2

trays in order to ensure uniform growth conditions.

Measurements during the cultivation period

During the 21 days cultivation period in growth chambers, 10 seedlings per light treatment were randomly selected, marked and measured for their growth rate and leaf number. Growth rate based on the height increment gained of each seedling every seven days.

Measurements at the end of the cultivation period

Leaf level

The impression approach was used to determine leaf stomatal density, which was expressed as the number of stomata per unit leaf area (Radoglou and Jarvis 1990). The abaxial epidermis of the leaf was cleaned first by using a degreased cotton ball, and then carefully smeared with nail varnish in the mid-area between the central vein and the leaf edge, for approximately 20 min. The thin film (approximately 15 mm × 10 mm) was peeled off from the leaf surface, mounted on a glass slide, immediately covered with a cover slip, and then lightly pressured with finepoint tweezers. All the impressions were taken from at least five leaves for each light treatment and examined under a light microscope with camera attachment (40×/0.75 magnification) using the Axio Vision program (47.1). Four fields per slide were randomly selected and photographed. Stomata and epidermal cells were counted on the photographs and the stomatal density (SD), stomatal index (SI%) and cell density (CD) were calculated. Stomatal index was estimated using the formula $[s/(e + s)]\cdot 100$ where s is number of stomata and e is number of epidermal cells (Salisbury 1927). The guard cells were not included in the number of epidermal cells. Cell density was calculated as the total number of cells ($e + s$) per unit area of leaf. Further leaf area was measured by the device LI-3000C Portable Area Meter (LI-COR Biosciences); Chlorophyll content index (CCI), defined as the ratio between leaf transmission percentages at 931 nm and 653 nm (Anonymous 2015), was measured by the portable CCM-200 (Opti-Sciences, Inc., NH 03051 USA). The CCM sensor area is 0.71 cm^2 and was placed randomly on the leaf mesophyll, avoiding the mid-vein. Five measurements were taken (per leaf) that were averaged to provide a single chlorophyll content index (CCI) per leaf. The saturation pulse method associated with the pulse-amplitude-modulation technique was applied for chlorophyll fluorescence measurements using a MINI-PAM fluorometer (Heinz Walz, Effeltrich, Germany). The tip of the fiber optics was located 1.0 cm from and 60 ° to the leaf surface. The effective quantum yield was calculated as $\Delta F/F_m' = (F_m' - F)/F_m'$, where F and F_m' are the fluorescence yield before and after the saturation pulse is applied on the leaf, respectively.

Plant growth parameters

Shoot height (SH), root length (RL), leaf dry weight (LDW), shoot dry weight (SDW) and root dry weight (RDW) were measured. SH and RL were defined as the distance from the top of the root plug to the upper and lower end of a seedling, respectively. LDW, SDW and RDW were assessed after oven drying at 70 °C for 48 h. The root-to-shoot ratio (R/S) was calculated on a dry weight basis.

Root architecture analysis

After being washed away gently, the remained soil particles from the roots of five selected *Castanea* seedlings for each of the light treatments were used. They were scanned and loaded in GiA Roots (Galkovskyi et al. 2012), which is a software tool to automate and facilitate the large-scale analysis of root networks. GiA Roots was used to quantify the structure of the plant root system by measuring the number of lateral roots in order to estimate root density. After counting the lateral roots, we proceeded computing their density with the following formula: Root density = Length of primary root / No of lateral roots (Bors-Oprişa et al. 2011). Following root architecture of seedlings was also defined by the number of FOLRs (First Order Lateral Roots) greater than 1 mm in diameter originating along the length of the taproot and at the base of the taproot. Further, selected seedlings were individually evaluated for their root fibrosity, classified on a1 to 5 scale by using an index designed by Hatchell and Muse (1990) and modified by Wilson et al. (2007) (Table 3).

Statistical analysis

Statistical analysis was conducted with IBM SPSS Statistics for Windows, Version 20.0. Collected data every seven days such as mean growth rate and leaf number under the different light treatments were analyzed using general linear model (GLM) repeated measurements. Each subject in the design was, hence, measured three times. At the end of the cultivation period collected data was analyzed using general linear model (GLM) multivariate analysis. Significant differences were established by multiply comparisons test with Bonferroni correction at $p < 0.05$.

Results and Discussion

Cultivation of the tested broad-leaved species showed significant morphological and physiological adjustments after 21 days of growing under different light qualities, reaffirming the reliance and the sensitivity of

Table 2. Growth rate of *Castanea sativa* seedlings grown under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments, every seven days (for a total of 21 days experimental period).

Light treatments	Growth rate			Leaf number		
	7^{th} day	14^{th} day	21^{st} day	7^{th} day	14^{th} day	21^{st} day
FL	1.58±0.54b	0.33±0.16a	0.15±0.08a	6.29±1.25a	7.71±0.95a	8±3.36b
L20AP67	2.81±0.60a	0.23±0.13a	0.29±0.24a	7.43±1.61a	8.57±2.14a	11.14±2.73a
AP673L	1.74±0.70b	0.28±0.20a	0.22±0.12a	6.43±2.14a	9.57±1.13a	10±1.52ab
G2	1.84±0.41ab	0.46±0.23a	0.07±0.05a	6.43±1.98a	8.71±1.38a	11.14±3.57a
AP67	1.78±0.23ab	0.21±0.14a	0.24±0.18a	6.43±2.63a	9.57±7.09a	10.43±2.77ab
NS1	1.39±0.27b	0.38±0.21a	0.21±0.21a	6.2±1.34a	8.57±1.90a	9.57±2.57ab

Note: Different small letters within the column indicate significant differences between the light treatments at $p < 0.05$. Data are mean values ($n = 10$) ± SE.

Table 3. Rating system for root fibrosity of Castanea sativa under FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.

Light treatments	Rating	Fibrosity class	Description of root system appearance
FL	3	Moderate	3–5 2nd order long roots; moderate density of higher order long and short roots
L20AP67	4	High	>5 2nd order long roots; moderate density of higher order long and short roots
AP673L	>5	Very high	5 >5 2nd order long roots; high density of higher order long and short roots
G2	5	Very high	5 >5 2nd order long roots; high density of higher order long and short roots
AP67	>5	Very high	5 >5 2nd order long roots; high density of higher order long and short roots
NS1	>5	Very high	5 >5 2nd order long roots; high density of higher order long and short roots

Note: The rating is based on visual assessment of the approximate number and type of high order lateral roots per 10 cm segment of primary first order lateral roots (those with a diameter > 1 mm, branching from the taproot). Rating: 1 – Very low (no second order long roots; zero or few short roots present); 2 – Low (one–three second order long roots; low density of higher order long and short roots); 3 – Moderate (three–five 2^{nd} order long roots; moderate density of higher order long and short roots); 4 – High (> five 2^{nd} order long roots; moderate density of higher order long and short roots); 5 – Very high (> five 2^{nd} order long roots; high density of higher order long and short roots). Long roots are > 5 mm and are likely to contain branches of the next highest order. Short roots are < 5 mm; they do not support roots of higher order (Hatchell and Muse 1990).

the seedlings to the inherent need of light especially in early developmental stages.

Castanea seedlings showed significant differences in height growth rate only the first week (7th day) of the experimental period (Table 2). L20AP67 showed significantly higher height increment of 67.61 %, 56.03 % and 47 % compared to NS1, FL and AP673L lights, respectively, while no significant differences were found for the rest of the lights (Table 2). Considering only the light quality effects, height increment was more enhanced by L20AP67 and G2 throughout the indoor cultivation, than NS1 and FL. A possible explanation for the higher values of the growth rate induced by the L20AP67 and the G2 could be the low R:FR ratios (2.9 and 2.5). Low R:FR ratio is an indication of shading by neighbouring plants and according to studies on the mechanisms that control the shade avoidance responses, the phytochrome system controls the distribution of auxin in different cell layers, thus shaded plants reduce cell expansion in their leaves and enhance cell elongation in the stems (Morelli and Ruberti 2002). Nevertheless, both L20AP67 and G2 showed also significantly faster leaf expansion of 11 leaves than the FL that showed the lowest of eight leaves (Table 2). According to Nhut et al. (2003), strawberry plantlets showed that the number of leaves was higher under blue plus red LEDs than of those treated with fluorescent or red LEDs alone.

It is well known that light causes also alternations in the stomata movements of plants. Unfortunately, there are few studies about the effects of light quality on this subject, especially for the parameters examined in this study. Stomatal opening and closure is affected by light; for instance Talbott et al. (2006) observed that blue and red light stimulated stomata opening, whereas green light inhibited it. It has been also reported that an increase in light intensity, decreases the number of epidermal cells and increases stomata number, index and size (Fernandez and Mujica 1973). Schoch et al. (1984) reported that blue and far-red light reduced the stomatal index, while red light increased it *in Vigna sinensis*. Kim et al. (2004) showed that blue and red light increased the stomata size and decreased the stomata number of chrysanthemum plantlets, while blue and far-red had the opposite effect. Furthermore, Lee et al. (2007) found that white light increased the stomata number and size, while blue light reduced the mentioned parameters of *Withania somnifera* plantlets. Treatments with UV-B can also affect stomatal conductance, altering the rate of water loss by transpiration and uptake rate of CO_2 for photosynthesis, while stomatal closure occurs rapidly even at low UV-B levels in some UV-B sensitive seedlings such as in Mono Maple (*Acer Mono Maxim*) (Tevini and Teramura 1989, Yao and Liu 2006). Nevertheless, G2 had the highest red (600–700 nm) and far-red (700–800 nm) percentages of 64.4 % and 25 %, respectively, while at the same time had the lowest blue (400–500 nm) percentage of 7.7 % that induced the highest impact on the hypostomatous character of *Castanea* leaves for the following parameters. Likewise, NS1 has the highest percentage of 38.9 % in blue-green region (500–600 nm) and it is the only that covered the UV band of 1 % (<400 nm) than the rest of lights. Consequently G2 showed significantly higher number of stomata (data not shown) and thus significantly higher SD of 251 stomata/mm^2, compared to the FL and L20AP67 (Fig. 1) that showed the lowest among all the lights of 137 stomata/mm^2 and 139 sto mata/mm^2, respectively (Fig. 1). The rest of the light treatments showed also higher

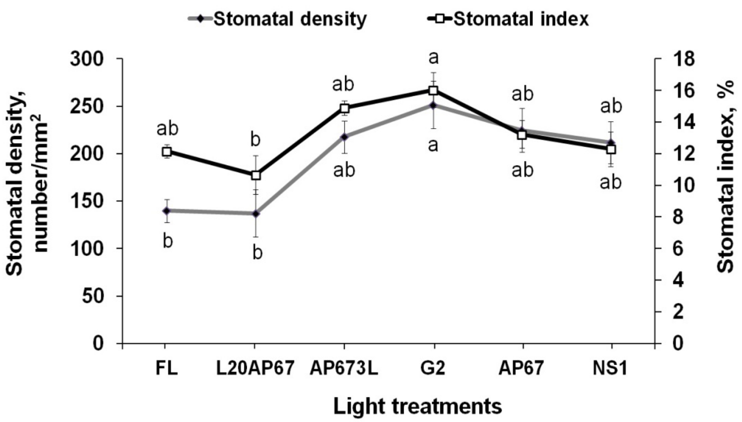

Fig. 1. Stomatal density (SD) and Stomatal index (SI) (%) on the abaxial leaf surface of Castanea sativa seedlings cultivated under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.

SD than the latter ones such as 225 stomata/mm^2, 217.7 stomata/mm^2 and 211.7 stomata/mm^2, for the AP67, AP673L and NS1 (Fig. 1). Furthermore, G2 also induced significantly higher SI% of 16.03 % compared to the L20AP67 that showed of 10.6 %. No significant differences were found for the SI% among the rest of the lights; however, they still showed higher values, such as 15 %, 13.2 % and 12.15 % for the AP673L, AP67 and FL lights, respectively, than the L20AP67 (Fig. 1). Additionally NS1 and AP67 had significantly created more epidermal cells compared to the FL conventional light (data not shown), thus significantly higher CD was also found (1714 and 1688.7) than 1140 induced by the FL (Fig. 2). Higher number of epidermal cells and CD were found for the rest of the light treatments than the FL of 1573.5, 1452, and 1276 induced by the G2, AP673L and L20AP67 (Fig. 2).

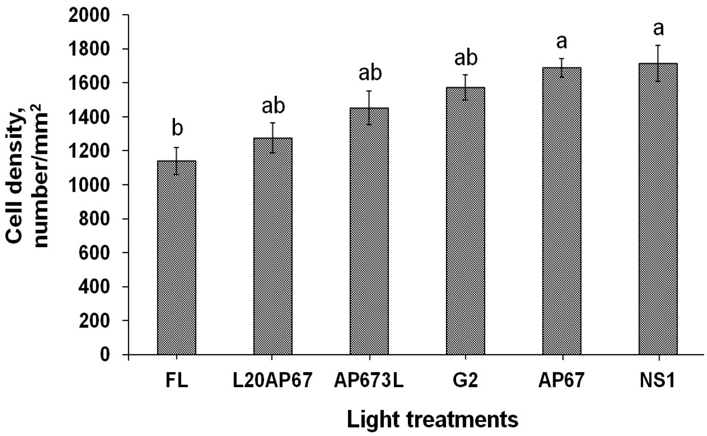

Fig. 2. Cell density on the abaxial leaf surface of *Castanea sativa* seedlings cultivated under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.

Note: Different letters within the columns indicate significant differences between the light treatments at $p < 0.05$. Data are mean values ($n = 5$) ± SE.

Leaf area of chestnut seedlings under different light treatments was similar; however, it was benefited more under LEDs than the FL light. Specifically, higher leaf area was formed under the

AP673L with 269.42 cm^2, followed by the NS1, L20AP67, AP67, G2, and FL with 216.75 cm^2, 215.8 cm^2, 203.37 cm^2, 188.55 cm^2 and 178,42 cm^2, respectively (Fig. 3). Johkan et al. (2010) found that red lettuce plants irradiated with red light increased their leaf area by 33 % than the fluorescent light, but those treated with blue LED lights decreased by more than 9 %. Also in another study on lettuce growth promotion under supplemental far red lighting was associated with the increase in leaf area (Kubota et al. 2012). Following L20AP67 had significantly higher CCI of 13.72 compared to the NS1 that had CCI of 6 (Fig. 4); while the rest of the lights had CCI values of 10.7, 9.42, 8.47 and 8.2 for the FL, G2, AP673L and AP67, respectively (Fig. 4). According to Randall and Lopez (2014), relative chlorophyll content for Pelargonium and Salvia seedlings was 21 % and 15 % higher when those grown under 70:30 red:blue LEDs than under high pressure sodium lamps (HPS). *Castanea* seedlings showed no significant differences for the effective quantum yield between the light treatments; however higher values of F_v/F_m close to 0.81 were found for t

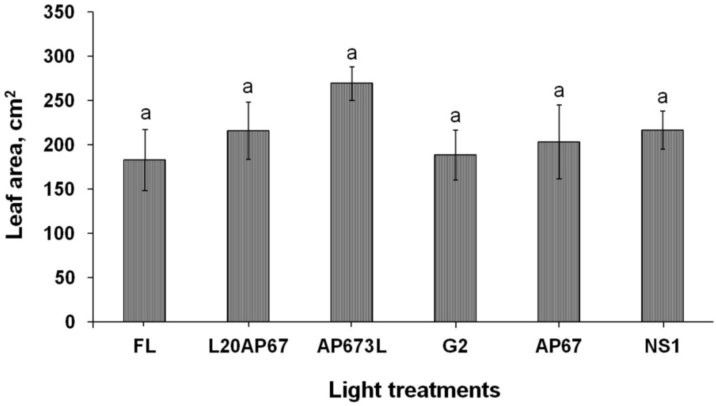

Fig. 3. Leaf area of Castanea sativa seedlings cultivated under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.

Note: No significant differences were found. Data are mean values (n = 10) ± SE.

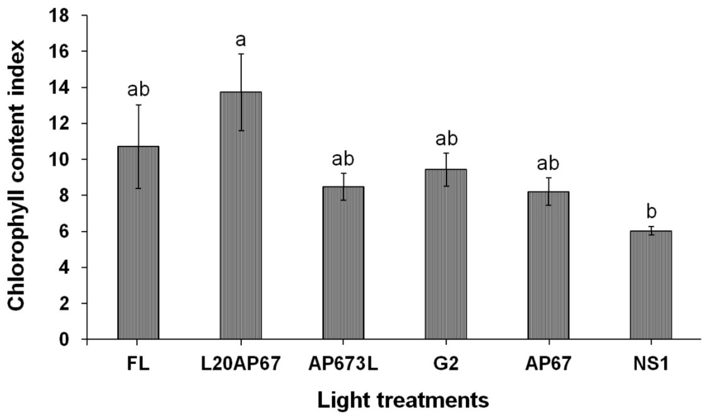

Fig. 4. Chlorophyll content index (CCI) of Castanea sativa seedlings cultivated under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.

Note: Different letters within the columns indicate significant differences between the light treatments at $p < 0.05$. Data are mean values ($n = 10$) ± SE.

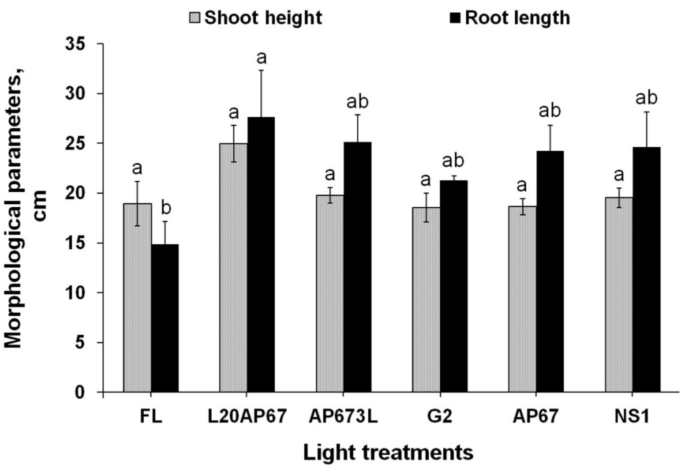

Fig. 5. Shoot height (SH) and root length (RL) of *Castanea sativa* seedlings cultivated under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.

Note: Different letters within the columns indicate significant differences between the light treatments at $p < 0.05$. Data are mean values ($n = 10$) ± SE.

he L20AP67 and the FL lights (data not shown). Despite the fact that the values found under the rest of the lights were less than the optimal value of 0.832 for F_v/F_{max}, typical of well-functioning photosynthetic apparatus (Björkman and Demmig 1987), however, this should not be a surprise as the parameter varies with species and environmental conditions (Björkman and Demmig 1987, Cha-um et al. 2010).

Shoot height of *Castanea* seedlings showed no significant differences between the different light environments; however, L20AP67 exhibited higher SH of 25 cm (Fig. 5). The rest of lights such as the AP673L, NS1, FL, AP67 and G2 showed similar shoot development of 19.78 cm, 19.53 cm, 18.94 cm, 18.64 cm and 18.53 cm, respectively (Fig. 5). Our findings were in accordance with the results of another study on the development of zygotic embryos and seedlings of *Castanea crenata* S. et Z., which showed that the hypocotyl development was not significantly affected by light quality as well (Park and Kim 2010). Shorter shoot height by 18 % was found for *Rosa × hybrida*, cv. Toril plants under LED lighting with 20 % blue and 80 % red compared to HPS lamps (Terfaa et al. 2013), which is in accordance with findings on several species on the inhibition of internodes growth caused by blue radiation (Folta et al. 2003, Dougher and Bugbee 2004). Maas and Bakx (1995) also showed that a decreased proportion of blue light increased the shoot length of 'Mercedes' roses. That was not the case in our findings, since the influence of light may vary from species to species, therefore the proportion of blue radiation for each of the different light treatments used showed no significant effect. However, lettuce seedlings treated with blue LEDs showed four-fold longer roots than those treated with broad-spectrum white LED or red LED alone (Kook et al. 2013). Our results revealed that L20AP67 showed significant effect on the root formation since induced the longest roots for sweet chestnut seedlings of 27.64 cm (60 % longer) compared to the FL light that induced the shortest of 14.87 cm (Fig. 5), while the rest of LEDs showed similar root length of 25.13 cm, 24.62 cm, 24.21 and 21.28 cm for the AP673L, NS1, AP67 and G2, respectively (Fig. 5).

Root architectural analysis reveals a formal description of root systems that has important ecological applications since it reflects root plasticity responses to environmental heterogeneity and edaphic constraints to plant productivity and determines the function of roots in mechanical support of the shoots (Fitter et al. 1991, Lynch 1995, McPhee 2005). Plants can actively sense light via their roots as well, by means of the analysis both of the spectrum and the intensity by using several photoreceptors such as phytochromes, which had maximum absorption wavelength in the red and far-red spectrum that ultimately affects the root elongation and lateral root formation in *Arabidopsis* seedlings (Takano et al. 2001; Raya-Gonzalez et al. 2014). Our findings revealed that *Castanea sativa* seedlings showed denser root system under the effect of LEDs, especially benefited by the G2 LED maybe due to its higher percentage in the red region (600–700 nm) and far-red region (700-800 nm), with a ratio of 1.52, than the FL light that showed the lowest ratio of 0.85 (Fig. 6); the rest of LEDs showed root density ratios of 1.45, 1.41, 1.30 and 1.13 induced by the NS1, AP67, AP673L and L20AP67, respectively. According to Nhut et al. (2003), the highest root number of strawberry plants was found under the effect of 70 % red plus 30 % blue LEDs. High root system fibrosity is considered a parameter that improves field survival and early growth of seedlings (Schultz and Thompson 1996, Wilson et al. 2007). Also the number of FOLR remained significantly higher (more than 30) under all LED treatments inducing threefold increase compared to the conventional FL light (Fig. 7). Further AP673L and NS1 LEDs obtained significantly higher number of FOLR with diameter >1 mm and categorized to the highest fibrosity class compared to the seedlings grown under the FL light that characterized with the least fibrous root system (Fig. 7 and Table 3).

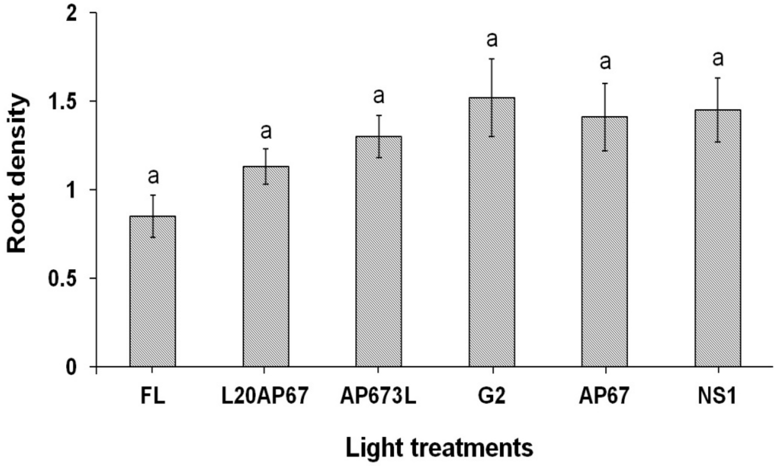

Fig. 6. Root density of *Castanea sativa* seedlings cultivated under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.
Note: No significant differences were found. Data are mean values (n = 5) ± SE.

The use of monochromatic LED lights supplemented to conventional light sources such as fluorescent or HPS that stimulated t he dry weight mass of several species was

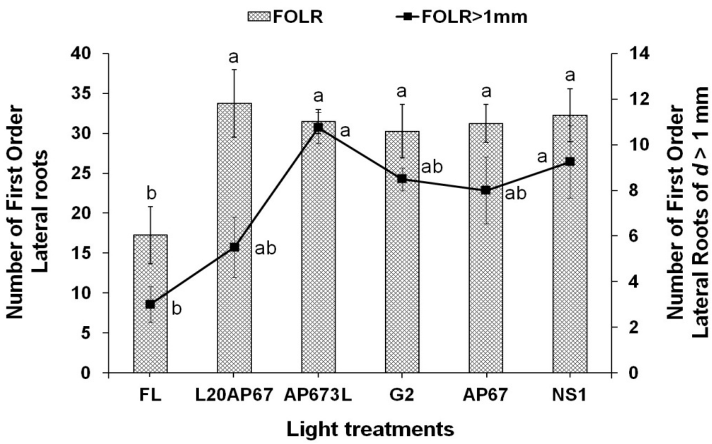

Fig. 7. Number of First Order Lateral Roots (FOLR) & number of First Order Lateral Roots of *d* > 1 mm of *Castanea sativa* seedlings cultivated under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.
Note: Different letters within the columns indicate significant differences between the light treatments at $p < 0.05$. Data are mean values ($n = 5$) ± SE.

investigated by several researchers (Hoenecke et al. 1992, Brown et al. 1995, Johkan et al. 2010, Li et al. 2012, Muneer et al. 2014). In this study the LED lights with continuous spectrum showed a predominately dry weight mass accumulation than the fluorescent con ventional light. Specifically, AP673L LED light induced significant increase of 85 % and 90.15 % in the SDW and RDW compared to the FL light (Table 4). The rest of LEDs also showed higher SDW and RDW than the FL (Table 4). As for the LDW, LEDs showed higher dry weight accumulation than the FL; however, no significant differences were found (Table 4). The relative allocation of dry weight matter to roots or shoots has been considered a key factor in plant strategies regarding water use and is very important for seedling performance and survival in the field (South 2000). Consequently

Table 4. Leaf, shoot, root dry weight (LDW,SDW,RDW) and root-to-shoot ratio (R/S) of *Castanea sativa* seedlings cultivated under the FL, L20AP67, AP673L, G2, AP67 and NS1 light treatments at the end of the 21 days experimental period in the growth chambers.

Light treatments	LDW	SDW	RDW	R/S ratio
FL	0.78±0.29a	0.53A±0.21b	0.99±0.28b	0.8±0.20b
L20AP67	0.99±0.34a	1.03±0.36ab	1.79±0.74ab	0.86±0.19ab
AP673L	1.41±0.24a	1.31±0.16a	2.61±0.44a	0.97±0.22ab
G2	0.98±0.34a	0.81±0.25ab	1.75±0.67ab	0.97±0.31ab
AP67	1.19±0.22a	1.00±0.22ab	2.21±0.41ab	1.01±0.18a
NS1	1.03±0.14a	0.95±0.37ab	1.80±0.88ab	0.88±0.27ab

higher allocation to the roots was obtained under LEDs than the FL, especially for the AP67 that showed significantly higher R/S ratio of 1 that characterized a more balanced allocation of dry weight mass both for the above and below parts of the plants (Table 4).

Conclusion

In the present study, the optimum light quality among LEDs of continuous spectrum and conventional fluorescent light for growing robust and healthier seedlings in controlled environment were investigated. Indeed sweet chestnut seedlings showed positive changes in growth, especially triggered by the LED light qualities compared to fluorescent light. Particularly, L20AP67 light stimulated the height growth rate, leaf, shoot and root development; G2 induced high cell division by means of higher stomatal density and index; NS1 and AP673L induced higher root fibrosity; AP673L increased significantly the shoot and root dry weight accumulation and LEDs in general induced more balance seedlings especially those cultivated under the AP67 by means of greater R/S ratio. However, further investigation needs to be done on the cultivation of forest tree species growing into growth chamber illuminated by LED lights and find out whether the possible differences between the treatments will be maintained after transplantation at the nursery or outplanting to a field site.

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COMPARISON OF PROBABILITY DISTRIBUTION FUNCTIONS APPLIED TO TREE DIAMETER AND HEIGHT OF THREE DEVELOPMENT STAGES IN A MIXED BEECH (*FAGUS ORIENTALIS* LIPSKY) FOREST IN HYRCANEAN REGION OF IRAN

Maryam Kazempour Larsary[1*], Kambiz Taheri Abkenar[1], David Pothier[2], Hassan Pourbabaei[1], and Farhad Fadaie Khoshkebijari[1]

[1]Department of Forestry, Natural Resources Faculty, University of Guilan, Somehsara, Guilan, P.O. Box 1144, Iran. [*]E-mail: kazempourmaryam@yahoo.com
[2]Centre d'étude de la forêt (CEF), and Département des sciences du bois et de la forêt, Pavillon Abitibi-Price, 2405 rue de la Terrasse, Université Laval, Québec, QC G1V 0A6, Canada.

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Abstract

Identification and assessment of quantitative characteristics of forest communities are the basic components of forest planning. Modeling frequency distributions of tree parameters such as diameter and height in various fields of forest sciences, including forest management, silviculture and forest biometrics plays an important role. The objective of this study was to determine the best probability distribution functions of tree diameter and height in each of three development stages (i.e., initial, optimal, and decay) in an unmanaged, uneven-aged mixed beech (*Fagus orientalis* Lipsky) forest. For this purpose, three sample plots of one hectare (100 m × 100 m), one in each of three development stages, were selected in the Hyrcanean region of Iran. Diameter and height of all trees with a diameter at breast height larger than 7.5 cm were measured. Beta, Johnson's S_B, lognormal, gamma and Weibull probability distribution functions were fitted to each diameter and height distribution. The comparison of observed and predicted probabilities was performed using Kolmogorov-Smirnov, Anderson-Darling and Chi-square tests. The goodness of fit of each distribution function varied among development stages. The three-parameter lognormal, the three-parameter Weibull and the Johnson's S_B functions showed the highest goodness of fit for tree diameter distribution at the initial, optimal and decay stages, respectively. For tree height distribution, the Johnson's S_B was the best fitted function for the optimal and decay stages, while none of these functions adequately explained the height distribution of trees at the initial stage. Best fitted models can be used to characterize the diameter and height distributions of similar stands and help achieve an optimal structure.

Key word: diameter and height distribution, forest development stage, Iran, mixed beech stand, probability distribution functions.

Introduction

Hyrcanean forests along the north-facing aspects of Elborz Mountains in Northern Iran are from natural origin and generally form uneven-aged stands composed of hardwood deciduous species (Sagheb-Talebi et al. 2004). Five main plant communities (i.e., *Querco-Buxetum*, *Querco-Carpinetum*, *Parrotio-Carpinetum*, *Fagetum* *hyrcanum*, and *Carpinetum orientale*) are distinguished along the altitudinal gradient of Elborz Mountains (Sagheb-Talebi et al. 2004). As for natural European beech (*Fagus sylvatica* L.), Oriental beech (*Fagus orientalis* Lipsky) forests are submitted to a wind disturbance regime that leads to a fine-scaled mosaic pattern of forests (Korpel 1995). This mosaic is composed of forest patches of three recognized development stages (i.e. the decay, initial, and optimal stages) that are spatially adjacent to each other or are developing in a cycle over time (Leibundgut 1993, Korpel 1995).

At the initial stage, the high growth rate of young trees produces a sustained increase in stand volume while canopy gaps are gradually closed. Although the stand structure at the early initial stage is still uneven-aged and multilayered, it changes gradually until the optimal stage is reached and then becomes regular and even-aged. The optimal stage is also characterized by a closed canopy, a scarce regeneration stratum and the presence of numerous suppressed small trees. At the decay stage, the increasing presence of dead wood and the establishment of tree regeneration in canopy gaps create vertical irregularities and uneven-agedness. Later, stem density and stand volume continue to decrease as a result of the death of many old trees that are replaced by a large number of young trees, and the forest gradually returns to the initial stage (Korpel 1995, Emborg et al. 2000). Each development stage is thus associated with a specific stand structure which can be represented by a tree diameter distribution (Es-hagh Nimvari and Mataji 2014).

Forest management based on close-to-nature silvicultural systems requires the characterization of forest resources for an adequate planning of future silvicultural interventions. Diameter distribution is a simple and yet powerful feature for characterizing forest stands and representing their sustainability potential (Bailey and Dell 1972, Rubin et al. 2006). For example, this tool can be used to predict whether the density of smaller trees is sufficient to replace the current population of larger trees in the future stand (Rubin et al. 2006). Also, knowledge of tree diameter distribution and its dependence on site, stand composition, age, and density is useful for economic and biological purposes (Bailey and Dell 1972). On one hand, knowing the number of trees in different diameter classes is essential to manipulate the stand structure and composition close to those of primeval forests. On the other hand, tree diameter distribution can be applied to determine the industrial use of wood, harvesting costs, expected yield, or price of the different products. Tree heights can be considered as the main morphometric variables of forest trees. In the forest biometrics, some variables such as height of forest trees can be used in several cases including the determination of tree and stand volume, site index, form factor, and slenderness coefficient 76 (Mohammadalizade et al. 2013). Awareness of tree diameter and height distributions are generally required for effective forest management planning and are particularly valuable in forest mensuration and inventory (Wang et al. 2008, Tsogt et al. 2013).

Many attempts have been made to describe tree diameter distribution using mathematical models: De Liocourt (1898) suggested a reversed J-shaped distribution for uneven-aged stands, which was later formulated as a negative exponential function that was widely used in forestry (Meyer 1952, Leak 1965). This negative exponential function was applied to mixed forests in Northern Pennsylvania as well as to many other forest stands (Shunzhong et al. 2006 quoted from Meyer 1952; Schmelz and Lindsey 1965). However, since that time, other statistical distribution functions were successfully applied to various stand types (Namiranian 1990). For example, the diameter distribution of Iranian beech forests has been modeled using several functions such as the beta, Weibull, normal, exponential, lognormal and gamma (e.g. Namiranian 1990, Mattaji et al. 2000, Fallah et al. 2000, Fallah et al. 2006, Mohammadalizade et al. 2009, Amanzadeh et al. 2011, Sheykholeslami et al. 2011, Fallahchai et al. 2012, Fallahchai and Shokri 2014, Mirzaei et al. 2015a). The beta function seems to be appropriate to estimate the diameter distribution of many stand types (e.g., Namiranian 1990, Mattaji et al. 2000; Liu et al. 2002). Fallahchai and Shokri (2014) concluded that the three-parameter Dagum distribution has more ability to determine the diameter distribution of *Alnus subcordata* trees.

However, Nord-Larsen and Cao (2006) considered that Weibull distribution functions are probably the most widely applied for modelling tree size distributions because of their high flexibility, while Siipilehto (1999) and Zhang et al. (2003) found that Johnson's S_B gave slightly better results than the Weibull function.

In the case of height distribution, various probability density functions (*pdf*s), e.g., Bur, Dagum, beta, normal, gamma, exponential, Johnson's S_B and Weibull have been successfully used (Tsogt et al. 2013, Kängsepp et al. 2014, Mohammadalizade et al. 2013, Mirzaei et al. 2015a, Mirzaei et al. 2015b). Tsogt et al. (2013) studied the height distribution of even-aged Scots pine (*Pinus sylvestris* L.) forests at three different age-classes (young, juvenile and old). They concluded that for the stand with the normal distribution shape, Johnson's S_B was better than Burr and Dagum functions. Mirzaei et al. (2015b) found that the beta and Weibull distributions were appropriate to explain the distributions of tree height, a diameter at breast height (DBH) and crown area.

Mixed beech forests are the richest and the most dominant tree community on the middle lands of Hyrcanean forests of Iran and as such, have the highest commercial value. In them, no studies were realized to model diameter and height distributions of natural mixed forests with beech and hornbeam predominance representing different development stages using probability density functions (*pdf*s). The specific objective of this study was to compare different statistical functions for describing tree diameter and height distributions of three forest development stages (initial, optimal, and decay) in an untouched uneven-aged mixed beech stands in the Hyrcanean region of Iran. The best fitted distributions in each of the three studied development stages can be used to effectively apply close to nature interventions in managed stands of similar composition in order to achieve stand stability and optimum biological and economic production.

Material and Methods

Study area

The study site was located in Caspian forest of Sistan district No 4 of Zeilaki watershed No 23 in Northern Iran (Longitudes 49°49´4″ and 49°53´15″, Latitudes 36°55´10″ and 36°58′16"). This study was conducted in a 59-ha forest area with an elevation range between 350 and 750 m a.s.l., an average annual temperature of 14 °C and an average annual precipitation 1273 mm. Mean stand density and growing stock volume are 212 stems ha^{-1} and 299.6 m^{3} ha^{-1}, respectively. Precipitation is well distributed so that there is generally no drought periods during a year. The soils are acidic brown or forest brown with soil textures varying from clay to clay-loam, mostly with single grain-soil structure and low permeability rate. The study area consists of a natural, mixed, deciduous forest dominated by (beech) *Fagus orientalis* and with less proportion of hornbeam (*Carpinus betulus* L.) minor presence of maple (*Acer velutinum* Boiss.), coliseum maple (*Acer cappadocicum* Gled.), caucasian alder (*Alnus subcordata* C.A.Mey), elm (*Ulmus glabra* Huds.), persimmon (*Diospyros lotus* L.), ironwood (*Parrotia persica* (Dc.) C.A. Mey) and lime (*Tilia begonifolia* Stev). Because the study area is subjected to periodic events of wind disturbances, it is composed of a mosaic of small forest patches, each corresponding to a specific development stage, but forming an uneven-aged forest when considered together. The study area has developed with minimal human disturbances and without silvicultural interventions during the last decades. Hence, this forest represents an untouched and unmanaged natural forest typical of native ancient forests of this region.

Data collection

We first conducted a forest survey in the study area to locate forest patches corresponding to different development stages. To determine them for each forest patch, the same criteria as those of previous studies were used (Korpel 1995, Sagheb-Talebi et al. 2003, Delfan Abazari et al. 2004, Mataji and Sagheb-Talebi 2007, Sefidi and Marvie Mohadjer 2010, Akhavan et al. 2012, Zenner et al. 2015). These criteria were the number and volume of stems, dead wood volume, proportion of dead wood volume by diameter class, proportion of volume by diameter class, presence of canopy gaps and number of tree layers (Table 1). Then, three forest patches were randomly selected to establish 1-ha (100 m × 100 m) sample plots during the summer of 2014 such as one plot was established in each of three develop-

Table 1. Minimum criteria for identification of plots pursuant to development stages.

Criterion	Development stages		
	Initial	**Optimal**	**Decay**
General age estimation	Young	Middle-aged	Old
Number of stand stories	> 2	Usually 1–2	> 2
Stem density	High	Medium	Low
Highest Proportion of trees by canopy stratum	In lower and middle stories	In upper story	In middle and upper stories
Highest proportion of trees by size	In small and medium sizes	In medium and large sizes	In large and extra-large sizes
Stand volume	Medium	High	Low
Dead wood volume	Medium	Low	High
Highest proportion of dead wood volume by size	In large and extra-large sizes	In small and medium sizes	In extra-large sizes
Gap	Present	Usually absent	Present
Regeneration	Clustered and present in gaps	Little and scattered over the whole area	Clustered and present in gaps

Note: Stage of development of plots was identified according to many of these criteria as possible.

ment stages (initial, optimal, and decay). Previous studies have confirmed the appropriateness of 1-ha plot to study the structure of Oriental beech stands in Iran (Eslami and Sagheb-Talebi 2007, Sagheb-Talebi and Schùtz 2002). Plots were all established in mixed beech stands with the same local climate, elevation, soils, aspect and slope. These plots had eastern aspect with an average slope of about of 45 %. In each plot, the species, height and diameter of all trees with DBH larger than 7.5 cm were recorded.

Data analysis

Five probability distribution functions (*pdf*s) were tested for characterizing the empirical DBH and height distribution of each forest patch. These *pdf*s were the beta, Johnson's S_B, three-parameter gamma, three-parameter lognormal and three-parameter Weibull (Table 2). These theoretical distributions were selected because:

1) They were often used in other studies to explain empirical diameter and height distributions (Amanzadeh et al. 2011, Amanzadeh 2015, Mohammadalizadeh et al. 2009, Podlaski 2006, Nord-Larsen and Cao 2006, Bullock and Boone 2007, Mohammadalizade et al. 2013, Mirzaei et al. 2015a);

(2) They generally provided accurate estimations of empirical diameter distribution in mixed forests of various structures (Amanzadeh 2015, Mattaji et al. 2000, Podlaski 2006, Shunzhong et al. 2006, Mohammadalizade et al. 2013, Mirzaei et al. 2015a); and

(3) The computation of their parameters is relatively easy (Kamziah et al. 1999, Podlaski 2006).

Parameters of *pdf*s were estimated with the maximum-likelihood method

Table 2. Probability distribution functions applied in the study area.

Distribution	Density function	Parameter
Beta	$f(x)=\dfrac{1}{\beta(\alpha_1,\alpha_2)}\dfrac{(x-a)^{\alpha_1-1}(b-x)^{\alpha_2-1}}{(b-a)^{\alpha_1+\alpha_2-1}}$ $a\le x\le b,\ \alpha_1>0,\ \alpha_2>0,$	α_1 and α_2 : continuous shape parameters a and b: continuous boundary parameters β is the beta function
Johnson's S_B	$f(x)=\dfrac{\delta}{\lambda\sqrt{2\pi}z(1-z)}\exp\left(-\dfrac{1}{2}\left(\gamma+\delta\ln\left(\dfrac{z}{1-z}\right)\right)^2\right)$ $z=\dfrac{x-\xi}{\lambda},\ \xi\le x\le\xi+\lambda,\ \delta>0,\ \lambda>0$	ξ : continuous location parameter λ : continuous scale parameter δ and γ : continuous shape parameters
Gamma	$f(x)=\dfrac{(x-\gamma)^{\alpha-1}}{\beta^\alpha\Gamma(\alpha)}\exp(-(x-\gamma)/\beta)$ $\alpha>0,\ \beta>0,\ \gamma\le x<+\infty$	α : continuous shape parameters β : continuous scale parameter γ : continuous location parameter Γ is the gamma function
Lognormal	$f(x)=\dfrac{\exp\left(-\dfrac{1}{2}\left(\dfrac{\ln(x-\gamma)-\mu}{\sigma}\right)^2\right)}{(x-\gamma)\sigma\sqrt{2\pi}}$ $\sigma>0,\ \gamma<x<+\infty$	μ and σ : continuous parameters γ : continuous location parameter
Weibull	$f(x)=\dfrac{\alpha}{\beta}\left(\dfrac{x-\gamma}{\beta}\right)^{\alpha-1}\exp\left(-\left(\dfrac{x-\gamma}{\beta}\right)^{\alpha}\right)$ $\alpha>0,\ \beta>0,\ \gamma\le x<+\infty$	α : continuous shape parameters β : continuous scale parameter γ : continuous location parameter

by using the distribution fitting software Easyfit 5.6 Professional (MathWave Technologies 2015) that was already used in several studies (e.g. Amanzadeh et al. 2011, Sohrabi and Taheri 2012, Khongor et al. 2011, Tsogt et al. 2013, Humphrey and Godwin 2014, Mirzaei et al. 2015a).

Comparison of actual DBH distributions with the theoretical *pdf* were evaluated by statistical tests that included Kolomogorov-Smirnov (K-S), Anderson-Darling (A-D), and χ^2 goodness-of-fit tests (Nord-Larsen and Cao 2006, Khongor et al. 2011, Tsogt et al. 2013, Amanzadeh et al. 2011, Humphrey and Godwin 2014).

The null and the alternative hypotheses in these tests were:

- H_0 – the data follow the specified distribution;
- H_1 – the data do not follow the specified distribution.

Kolmogorov-Smirnov statistic (Chakravarti et al. 1967) is a nonparametric test that is applicable when the population distribution function is continuous and can thus be used to investigate the significance of the difference between an observed distribution and the theoretical probability distribution (Kanji 2006, Jäntschi and Bolboaca 2009). It is based on the maximum vertical difference between the theoretical and the empirical cumulative distribution function (ECDF). The K-S statistic (D) corresponds to:

$$D = \max\,(F - Sn),$$

where F is the observed cumulative frequencies and Sn is the theoretical cumulative frequencies.

A-D test (Stephens 1974) is also a nonparametric test that is used to test if a data sample came from a population with a specific distribution. It is a modification of K-S and gives more weight to the tails of a distribution than does K-S test. A-D statistic (A^2) is defined as:

$$A^2 = -N - S,$$

where N is the sample size,

$$S = \sum_{i=1}^{N} \frac{(2i-1)}{N}\left[\ln F(X_i) + \ln\left(1 - F\left(X_{N+1-i}\right)\right)\right]$$

and F is the cumulative distribution function of the specified distribution. Note that X_i are the ordered data.

The χ^2 test (Snedecor and Cochran 1989) is used to test if a data sample coming from a population with a specific distribution. This test is applied to categorical data (i.e. data put into classes). This is actually not a restriction since classes can be easily formed from continuous data before applying the chi-square test. However, the value of the χ^2 test statistic is dependent on how the data are classified. Another disadvantage of the chi-square test is that it requires a sufficient sample size in order for the chi-square approximation to be valid. This test is sensitive to the choice of classes. For the χ^2 approximation to be valid, the expected frequency in each class should be at least five or more data points. This test is not valid for small samples, and if some of the counts are less than five, classes must be combined.

The χ^2 test statistic is defined as:

$$x^2 = \sum_{i=1}^{k} \left(O_i - E_i\right)^2 \Big/ E_i$$

where O_i is the observed frequency for class i and E_i is the expected frequency for class i.

Hypothesis tests of the distribution functions were performed by examining the *p* value that was associated with a goodness-of-fit statistic. If the test statistic was greater than the critical value obtained from a table or the *p* value was less than a predefined critical value, the null hypothesis (H_0) was rejected at the chosen significance level (0.01, 0.05, etc.), and accepted functions were ranked according to the test statistic.

The stem density of *F. orientalis* was higher than that of *C. betulus* in all development stages while the DBH of *F. orientalis* was larger than that of *C. betulus* except in the initial stage, indicating that small diameter trees in this stage were mostly *F. orientalis* (Table 3). The greatest coefficient of variation of DBH occurred in the initial stage, while in the op-

Results

In total, the diameter and height of 857 trees in the three sampled plots were measured. The initial stage had the highest frequency in small trees whereas the decay stage was mostly composed of mature trees. As expected, the optimal stage was associated with a bell-shaped, skewed distribution (Fig. 1 and 2). Mean tree DBH and height increased from initial to decay stages, while the number of trees followed the opposite trend (Table 3). The initial stage was associated with the largest stem number, and the smallest average DBH. The decay stage can be recognized by the lowest stem number and basal area (Tables 3 and 4).

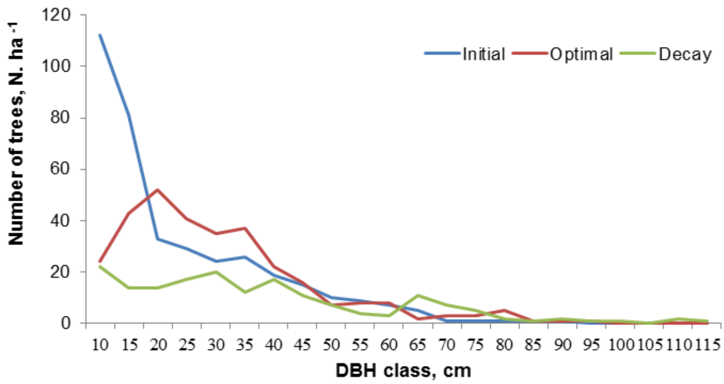

Fig. 1. Stem diameter distribution in each developmental stage.

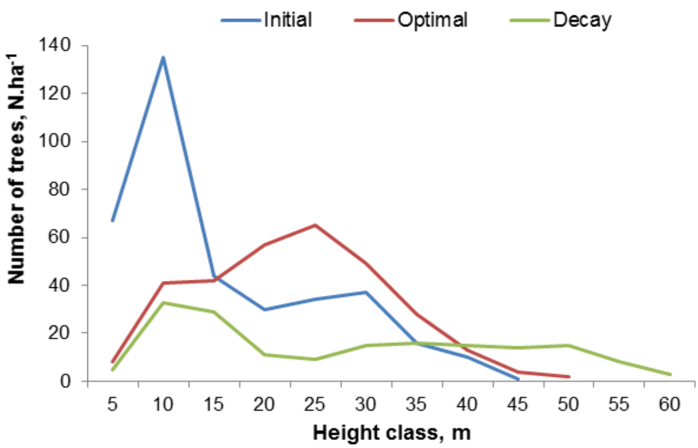

Fig. 2. Stem height distribution in each developmental stage.

Table 3. Species characteristics of each development stage (based on 1 ha area).

Indicator	Sample plot											
	Initial				Optimal				Decay			
	Beech	Hornbeam	Others	Total	Beech	Hornbeam	Others	Total	Beech	Hornbeam	Others	Total
Number of stems	217	127	30	374	175	132	2	309	91	57	26	174
Frequency, %	58	34	8	100	56.6	42.7	0.7	100	52.3	32.7	14.9	100
Mean dbh, cm	20	28	34	23.8	31	27.5	30	30.4	40	36	36	38
DBH CV, %	68	51	71	66	57	45	-	54	72	41	48	61
Mean height, m	14	18	20.5	15.9	23.7	22.1	23	23	27	27.5	31	27.8
Basal area, $m^2 \cdot ha^{-1}$	15.04	14.61	4.25	33.9	19.3	9.4	0.18	28.9	17	7	3.25	27.25
Volume, $m^3 \cdot ha^{-1}$	160.6	107.7	46.7	315	246.9	86.8	1.7	335.4	211.5	64.5	29.6	305.6
Number of snags	2	1	1	4	1	6	-	7	4	5	-	9
Number of snags60 cm dbh	-	-	-	-	1	1	-	2	2	-	-	2

Note: Beech (*Fagus orientalis*); Hornbeam (*Carpinus betulus*) and others, tree species including *Acer velutinum*, *Acer cappadocicum*, *Alnus subcordata*, *Ulmus glabra*, *Diospyros lotus*, *Parrotia persica* and *Tilia begonifolia*; CV coefficient of variation.

timal stage, all trees had about the same height and DBH, as indicated by the lowest CV of DBH and height (Table 4). In the decay stage due to the establishment of regeneration in gaps created by dead standing or downed mature trees, CV of DBH and height were quite large (Table 4). In addition, the initial stage was characterized by the smallest DBH standard deviation (Table 4). The greatest diameter and height variation occurred in the decay stage (Table 4). Tree distributions displayed positive skewness, i.e., asymmetry towards positive values for both DBH and height, positive kurtosis for DBH and negative kurtosis for height at all three stages (Table 4). The greatest asymmetry occurred in diameter and height distributions at the initial stage, while the optimal stage was associated with the less asymmetrical height distribution (Table 4).

The results of the goodness-of-fit tests for each of the five distribution functions applied to the observed DBH and height data of the initial stage are presented in Table 5. K-S and A-D tests indicate that the three-parameter lognormal function can adequately fit the observed DBH as suggested by their lower calculated statistics compared to the tabulated critical values. Other distribution functions were unable to adequately represent tree diameter distribution at this stage. Using DBH classes of 5 cm, Figure 3 shows the observed DBH distribution and the estimated probabilities of the distribution functions of DBH class. None of

Table 4. Descriptive statistics for DBH and height of each development stage (based on 1 ha area).

Development stages	Sample size	Variable	Mean	Standard deviation	Maximum	Minimum	Coefficient of variation, %	Skewness	Kurtosis
Initial	374	DBH, cm	23.8	15.71	91	5	66	1.36	1.55
		Height, m	15.9	9.77	43	3	61.6	0.92	-0.32
Optimal	309	DBH, cm	30.4	16.4	95	5	54	1.31	1.86
		Height, m	23	9.24	52	3	40	0.24	-0.28
Decay	174	DBH, cm	38	23.3	115	8	61	1.00	0.67
		Height, m	27.8	15.5	60	5	56	0.31	-1.2

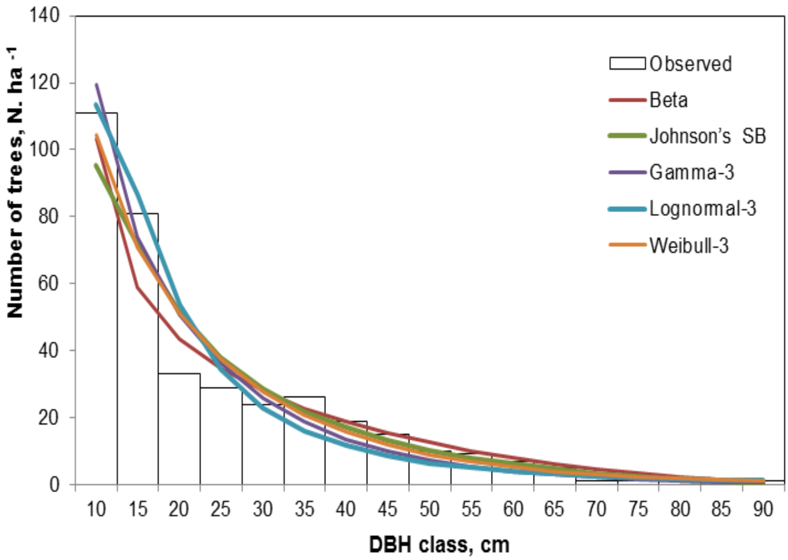

Fig. 3. Actual and theoretical DBH distributions in the initial stage.

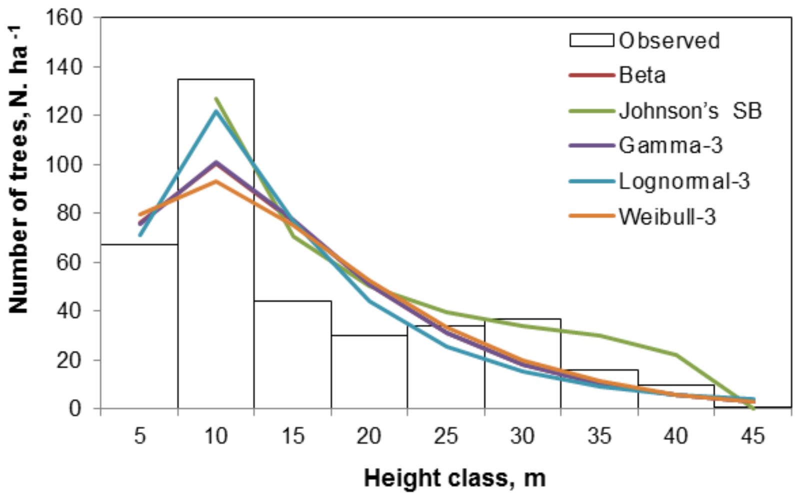

Fig. 4. Actual and theoretical height distributions in the initial stage.

the distribution functions were able to adequately fit the height observations of the stand representing the initial stage (Table 5). Using height classes of 5 m, Figure 4 shows the observed height distribution and the estimated probabilities of the five distribution functions.

Table 6 presents the parameter values of the distribution models illustrated in Figures 3 and 4.

Table 7 presents the results of goodness-of-fit tests of each DBH and height distribution function for the optimal stage. All three tests indicated that all distributions were significantly related to the observed DBH data. Among these five functions, K-S and A-D tests indicate that Johnson's S_B and three-parameter gamma distributions were more closely related to the observed data as indicated by the lower values of their statistics. However, the χ^2 test indicated that the three-parameter Weibull distribution

Table 5. Goodness-of-fit and ranking of the five distributions in the initial stage.

Variable	Distribution	Kolmogorov Smirnov			Anderson Darling			Chi-squared		
		Statistic	*P* value	Rank	Statistic	Critical value	Rank	Statistic	*P* value	Rank
DBH	Beta	0.12431	0.00**		10.962	3.9074**		60.556	0.00**	
	Johnson's S_B	0.10603	0.00**		3.742	3.9074 *ns*	2	16.374	0.03 *ns*	1
	Gamma-3	0.11031	0.00**		46.607	3.9074**		-	-	
	Lognormal-3	0.08413	0.055 *ns*	1	3.1582	3.9074 *ns*	1	58.302	0.00**	
	Weibull-3	0.09542	0.00**		44.793	3.9074**		-	-	
Height	Beta	0.12047	0.00**		6.8092	3.9074**		60.323	0.00**	
	Johnson's S_B	0.09666	0.00**		58.681	3.9074**		-	-	
	Gamma-3	0.1207	0.00**		6.8988	3.9074**		71.269	0.00**	
	Lognormal-3	0.09513	0.00**		4.9316	3.9074**		55.134	0.00**	
	Weibull-3	0.1245	0.00**		7.2893	3.9074**		112.04	0.00**	

Note: * & ** – the null hypothesis rejected at α = 0.05 and α = 0.01, respectively and ns – no significant.

Table 6. Distribution parameter estimates for initial stage.

Variable	Distribution	Parameters			
DBH	Beta	$\alpha_1 = 0.63035$	$\alpha_2 = 2.6744$	$a = 8.0$	$b = 100.1$
	Johnson's S_B	$\gamma = 1.4399$	$\delta = 0.79781$	$\lambda = 90.967$	$\xi = 5.9557$
	Gamma-3	$\alpha = 0.85437$	$\beta = 16.961$	$\gamma = 8.0$	
	Lognormal-3	$\sigma = 1.0367$	$\mu = 2.3402$	$\gamma = 7.1483$	
	Weibull-3	$\alpha = 0.93895$	$\beta = 16.404$	$\gamma = 8.0$	
Height	Beta	$\alpha_1 = 1.7889$	$\alpha_2 = 2.3206E+6$	$a = 2.928$	$b = 1.6822E+6$
	Johnson's S_B	$\gamma = 0.75061$	$\delta = 0.50582$	$\lambda = 35.687$	$\xi = 5.669$
	Gamma-3	$\alpha = 1.8029$	$\beta = 7.1779$	$\gamma = 2.9255$	
	Lognormal-3	$\sigma = 0.75145$	$\mu = 2.3289$	$\gamma = 2.4261$	
	Weibull-3	$\alpha = 1.3736$	$\beta = 14.174$	$\gamma = 2.9718$	

was more closely related to the observed data. Figure 5 shows the observed data and the estimated probability functions of DBH class whose parameter values are presented in Table 8. K-S and A-D tests indicate that Johnson's S_B and three-parameter Weibull function can adequately fit the observed height data as suggested by their *P* value and by lower calculated statistics compared to tabulated critical values (Table 7). However, the χ^2 test indicated that the three-parameter Gamma and lognormal distribution were not able to fit height observation (Table 7). Figure 6 shows the observed data and the estimated probability functions of height class whose parameter values are presented in Table 8.

The results of goodness-of-fit tests of the distribution functions applied to the decay stage are shown in Table 9. K-S and A-D tests indicated that all distribution functions can significantly fit the actual di-

Table 7. Goodness-of-fit and ranking of the five distributions in the optimal stage.

Variable	Distribution	Kolmogorov Smirnov			Anderson Darling			Chi-squared		
		Statistic	*P* value	Rank	Statistic	Critical value	Rank	Statistic	*P* value	Rank
DBH	Beta	0.04936	0.42 ^{ns}	4	0.40794	3.9074 ^{ns}	2	11.306	0.18 ^{ns}	3
	Johnson's S_B	0.04362	0.58 ^{ns}	1	0.77261	3.9074 ^{ns}	5	9.3294	0.31 ^{ns}	2
	Gamma-3	0.04934	0.42 ^{ns}	3	0.39815	3.9074 ^{ns}	1	11.321	0.18 ^{ns}	4
	Lognormal-3	0.05415	0.31 ^{ns}	5	0.51561	3.9074 ^{ns}	3	11.789	0.16 ^{ns}	5
	Weibull-3	0.04769	0.46 ^{ns}	2	0.66136	3.9074 ^{ns}	4	8.027	0.43 ^{ns}	1
Height	Beta	0.05644	0.27 ^{ns}	3	0.72219	3.9074 ^{ns}	3	13.24	0.10 ^{ns}	3
	Johnson's S_B	0.05032	0.40 ^{ns}	1	0.65684	3.9074 ^{ns}	1	12.187	0.14 ^{ns}	1
	Gamma-3	0.06043	0.20 ^{ns}	5	0.94972	3.9074 ^{ns}	5	17.537	0.02^{*}	
	Lognormal-3	0.05825	0.23 ^{ns}	4	0.91235	3.9074 ^{ns}	4	17.259	0.02^{*}	
	Weibull-3	0.05397	0.05 ^{ns}	2	0.68596	3.9074 ^{ns}	2	12.501	0.13 ^{ns}	2

Note: * & ** – the null hypothesis rejected at α = 0.05 and α = 0.01, respectively and ns – no significant.

ameter data. However, for all three goodness-of-fit tests, Johnson's S_B distribution was more closely related to the observed DBH distribution. Figure 7 shows the ob-

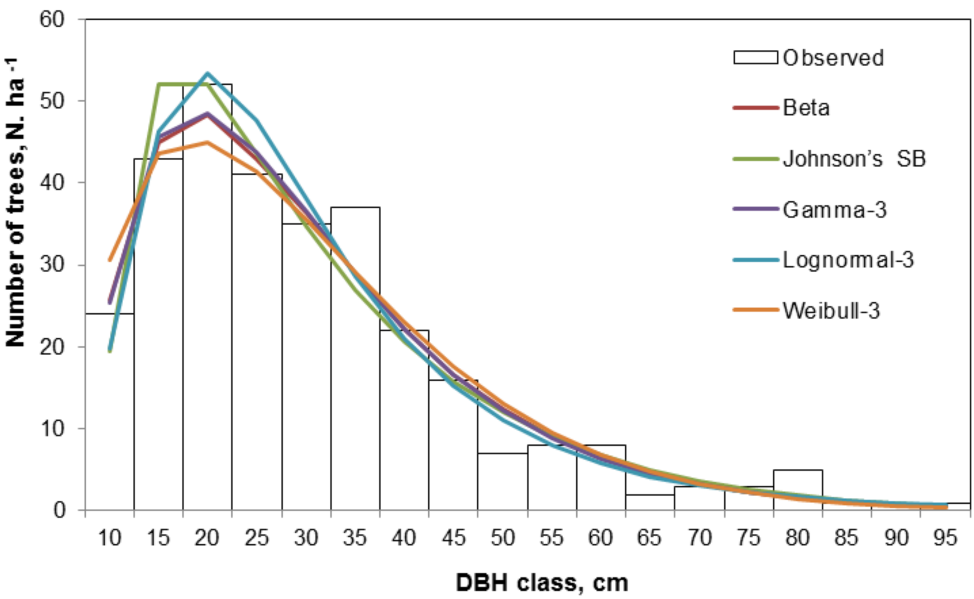

Fig. 5. Actual and theoretical DBH distributions in the optimal stage.

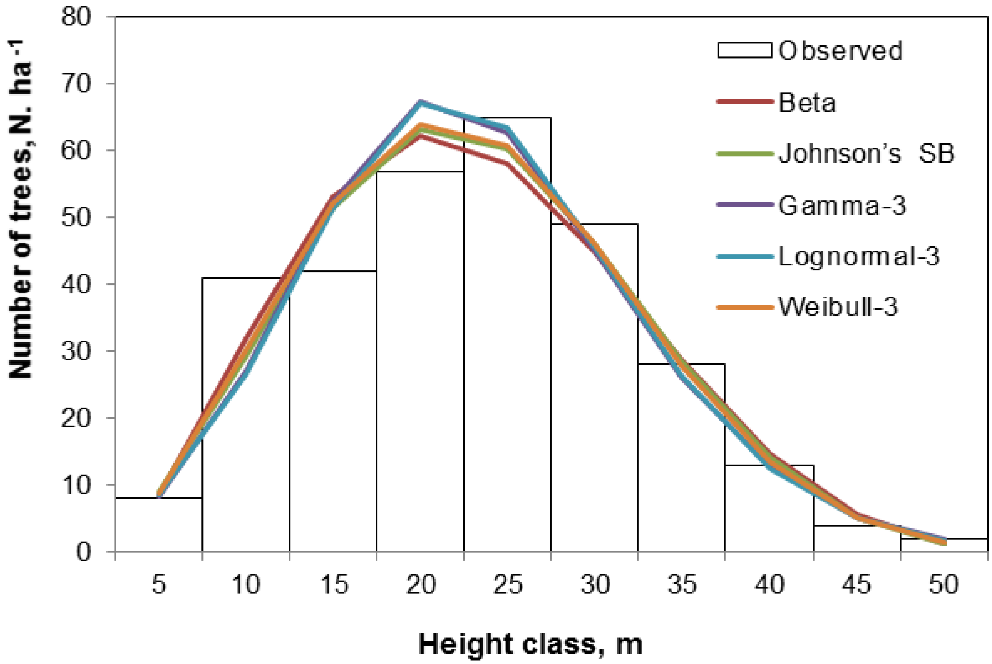

Fig. 6. Actual and theoretical height distributions in the optimal stage.

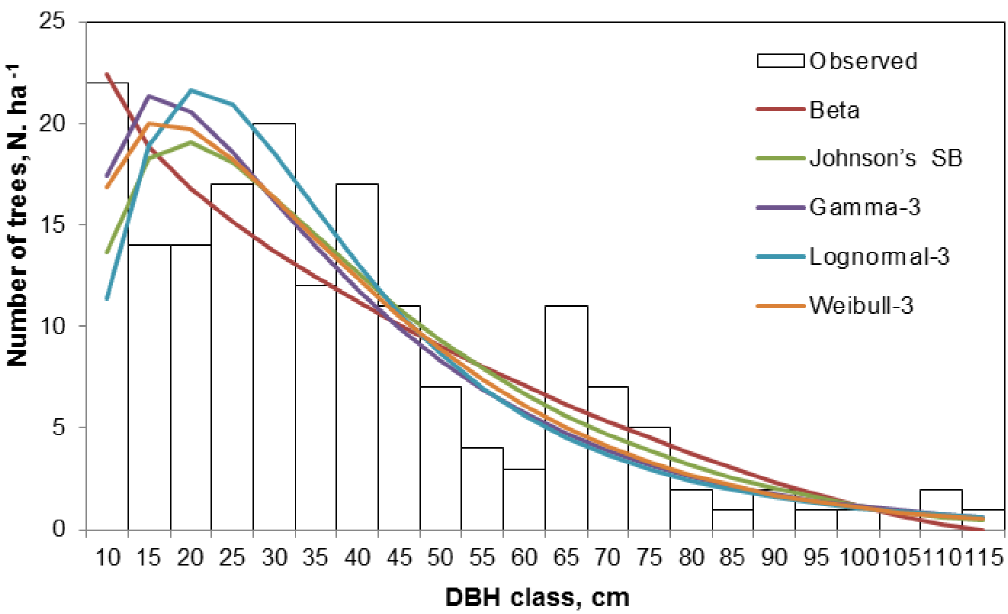

Fig. 7. Actual and theoretical DBH distributions in the decay stage.

Table 8. Distribution parameter estimates for optimal stage.

Variable	Distribution	Parameters			
DBH	Beta	$\alpha_1 = 1.9447$	$\alpha_2 = 119.94$	$a = 7.2644$	$b = 1456.6$
	Johnson's S_B	$\gamma = 1.9088$	$\delta = 1.0811$	$\lambda = 128.14$	$\xi = 7.3804$
	Gamma-3	$\alpha = 1.9867$	$\beta = 11.664$	$\gamma = 7.2145$	
	Lognormal-3	$\sigma = 0.57173$	$\mu = 3.1762$	$\gamma = 2.2901$	
	Weibull-3	$\alpha = 1.4225$	$\beta = 24.92$	$\gamma = 7.7553$	
Height	Beta	$\alpha_1 = 3.2793$	$\alpha_2 = 5.5323$	$a = 0.66128$	$b = 60.746$
	Johnson's S_B	$\gamma = 0.80996$	$\delta = 1.9361$	$\lambda = 78.677$	$\xi = 8.6517$
	Gamma-3	$\alpha = 27.271$	$\beta = 7.1779$	$\gamma = -25.373$	
	Lognormal-3	$\sigma = 0.11346$	$\mu = 4.3916$	$\gamma = -58.253$	
	Weibull-3	$\alpha = 2.5889$	$\beta = 24.61$	$\gamma = 1.1972$	

Table 9. Goodness-of-fit and ranking of the five distributions in the decay stage.

Variable	Distribution	Kolmogorov Smirnov			Anderson Darling			Chi-squared		
		Statistic	*P* value	Rank	Statistic	Critical value	Rank	Statistic	*P* value	Rank
DBH	Beta	0.0844	0.16 ^{ns}	4	1.138	3.9074 ^{ns}	3	7.5172	0.37 ^{ns}	2
	Johnson's S_B	0.06807	0.37 ^{ns}	1	0.67241	3.9074 ^{ns}	1	5.6985	0.57 ^{ns}	1
	Gamma-3	0.09474	0.08 ^{ns}	5	1.1721	3.9074 ^{ns}	4	22.42	0.00 **	
	Lognormal-3	0.07253	0.30 ^{ns}	2	1.233	3.9074 ^{ns}	5	10.213	0.17 ^{ns}	3
	Weibull-3	0.07944	0.21 ^{ns}	3	0.93211	3.9074 ^{ns}	2	14.973	0.03 *	
Height	Beta	0.081113	0.19 ^{ns}	2	9.1416	3.9074 **		-	-	
	Johnson's S_B	0.05938	0.55 ^{ns}	1	12.241	3.9074 **		-	-	
	Gamma-3	0.11135	0.02 *		3.0931	3.9074 *		24.974	0.00 **	
	Lognormal-3	0.11939	0.01 *		3.8496	3.9074 *		38.645	0.00 **	
	Weibull-3	0.02034	0.02 *		3.1169	3.9074 *		37.434	0.00 **	

Note: * & ** – the null hypothesis rejected at α = 0.05 and α = 0.01, respectively and ns – no significant.

served and estimated values for the dist ribution functions whose parameters are presented in Table 10. A-D and χ^2 tests indicate that none of the distribution functions were able to fit height observations, while K-S test indicated that the Johnson's S_B and beta distributions were significantly related to the observed data (Table 9). Figure 8 shows the observed data and the estimated probability functions of height class whose parameter values are presented in Table 10.

Discussion

Stand development stages are generally characterized by several structural

Table 10. Distribution parameter estimates for decay stage.

Variable	Distribution	Parameters			
DBH	Beta	$\alpha_1 = 0.9149$	$\alpha_2 = 2.3462$	$a = 8.0$	$b = 115.0$
	Johnson's S_B	$\gamma = 1.422$	$\delta = 1.0443$	$\lambda = 148.29$	$\xi = 2.4148$
	Gamma-3	$\alpha = 1.3551$	$\beta = 22.259$	$\gamma = 7.837$	
	Lognormal-3	$\sigma = 0.62824$	$\mu = 3.4664$	$\gamma = -0.55885$	
	Weibull-3	$\alpha = 1.2399$	$\beta = 32.169$	$\gamma = 7.895$	
Height	Beta	$\alpha_1 = 0.85416$	$\alpha_2 = 1.2105$	$a = 5.0$	$b = 60.041$
	Johnson's S_B	$\gamma = 0.26519$	$\delta = 0.48972$	$\lambda = 49.791$	$\xi = 6.8766$
	Gamma-3	$\alpha = 1.7581$	$\beta = 13.283$	$\gamma = 4.4161$	
	Lognormal-3	$\sigma = 0.58752$	$\mu = 3.2198$	$\gamma = -1.5762$	
	Weibull-3	$\alpha = 1.4483$	$\beta = 25.451$	$\gamma = 4.6297$	

and biological criteria such as species composition and distribution, horizontal and vertical structure, canopy status, forest health and rate of tree mortality (Chernyavskyy 2003, Hassani and Amani 2010). Distribution functions are powerful mathematical tools for describing forest stand diameter structure (Mohammadalizadeh et al. 2009, Duan et al. 2013) and predicting the growth of stands of various structures (even- and uneven-aged) and compositions (pure and mixed) (Palahi et al. 2007, Gorgoso et al. 2007). This study was carried out to compare the performance of different probability distribution functions in estimating the diameter and height distribution of trees from different development stages in uneven-aged natural mixed beech forest. Fitting results showed that the best theoretical distributions differed between development stages. Structural differences between development stages can explain these different results.

At the initial stage, the observed DBH distribution was characterized by few trees larger than 70 cm in DBH, a high proportion of trees with DBH below 20 cm and the greatest coefficient of variation of DBH (Fig. 1, Table 4). The wide range of DBH and the reverse-J shape of the actual DBH distribution indicate that the initial stage was characterized by an irregular uneven-aged structure. For such a diameter distribution, three-parameter lognormal and Johnson's S_B distribution were the best fitted functions. These functions resulted in positively skewed distributions and their parametric distributions were very flexible (Hafley and Schreuder 1977, Zwillinger and Kokoska 2000). Both functions were thus able to take into account the presence of many small trees together with some large trees that displayed asymmetry towards positive values (Table 4). Similar results were obtained by Amanzadeh et al. (2011) in natural beech stands and by Amanzadeh (2015) in natural mixed hornbeam forests of Iran, both at the initial development stage. Nanang (1998) also observed that lognormal distribution was appropriate to represent the diameter distribution of Neem (*Azadirachta indica* A. Juss. [Meliaceae]) using the K-S test. In addition, Sheykholeslami et al. (2011) observed the good performance of the lognormal function based on the results of K-S and χ^2 test in uneven-aged forests of Iran, but without taking into account different development stages. The tree height distribution at the initial stage was characterized by an outstanding peak in the 10-m height class and a marked positive skewness (Fig. 4). All modeled functions displayed a peak around the 10-m height class but they overestimated the tree frequency observed in height classes 15 and 20 and underestimated that observed in classes 25, 30, 35 and 40 m, while the Johnson's S_B followed the opposite trend (Fig. 4). In this stage, none of the tested functions adequately fit the height distribution likely because the competition dynamics among trees for light stimulated the high growth of young trees (Akhavan et al. 2012).

The empirical DBH distribution at the optimal stage was characterized by a large number of trees with DBH below 35 cm and by a moderate proportion of medium diameter classes (Fig. 1 and 5). Overall, homogeneous diameter and height distributions (lowest DBH and height CV, Table 4) were observed at this stage which was very similar to even-aged stand. Tree DBH distribution displayed a peak at the 20 cm class with a positive skewness. The DBH distribution was best fitted by the three-parameter Weibull, Johnson's S_B and three-parameter gamma functions and, to a lesser degree, by the beta and three-parameter lognormal functions. This is in agreement with the findings of Amanzadeh et al. (2011) who obtained similar appropriate models for fitting the diameter distribution at this stage although the ranking of these functions was different. Similarly, Amanzadeh (2015) reported that Weibull, gamma, and lognormal distribution functions were more closely related to the diameter distribution of that type of stand. Consequently, Weibull and gamma functions seem to have the greatest flexibility and universality to represent the diameter distribution of trees at the optimal stage. Tree height distribution displayed a peak at the 25 m class, whereas, the lower negative kurtosis of height distribution indicated that the stand representing this stage was a little bit flatter than a normal distribution (Fig. 6, Table 4). For such a height distribution, Johnson's S_B, three-parameter Weibull and beta distribution were the best fitted functions. Mohammadalizadeh et al. (2013) found that the Weibull function was more closely related to height distribution in uneven-aged stands. Mirzaei et al. (2015a) observed the good performance of the beta and Weibull distributions based on the results of K-S test in open oak forests of Iran.

At the decay stage, a higher proportion of old trees with DBH above 50 cm was observed compared to previous stages (Fig. 1 and 7). The natural mortality of some of these old trees created canopy gaps that were gradually filled by small diameter trees. Accordingly, the observed DBH distribution has a moderate downtrend indicating an uneven-aged structure. This diameter distribution was best modeled by the Johnson's S_B and, to a lesser degree, by the three-parameter lognormal, beta and three-parameter Weibull distribution. Similar results have been reported by Amanzadeh et al. (2011) and Amanzadeh (2015) who noticed the greatest usefulness of the Johnson's S_B distribution at this stage. In addition, Tesgot et al. (2013) observed that the Johnson's S_B function adequately represent ed the diameter distribution of old forest stands while the three-parameter Weibull function was useful in old-growth Norway spruce stands (Karczmarski 2005). The tree height distribution was characterized by an outstanding peak in classes 10–15 and by lower peaks in classes 30, 35, 40, 45 and 50 (Fig. 8). This particular height distribution observed at the decay stage was adequately fit by the Johnson's S_B and beta distributions. Mirzaei et al. (2015b) also observed that the beta distribution was appropriate to represent the height distribution of uneven-aged stand from the Ilam region of Iran.

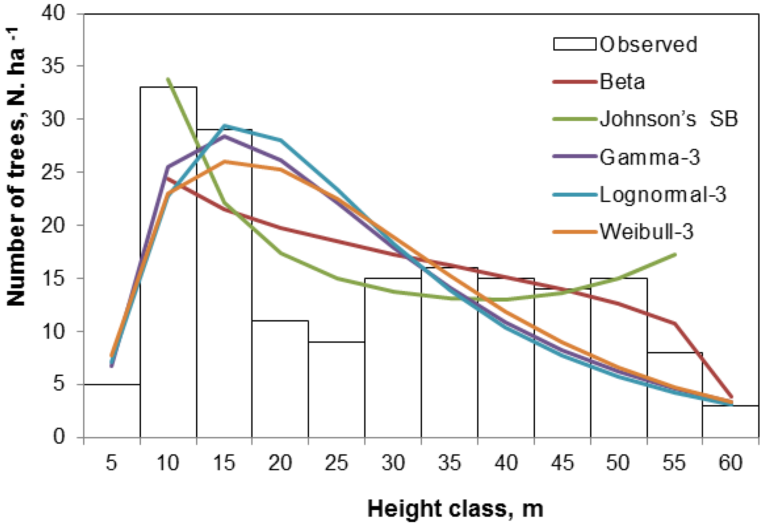

Fig. 8. Actual and theoretical height distributions in the decay stage.

Our results indicate that the three-parameter lognormal function can be appropriate for DBH distribution at all three stages in these mixed stands. Similar results have been reported by Amanzadeh et al. (2011) for the same three stages in beech stands. However, the gamma (Mohammadalizadeh et al. 2009) and the beta (Namiranian 1990, Mattaji et al. 2000, Fallah et al. 2006) distribution models were also successfully used for beech stands, but without taking into account different development stages. In this study, Beta and Johnson's S_B functions performed better to describe the height distributions of the optimal and decay stages. The Weibull distribution was found as the most appropriate model for beech stands (Mohammadalizadeh et al. 2013) while the beta distribution was the best fitted function for Persian oak (*Quercus persica* Jaub. et Spach) stands (Mirzaei et al. 2015a, Mirzaei et al. 2015b), but without taking into account different development stages.

Conclusion

Diameter distribution gives information about stand and age structure, stand stability, etc., and enables optimal planning of silvicultural treatments. In addition, the height distribution should be considered and determined simultaneously for a better interpretation of stand structure and a more accurate estimation of forest volume stocks. This information is essential for understanding how the structure of natural forests change over development stages as influenced by various factors (e.g., stem density, canopy gaps, light competition, etc.).

In accordance with our results, we conclude that a single theoretical distribution function can produce satisfactory results to model DBH distributions of stands at the optimal and decay stages. However, in stands with a large proportion of trees with DBH ≤ 20 cm (60 %) and a small proportion of trees between 20 and 30 cm in DBH (14 %) and a negative binomial distribution (e.g. initial stage), the use of a finite mixture distribution may provide accurate approximation of diameter distributions as was observed in this study. This is also the case for height distributions characterized by a large proportion of trees with height ≤ 15 m (63 %) and a small proportion of trees with height ≥ 35 (8 %). It thus seems that self-thinning is the principal process among trees in the lower and middle stories because of dense regeneration under canopy gaps at the initial stage. Consequently, the three-parameter lognormal or the finite mixture distributions can be used to effectively apply close to nature interventions (crop tree thinning) in the lower and middle stories to accelerate this natural process and, alternatively, to promote tree diversity in managed stands of similar composition. More homogenous conditions (DBH and height distributions) and high stand volume (Table 2) were observed in the optimal stage. In addition, tree regeneration density decreased because of canopy closure and sapling density declined as competition-induced mortality thinned suppressed trees. Accordingly, for both DBH and height distributions, the three-parameter Weibull and Johnson's S_B were the best model to represent this self-thinning process. However, to represent the increasing number and size of persistent canopy gaps that allow the regeneration and recruitment of shade-tolerant species and, to a lesser degree, mid-shade tolerant tree species at the decay stage, the Johnson's S_B could be used successfully for both DBH and height distributions.

Since the number, basal area and volume of *F. orientalis* trees were higher than *C. betulus* and other species at all three stages (Table 3), this dominance should be maintained if silvicultural treatments are applied (Fallah et al. 2000). Because uneven-aged mixed forests are generally resistant against natural disturbances while providing sustainable production (Alijani et al. 2014), diameter distribution functions have been widely applied to develop forest growth models. These models provide foresights of forest stand development that may simplify management decisions (Merganic and Sterba 2006).

Finally, there is no theoretical reason for the fact that a particular distribution model should be used for all situations (Wang and Rennolls 2005, Amanzadeh et al. 2011). It seems that the performance of theoretical distributions is affected by the structure (even- and uneven-aged, and pure and mixed stands), density, development stages and habitat conditions of studied stands (Bailey and Dell 1972, Amanzadeh et al. 2011, Amanzadeh 2015, Podalski 2006).

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GEOMETRICAL CONSTRUCTIONS AS A BASIS FOR COMPOSITION TYPES IN LANDSCAPE ARCHITECTURE

Veselin Shahanov

Department of Park and Landscape Design, University of Forestry, 10 St. Kliment Ohridski Blvd., 1797 Sofia, Bulgaria. E-mail: vshahanov@yahoo.com

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Abstract

Different kinds of compositions are used in landscape architecture practice, despite the fact that composition types are not deeply theorized or defined precisely as composition styles. The conception of styles concerned in landscape history and theory is a general issue. The current paper aims to explore the geometrical construction types as a concept which gives to composition specific and aesthetic qualities. The research methodology includes the following three steps: several compositions have been analyzed and evaluated by different experts; a model for typology has been made, and as a result a conception for typology has been created. Exemplary compositions are explored through their drawings, plans or satellite images and are perceived as abstract, plane, graphic images. In this way their aesthetic qualities are explored isolated. The evaluation has been statistically checked. On the basis of comparative analysis of the best compositions, criteria for creation of a type model have been suggested. As a combination between the variables of the particular criteria certain types of compositions have been finally established. Thus the geometrical patterns could be understand better and effectively use in the creation of plan drawings.

Key words: design approach, geometrical pattern, type model, visual quality.

Introduction

The composition is a fundamental concept in the arts. It is defined as aesthetic activities towards a harmonious organization of several components (Tkachev 2006). In every field – art, design, architecture, and landscape architecture – there is a theoretical basis that serves to build the composition of the work. In landscape architecture (LA) are used a variety of approaches and composition methods. Typology is one of them.

Achieving harmonious and aesthetic compositions in LA is based generally on geometric constructions and universal artistic laws and principles. By their use in the past masterpieces of garden and park art have been created. The actuality of the study lies in the question whether contemporary landscape-architectural compositions (LAC), influenced by modern art trends obey these principles.

This study aims to identify the most common types of LAC, based on geometrical constructions and characterized by high aesthetic qualities and expressiveness. To realize the objective have been set out the following tasks:

- after selection of exemplary LAC the relationship between aesthetic qualities and geometric constructions to be analyzed;
- based on geometric constructions a model of typology to be made;
- using the model specific types of LAC to be defined.

The scope of the study is determined by the ability to analyze large amounts of exemplary projects. In this case, they are not an illustration, but rather raw material that serves to build the model. The projects are selected from Landezine (2015). This website is among the best ten websites for landscape architecture in the last three years according to the international company Global Site Plans and Alexa International Rankings. The used projects are built after the beginning of the XXI century, as it is assumed that they most closely reflect the impact of various contemporary trends in art. Geographically are covered almost all continents, although dominate projects located in countries with a developed landscape-architectural practice. For the above reasons and methods of work, the analysis of sample projects is done in graphic data – plans, drawings, satellite images, i.e., have been taken into consideration their plane compositions. The plan drawings are more appropriate of geometric analysis.

To conduct the study has been used the following methodology (Fig. 1). The aesthetic qualities of LAC examples are determined by the method of expert evaluation. Establishing the relationship between these qualities and the degree of complexity of the compositions followed. Geometric constructions serve as a basis for creating a model for typology of LAC. It helps to synthesize specific types of LAC. The study concludes by identifying the most common types of compositions, which experts determined that have the highest aesthetic qualities.

For the implementation of the first task an interdisciplinary approach has been

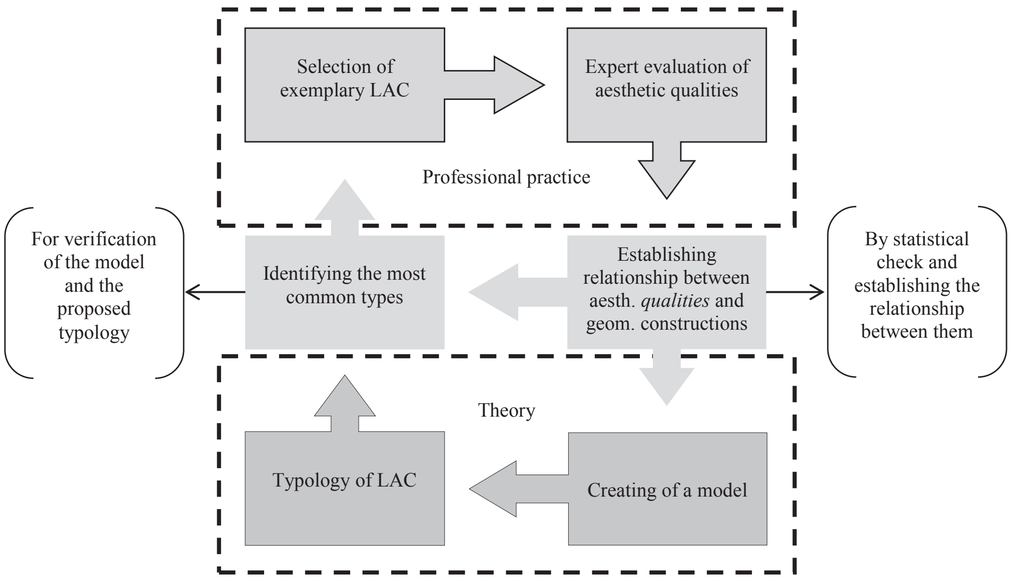

Fig. 1. Methodology and sequential steps.

used. Seventy selected examples of LAC are evaluated by forty experts in the field of landscape architecture, architecture and urban planning, engineering design and fine arts. Half of the experts are in the professional design practice, most of whom with more than ten years of experience. The rest of experts are teachers and researchers from four universities, most of whom with solid design experience too. The projects are analyzed as graphic plain compositions, without considering context, existing features, purpose, size, i.e., in an abstract way. The aim is to be assessed the artistic and aesthetic qualities of the compositions isolated from other functional and technical aspects. This allows the defined problem to be considered from different angle, so that the interdisciplinary approach could be applied.

Argument for this is that the aesthetic qualities of the composition are essential. From psychological point of view 90 % of the information about the surrounding environment of the man is perceived by eyes (Stepanov 2007). According to Rappaport (1970) the importance of the visual characteristics of the environment requires placing the form-creation process on objective scientific basis, in order to avoid subjective interpretations (as cited in Stepanov 2007).

On the other hand since the visual qualities of a work are associated with different effects that it causes in the perceiver, it is incorrect to talk about objective aesthetic evaluation. Even less is possible by quantitative criteria to assess qualities associated with harmony, unity, etc. However, there are two concepts for defining the visual qualities of the landscape – 'objective', based on its physical characteristics and 'subjective', which evaluates the landscape in its perception (Lothian 2000, van Etteger 2008). If these two approaches are adopted in assessing the aesthetic qualities of LAC, the current study is based on the second concept.

After evaluating the sample compositions and determining the relationship between aesthetic qualities and geometric constructions, has been proceed towards solving the second problem of the study – the creation of a model for typology of LAC. The selection of criteria for the model is related to the way in which has been looked at the example compositions – as flat abstract compositions. In this connection, the plane composition, which is relatively two-dimensional and includes clarification of alleys, playgrounds, lawns, flower beds and water areas (Shtiliyanov 2009) is seen as a graphical composition which elements are point, line, spot and colour (Ustin 2007). The criteria are formulated namely on the basis of interactions between these graphic elements and the possibilities for structuring, constructing and form-creation.

Comparing landscape design with other arts: painting, architecture, etc., Fomina (1988) stresses that the creation of LA works is through composing and constructing, and that in the basis of design are the geometric constructs. Precisely constructions based on a logical basis help to define objective criteria for creating typological model.

The third task of the study is related to the definition of specific types of LAC. This is the stage of synthesis, in which the types are obtained as a combination of selected criteria and their variables. There are several studies on the typology and its application in science and practice.

The idea of Type like the idea of Form is philosophical question, which can be viewed from different perspectives (Madrazo 1995). In many philosophical texts and literary materials for psychology of perception, the term 'type' is used to mean, close to the meaning of 'model'

(Güney 2007). On the other hand, comparing the ’type‘ and ’model‘, Quatremere de Quincy (1977) found that the type is associated not so much with the image of a thing which can be copied or imitated, but rather with the idea that an element itself may serve as a rule when creating a model (as cited in Güney 2007).

In this sense, according to Rossi (1982) type is something permanent and complex – a logical principle that precedes the form and creates the form (as cited in Güney 2007). Other authors also considered the type as a principle, which results in the creation of a shape (Sandalack and Uribe 2010).

Unlike art and architecture, in the field of landscape architecture theoretical studies for typology of composition are few. Historically, the concept of type in garden design is used to denote gardens with different composition – Baroque, Renaissance, etc. One of the earliest definitions of different types of gardens is from ancient China. There are three main landscapes created in Chinese gardens – ‘laughing’, ‘threatening’ and ‘idyllic’ (romantic) (Dobrev 2011). These types differ in the use of different compositional methods, different kind of landscape components as well as different way of their use. ‘Laughing’ type includes the use of many flowering species in the open space, while in the ‘threatening’ type prevail high cliffs, artificial hills, weird trees, etc. ‘Idyllic’ type normally includes construction of an island in a lake, arched bridge, weeping willow, etc.

Materials and Methods

1. Comparative analysis of exemplary LAC

Some of the main issues at this stage of the study are:

- Whether aesthetic qualities of the composition are due to simple interactions between compositional elements or they are result from complex composition constructs?
- Does simplification of operations linking the compositional elements lead to enhance the aesthetic qualities of the composition as a whole?
- Does relationship between these two judgments inversely proportional and generally whether linear or a complex curve?

The arising issues lead to the formulation of the following thesis. If the composition is considered as a system of interrelated elements, the aesthetic qualities of the work may be tested by analysis of the elements connections in the system. When the links are through forceful and clear structure, the integrity of the system is greater. Since integrity is one of the most significant compositional and artistic principles in art, seemingly more explicit linking elements should lead to increasing the aesthetic qualities of the composition. In this regard have been analyzed:

- the aesthetic qualities of exemplary projects;
- the degree of complexity of compositional constructions;
- the relationship between them.

1.1. Determination of the exemplary LAC aesthetic qualities

Aesthetic qualities of exemplary LAC are evaluated by 40 experts dealing with creation of different type of compositions – landscape architects, architects, designers and artists. Assessment is performed on five-point scale:

1. Very low aesthetic evaluation – 1 point;
2. Low aesthetic evaluation – 2 points;

3. Average aesthetic evaluation – 3 points;

4. High aesthetic evaluation – 4 points;

5. Very high aesthetic evaluation – 5 points.

Average aesthetic evaluation, a sample of which is shown in Table 1, is giveby the formula: $AE = \frac{E_1 + E_2 + E_n}{n}$,

where: *AE* – average aesthetic evaluation; E_n – individual expert assessment; *n* – the number of experts.

1.2. Determination of the compositional constructions degree of complexity

Compositional constructions are actually geometric constructions. Their degree of complexity is associated with three main aspects:

A. Geometrical type of compositional elements;

B. Interaction between the compositional elements themselves;

C. Interaction between compositional elements and the background (visual field).

Each of these aspects affects the possibilities of linking compositional elements and can be evaluated in three degrees of complexity – low, medium and high. Geometric type is determined by the forms used. According to them the following fundamental differences can be determined:

- using elements with identical geometric type – a relatively common forms;
- using elements with geometric forms of the same type – similar forms with slight variations in geometry;
- using different in their geometric form elements – different forms without domination of any of them.

The interaction between compositional elements is determined by their location and orientation. According to them the following fundamental differences can be determined:

- connecting all elements by certain grid, main axes, similar orientation (direction), etc.;
- connecting some elements by one or another type of connections;
- lack of connection between elements.

The interaction between compositional elements and the background is deter mined by their number and size. Accord ing to them the following fundamental differences can be determined:

- using a small number of large compositional elements, which are easily

Table 1. Assessment of aesthetic qualities of exemplary compositions (fragment), points.

No	Exemplary composition	Average assess-ment	Individual expert assessment										
			1	2	3	4	5	6	7	8	9	…	40
42	Playground at Zorlu Centre	3.43	3	3	1	2	4	4	5	5	5	…	2
43	Garden of 10,000 Bridges	3.73	4	5	5	3	2	3	4	3	5	…	4
44	Hoekenrode Square	3.93	3	2	4	2	4	3	5	4	5	…	5
45	Hoekenrode Square Clos Layat Park	3.33	2	4	1	3	2	4	4	3	4	…	5
46	Central Garden Block B4	3.63	4	2	1	4	3	3	4	2	3	…	5

Table 2. Determination of the degree of complexity of exemplary compositions (fragment), points.

No	Exemplary composition	Overall assessment	Criteria: A. Geometrical type of elements	B. Elements interaction	C. Element-background interaction
42	Playground at Zorlu Centre	9	3	3	3
43	Garden of 10,000 Bridges	6	2	3	1
44	Hoekenrode Square	8	2	3	3
45	Hoekenrode Square Clos Layat Park	7	3	3	1
46	Central Garden Block B4	8	2	3	3

distinguished from the background – clear distinction between element and background;

- using many compositional elements in different sizes, whereat the background is lost – the elements are densely arranged together;
- using a small number of large compositional elements, which are difficult to be distinguished from the background – there is no clear idea of element and background.

Through these three aspects of linking compositional elements, each with three levels of complexity (organization), has been determined the general scale for assessing the complexity of compositional constructions. Low complexity is evaluated with 1 point, average – with 2 and high complexity – with 3 points. By summing up the three aspects has been received an overall score of 3 to 9, so that evaluation of 9 points means too complicated decision and evaluation of 3 points – the most simple one, Table 2.

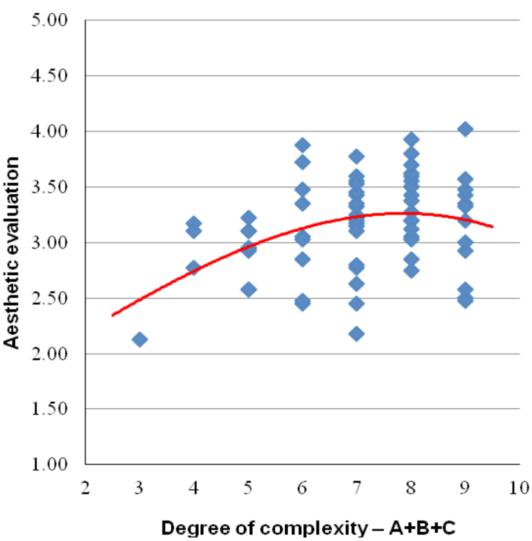

Fig. 2. Relationship between aesthetic qualities and complexity of geometric constructions in the composition.

1.3. Determination of the relationship between the aesthetic qualities and the complexity of geometric constructions in the composition

As comparing the results of the aesthetic evaluation and the complexity evaluation of the exemplary compositions, can be seen that there is a correlation between these two aspects. The correlation coefficient $r = 0.3$, which means that the correlation dependence between studied values is low to moderate. By increasing complexity of geometric constructions, the aesthetic qualities of composition increase as well (Fig. 2).

The figure shows that the compositions which received aesthetic assessments between 2.0–3.5 points have different degree of complexity – they have relatively even distribution. The composition received the lowest aesthetic evaluation – 2.13 points has the lowest degree of complexity as well – 3.0 points. Those compositions with aesthetic evaluation over 3.5 points have moderate to high complexity. The composition received the highest aesthetic evaluation – 4.03 points has the highest degree of complexity as well – 9.0 points.

2. Model for composition typology

The model consists in the selection of several characteristics of the composition, which can always be found in each LA project. These characteristics are defined as compositional criteria. Each of them varies in certain range, i.e. each characteristic can be represented by a different variety. Thus a type of LAC can be determined by the combination of different varieties of each of the criteria, Table 3.

Table 3. Principal scheme for model typology.

Compositional criterion	Variety 1	Variety 2	…	Variety, m
1	1.1	1.2	…	1.m
2	2.1	2.2	…	2.m
…	…	…	…	…
n	n.1	n.2	…	$n.m$

A certain type LAC can be expressed as follows: CT (n,m) = 1.m+2.m+…+$n.m$, where: CT – compositional type; n – the number of criteria; m – the number of criteria varieties.

3. Criteria for LAC typology

Depending on the aspect in which the composition is examined, various criteria can be used in order to receive a particular typology. Since geometric constructions allow the compositions to be compared on an objective basis, this gives rise to criteria selection. In relation to the research aim, have been selected the same three criteria used to determine the degree of complexity of compositional constructions. They define the plane composition as an abstract two-dimensional image and are as follows:

- criterion ’form creation‘ defined by the geometrical type of compositional elements;
- criterion ’structure‘ defined by the interaction between compositional elements;
- criterion ’element-background‘ defined by the interaction between compositional elements and the background.

There are three possible varieties of each of the criteria (again by analogy to the three degrees of complexity), Table 4.

Table 4. Compositional criteria and their kinds.

	Criteria		Criteria variety 1		Criteria variety 2		Criteria variety 3
A	Form creation	a_1	identical type of forms	a_2	similar type of forms	a_3	different type of forms
B	Structure	b_1	connection of all elements	b_2	connection of some elements	b_3	lack of connection between elements
C	Element-background	c_1	elements with background	c_2	elements without background	c_3	elements and background merging

Results and Discussion

On the basis of exemplary compositions comparative analysis, the proposed typological model, and the adopted criteria and their varieties, certain types of compositions have been finally established.

The possible combinations of the selected three criteria, each with three varieties, allow for the formation of a total of 27 types of compositions. In the surveyed exemplary compositions are explored 21 of the defined types. Composition types are recorded with letters and numbers. The letter shows the certain criterion and the number index recorded after the letter – its variety. For example composition type $a_1b_3c_2$ represents a composition which includes elements with identical forms (a_1), with no connection between them (b_3), occupying the whole space (c_2).

Composition types have some important indicators – aesthetic value, complexity value, frequency of use, rating value. The aesthetic evaluation of compositional types is obtained as an average value from the aesthetic assessments of those compositions, which use the particular type.

The criteria varieties are identical to the degrees of complexity of the geometrical constructions, e.g.: the first variety of 'form creation' criterion – a_1, which comprises the use of identical types of forms has a low complexity; its second variety – a_2, which includes the use of similar forms has average complexity; its third variety – a_3, which comprises the use of different forms has high complexity. Thus the sum of the numeric indices of a certain compositional type determines its complexity index. It varies from 3 to 9, for example compositional type $a_2b_2c_3$ has complexity index 7.

Table 5. Total evaluation of the used composition types.

No	Compositional type	Total evaluation	Aesthetic evaluation × 4	Complexity index × 3	Frequency of use × 2	Rating index × 1
1	$a_3b_3c_3$	67.26	12.62	27.00	24.00	3.65
2	$a_2b_3c_3$	60.69	13.53	24.00	20.00	3.16
3	$a_2b_2c_3$	56.46	13.18	21.00	20.00	2.28
4	$a_3b_2c_3$	53.77	13.30	24.00	12.00	4.47
5	$a_3b_3c_1$	48.91	11.97	21.00	12.00	3.94
6	$a_3b_3c_2$	47.70	13.40	24.00	4.00	6.30
7	$a_2b_3c_1$	38.78	13.55	18.00	4.00	3.23
8	$a_3b_2c_2$	38.08	13.70	21.00	2.00	1.38
9	$a_2b_1c_3$	37.53	12.27	18.00	6.00	1.26
10	$a_2b_2c_2$	37.34	13.90	18.00	2.00	3.44
11	$a_2b_3c_2$	37.09	11.20	21.00	4.00	0.89
12	$a_1b_3c_1$	36.54	10.30	15.00	2.00	9.24
13	$a_3b_2c_1$	35.51	11.60	18.00	4.00	1.91
14	$a_1b_1c_3$	34.67	12.20	15.00	6.00	1.47
15	$a_1b_2c_3$	33.16	12.10	18.00	2.00	1.06
16	$a_2b_2c_1$	32.68	12.35	15.00	4.00	1.33
17	$a_2b_1c_1$	30.66	12.55	12.00	4.00	2.11
18	$a_1b_2c_2$	29.71	11.70	15.00	2.00	1.01
19	$a_2b_1c_2$	27.70	10.30	15.00	2.00	0.40
20	$a_1b_1c_2$	26.15	11.10	12.00	2.00	1.05
21	$a_1b_1c_1$	24.76	8.50	9.00	2.00	5.26

The composition rating value is obtained as the number of viewers is referred to the accumulated days from the date of publication on the website http://www.landezine.com until the time of calculation (on 22.08.2015). The rating indicator of each compositional type is calculated as an average value of the ratings of all compositions, which use the particular type. The resulting value determines its rating index.

The total evaluation of the compositional types is prepared in the following manner. According to its importance, examined indicators receive different number of points: aesthetic evaluation – 4 points; complexity index – 3; frequency of use – 2; rating index – 1. The total evaluation of each compositional type is obtained as sum of the values of all indicators multiplied by the corresponding number of points, Table. 5

In figures 3a, 3b, 3c, 3d are shown exemplary compositions in which are used compositional types, received the highest total evaluation. Composition No 36 has aesthetic evaluation 4.03 and the compositional type a3b3c3 has total evaluation 67.26, the highest complexity index, the highest frequency of use, and some of the highest values of aesthetic evaluation and rating index (Fig. 3a). Composition No 44 has aesthetic evaluation 3.93 and the compositional type a2b3c3 has total evaluation 60.69 (Fig. 3b). Composition No 48 has aesthetic evaluation 3.78 and

Fig. 3a. Exemplary composition No 36 Asnières Residential Park (aesthetic evaluation 4.03), Compositional type a3b3c3 (total evaluation 67.26).

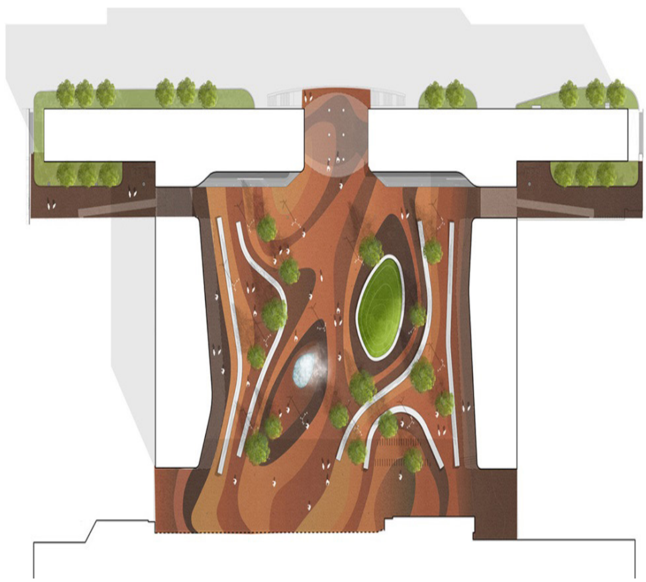

Fig. 3b. Exemplary composition No 44 Hoekenrode Square (aesthetic evaluation 3.93), Compositional type a2b3c3 (total evaluation 60.69).

Fig. 3c. Exemplary composition No 48 Scholars' Green Park (aesthetic evaluation 3.78), Compositional type a2b2c3 (total evaluation 56.46).

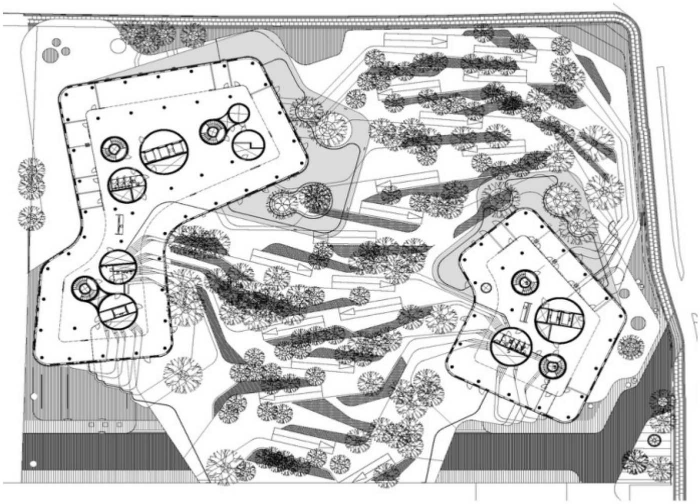

Fig. 3d. Exemplary composition No 53 The City Dune / SEB Bank (aesthetic evaluation 3.70), Compositional type a3b2c3 (total evaluation 53.77).

the compositional type a2b2c3 has total evaluation 56.46 (Fig. 3c). Composition No 53 has aesthetic evaluation 3.70 and the compositional type a3b2c3 has total evaluation 53.77 (Fig. 3d)

Conclusions

The implementation of the research aim and tasks allows conceptualizing the typology of composition in landscape architecture and leads to the formulation of the following conclusions:

1. It is confirmed that the use of a typology as a compositional tool increases the aesthetic properties – the most commonly used types have the highest aesthetic evaluation.

2. Compositional type $a_3b_3c_3$ has most qualities – it is a composition created by different types of forms, without links between them, with merging of elements and background. These combinations as well as other ones that obtain compositional types with high total assessment are a prerequisite for creation of landscape-architectural compositions with profound geometrical constructions that meet modern ideas about shaping the open space and ultimately to achieve high aesthetic qualities.

3. The compositional type received the lowest aesthetic evaluation – 2.58 is $a_1b_3c_1$ – a composition created by the same type of forms, without connections between them, with elements and background.

4. In general the aesthetic composition is a result of deeper geometric constructions. The essential thing is linking a quantitative indicator of the composition such as the degree of complexity with a qualitative characteristic such as its aesthetic assessment. The choice of compositional type with more complex constructions, which is relatively easily, allows the creation of landscape-architectural compositions with high aesthetic qualities.

5. The research shows the relationship between the aesthetic qualities and the geometrical constructions, but to have a practical application such a study it is advisable the compositional types to be presented in appropriate graphic form.

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REGENERATION AND GROWTH OF WILLOW CLONES IN A BIOMASS PLANTATION

Aneliya Dimitrova*, Kancho Kalmukov, Ilka Yonovska, and Miroslav Mikov

Experimental Station for Fast-Growing Forest Species, 18 Nove str., Svishtov 5250, Bulgaria.
E-mails: elina04@abv.bg*, kkalmukov@abv.bg, iyonovska@mail.bg, mmikov2008@gmail.com

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Abstract

The study investigates the shoot regeneration ability of ten willow clones in an experimental plantation planted in the same initial density scheme (2.0 × 0.30 m). They were planted in lots with identical soil characteristics on the second terrace of the Danube River. The soil is Haplic Kastanozem with alkalinity (pH) between 7 and 8 and underground water below 8 m. The climatic conditions are typical for the continental climate of Northern Bulgaria. Development of new shoots was recorded in three consecutive years – from the seventh to the ninth year of their growth. The degree of preservation of the coppiced stumps, the number of shoots and their biometric data (height, basal diameter, fresh and dry weight, as well as their health status (live or withered), were recorded and compared. The results show that the shoot regeneration ability and the productivity of the various clones depend on the climatic conditions and the age of the plants. This shows that certain clones are more suitable for biomass production depending on the type of wood needed and the specific soil and climatic conditions of the designated planting area.

Key words: coppice, lignocellulosic crops, *Salix*, sprouts.

Introduction

The environmental and economic importance of wood biomass for the national energy balance requires an efficient and rational production of wood mostly in biomass plantations of suitable tree species planted on appropriate soils and cultivated using modern sylvicultural methods and techniques.

The suitability for production of biomass of the tree species depends on their biological characteristics – growth rate, propagation, yield, natural resilience, etc. A review of the literature revealed that woody crops for energy production include several forest tree species notably sharing the following characteristics: fast growth and high biomass yield, potential to be managed as a coppice and high management intensity (Guidi et al. 2013).

The species of genus *Salix* L. have a relatively fast growth rate, good shoot-regeneration ability and a high productivity. Combined with their outstanding ecological flexibility, genus diversity and resilience to adverse environmental conditions, these characteristics make them preferred tree species for biomass plantations (Zahariev 1977; Zaharinov and Naydenov 2012; Kalmukov 1995, 2013; Tsanov 1965, 1970, 1974).

The study aims to determine the regeneration and growth dynamics and the age-related productivity of ten willow clones in a biomass plantation.

Object and Methods

The observations were conducted in an experimental plantation of willow clones planted in 2005 in the nursery of the Experimental Station for Fast-growing Forest Species near the village of Vardim – 43°36'28.0" N, 25°29'30.5" E. The terrain is flat, on the second terrace of the Danube River. The soil is Haplic Kastanozem with alkalinity (pH) between 7 and 8 and underground water below 8 m. The surface soil layer is more than 50 cm deep, with low contents of nutrient nitrogen and phosphorus and sufficient content of potassium. The mechanical composition is heavy sandy-clayey, with good water-retaining capacity. The active moisture content in the one-meter layer is 217 mm, and in the two-meter layer is 452 mm. The average annual precipitation for the last 25 years is 510.9 mm. The average precipitation during the period (April – August) is 236.6 mm, which is 46.3 % of the average annual precipitation.

The cuttings were planted in spring, after a complete cultivation of the soil. The propagules were planted as standard cuttings from one-year shoots at a planting density of 2.0 × 0.30 m (16,667 cuttings per hectare). At the end of the first year the surviving saplings were counted and the sample was replenished in the following spring. Each year, prior to growing, they were coppiced and the cut shoots were removed from the plantation. The ridges were hoed manually and the furrows were disk-harrowed during the growing period. Of a total 130 willow clones, 10 were selected – 7 deciduous tree and 3 shrub (wicker) clones. Observations of the degree of rooting and growth during the first growing period and at the age of 6, 8 and 9 were made to collect empirical data. The degree of preservation of the stumps of the different clones, as well as the status and growth of shoots per stump, were monitored during the entire period. The length of the shoots was measured with an accuracy of 1.0 cm and their basal diameter – with an accuracy of 0.1 cm. The fresh and dry weight of the biomass produced annually by each clone was determined. The effect of the climatic conditions was determined through daily meteorological observations.

Results and Discussion

During the nine years of the study the amount of precipitation both on annual basis and during the growing period varied. The highest annual precipitation was recorded in 2005 (the year when the cuttings were planted) – 940.0 mm and the lowest annual precipitation was recorded in the seventh year (2011) – 432.7 mm. The precipitation during the vegetation period (April – August) was highest in the ninth year (2013) – 400.6 mm and lowest in the fourth year (2008) – 165.2 mm. The precipitation during the vegetation period in 2012 (8^{th} year) was 61.75 % of that in 2010 – 316.2 mm (6^{th} year) and 48.72 % of that in 2013 (9^{th} year) – see Table 1.

The intensity of shoot regeneration depends on the climatic conditions and the age of the saplings.

During the first year after the cuttings are planted, the degree of rooting depends on the characteristics of each clone, the quality of cuttings and certain subjective factors. This explains the differences in the degree of rooting. In the first year after the initial planting, the different

Table 1. Meteorological data for the period 2005–2014 at the test plantation in Vardim.

Year	Precipitation, mm			Average annual ambient temperature, °C	Absolute lowest ambient temperature, °C			
	annual	April – August			Jan.	Feb.	Nov.	Dec.
		mm	part of the annual, %					
2005	940.0	367.5	39.00	12.74	-5.0	-18.0	-	-
2006	445.0	204.8	45.27	11.49	-16.0	-13.0	-	-
2007	746.0	268.3	35.97	13.02	-6.0	-	-	-
2008	501.5	165.2	32.94	13.01	-16.0	-8.0	-	-
2009	562.2	178.9	31.82	12.70	-10.8	-2.3	-	-8.0
2010	720.5	316.2	43.90	11.70	-14.8	-9.0	-	-8.5
2011	432.7	351.1	81.14	11.80	-8.8	-7.5	-	-3.4
2012	517.8	195.2	37.70	13.01	-16.5	-23.0	-	-7.8
2013	690.8	400.6	58.00	12.90	-12.3	-5.0	-	-2.8
2014	757.1	450.8	59.56	12.70	-7.8	4.3	-1.8	-11.5
Average	631.4	289.86	45.91	12.52	-16.5	-23.0	-1.8	-11.5

clones had different number of shoots per stump. Most of the clones had up to three shoots, the number of shoots per stump varied according to the species. Four species had up to two shoots and only one species (*Salix excelsa* '84/8') had up to four shoots (Table 2).

Of the seven deciduous tree clones (rows 1 through 7 in the Table 2), the greatest number of shoots was recorded for *Salix alba Si* '2/64' and the smallest – for *Salix excelsa* '62/8'. The slowest growth rate was recorded for the *Salix alba* '351'. Of the 3 bush clones (rows 8 through 10 in the table) the highest growth rate during the first year was recorded for the *S. viminalis purpurea* 'X/43'. The highest growth rate of all clones during

Table 2. Biometric data for one-year-old biomass plantations of willow clones.

No	Clone	Different number of shoots per stump, %				Rooting, %	Shoots per ha	D_0, mm			H, m		
		1	2	3	4			av.	min	max	av.	min	max
1	*Salix alba* 'IX/4'	15.0	70.0	15.0		100	18182	27.5	15	44	2.71	1.70	3.20
2	*Salix alba* '351'	52.9	41.2	5.9		85	11818	16.8	6	25	2.20	1.45	3.00
3	*Salix alba Si* '2/64'	15.0	30.0	55.0		100	19545	21.9	12	33	2.50	1.60	3.10
4	*Salix Humboldtiana B.* '112'	5.0	5.0	90.0		100	18182	22.1	10	36	2.32	0.70	3.10
5	*Salix alba* '82/11'	57.9	42.1			95	12273	22.2	14	34	2.79	1.75	3.40
6	*Salix excelsa* '84/8'	36.8	36.8	21.0	5.4	95	16818	19.3	7	31	2.74	1.20	3.20
7	*Salix excelsa* '62/8'	61.1	38.9			90	11364	18.0	9	27	2.53	1.70	3.15
8	*Salix bregensis* 'IX/45'	47.4	52.6			95	13182	20.3	14	30	2.35	1.85	2.80
9	*Salix viminalis rubra* ‚X/3'	66.7	26.7	6.6		75	9545	19.5	10	32	1.94	1.70	2.65
10	*Salix viminalis purpurea* 'X/43'	60.0	40.0			100	12727	24.0	10	36	2.71	1.60	3.80

a b

Fig. 1. Plantations of the *Salix alba* 'IX/4': a) one-year-old; b) nine-year-old.

the first year was recorded for *Salix alba* 'IX/ 4' (Fig. 1).

The average diameter at the stump of this clone's shoots was 27.5 mm, and the largest absolute diameter was 44 mm (Table 1). Its productivity was 11.83 t/ha of green biomass and 4.67 t/ha of dry biomass (Table 3).

All 10 clones had fast initial growth of their shoots (Table 3). As the stumps aged, the survival rates of the various clones began to decline at various degrees for the different species. The fastest rates of decline were recorded for the shrub species and especially for the *S. viminalis purpurea* 'X/43' (Table 3). The number of shoots increased until the sixth year of the plantation and after that started to decline gradually. Of all shrub species the *S. viminalis rubra* 'X/3' had the highest number

a b

Fig. 2. Plantation of the shrub clone *S. viminalis rubra* 'X/3' in year one (a) and year nine (b) with annual coppicing.

Table 3. Biometric data dynamics of biomass plantations of various species of willow.

No	Clone	Stump age, yrs	Sur-vival, %	Shots, number per ha	Dimensions		Yield, t/ha	
					D_0 av., mm	H av., cm	fresh	dry
1	*Salix alba* 'IX/4'	1	100	18,182	27.50	271	11.83	4.67
		6	88	296,153	12.00	207	68.40	
		8	84	185,577	15.60	243	39.48	
		9	80	216,667	9.70	219	45.14	31.27
2	*Salix alba* '№351'	1	85	11,818	16.80	202	2.70	1.17
		6	88	437,179	8.54	187	37.84	
		8	88	289,103	8.40	180	22.15	
		9	88	415,705	6.70	152	19.77	9.77
3	*Salix alba Si* '2/61'	1	100	19,545	21.90	160	9.56	4.22
		6	64	358,974	10.64	205	57.10	
		8	58	258,333	8.10	168	24.66	
		9	50	307,051	8.10	163	25.08	12.56
4	*Salix alba Humboldtiana* 'B-112'	1	100	18,182	22.10	232	7.23	2.91
		6	86	192,948	13.00	215	45.94	
		8	80	175,000	14.50	237	55.13	
		9	80	248,718	9.30	195	45.38	21.43
5	*Salix alba* '82/11'	1	95	12,273	22.20	279	5.27	2.36
		6	78	175,000	14.36	255	45.41	
		8	78	60,897	10.10	193	6.83	
		9	76	107,692	10.00	206	6.97	3.49
6	*Salix excelsa* '84/8'	1	95	16,818	19.30	274	5.23	2.67
		6	86	192,948	12.33	233	39.28	
		8	78	143,590	10.10	199	17.41	
		9	78	204,167	10.10	199	19.92	10.97
7	*Salix excelsa* '62/8'	1	90	11,364	18.00	253	2.91	1.45
		6	86	372,115	9.31	200	53.75	
		8	86	124,679	10.40	196	17.27	
		9	82	222,436	9.60	165	20.07	12.06
8	*Salix bregensis* 'IX/45'	1	95	13,182	20.30	235	4.29	1.94
		6	68	305,128	11.96	220	49.40	
		8	66	187,821	10.60	204	14.25	
		9	64	299,359	7.80	180	11.94	6.18
9	*Salix viminalis rubra* 'X/3'	1	75	9,545	19.50	194	5.48	2.55
		6	52	708,333	8.22	192	40.64	
		8	46	499,679	7.70	174	26.30	
		9	38	565,385	6.60	145	17.05	8.46
10	*Salix viminalis purpurea* 'X/43'	1	100	12,727	24.00	271	7.69	3.32
		6	36	548,077	8.89	189	53.27	
		8	36	233,013	7.60	148	19.66	
		9	32	277,564	5.40	129	8.30	3.97

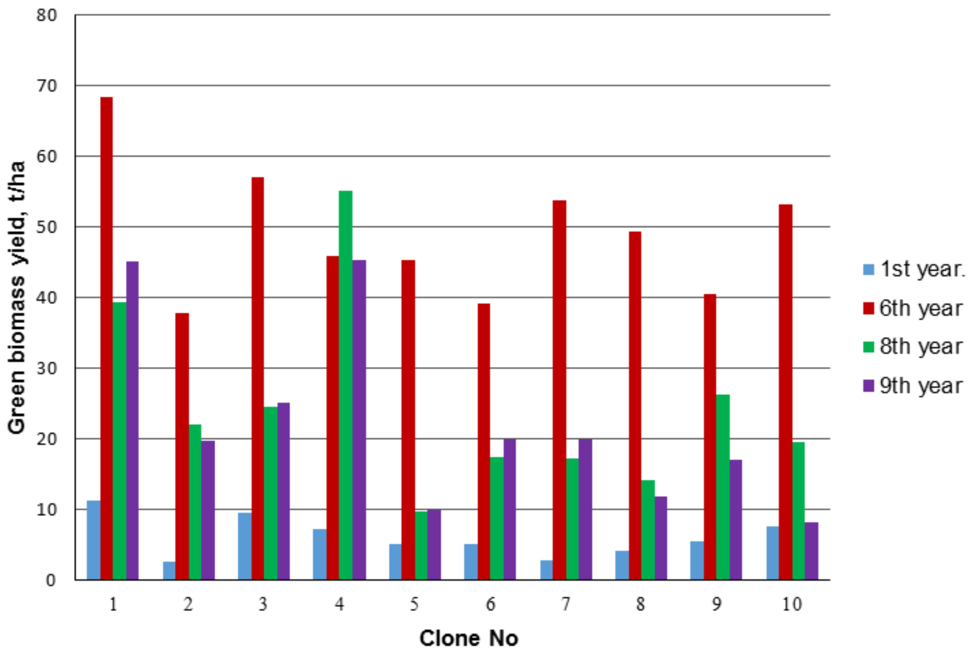

Fig. 3. Green biomass yield of the ten clones at different ages.

of shoots per hectare for the whole period of the study after the first year (Table 2).

In the eight year of the study (2012), all clones had the smallest number of shoots compared to the number of shoots in the sixth and the ninth year due to the adverse climatic conditions in that year: low amount of precipitation, particularly during the vegetation period, preceded by cold winter (Table 1). The largest number of shoots and productivity were recorded in the sixth year. The diameter growth in that year is proportional to the number of shoots. As the clones got older the basal diameter of the shoots decreased (Table 3).

As the clones got older, their productivity increased at various rates until the sixth year and after that began to decrease. Similar results have been reported by Arevalo et al. (2007), Aravanopoulos (2010) and Hangs et al. (2011). The best overall productivity throughout the study was calculated for *Salix alba* 'IX/4′, followed by *Salix alba* 'Si 2/61′. The productivity (yield) of *Salix alba Humboldtiana* 'B-112′ was relatively stable throughout the study and did not decrease after the sixth year unlike the other clones.

The yield of the shrub clones peaked in their sixth year and after that declined. During the first and the sixth year the highest yields were recorded for *Salix viminalis purpurea* 'X/43′ and during the eighth and the ninth year the highest green biomass yield was recorded for *Salix viminalis rubra* 'X/3′ (Fig. 3).

Conclusions

As a result of this research, the following conclusions were made:

– Deciduous tree and shrub willows

have specific shoot regeneration characteristics, growth rates and yields, which depend also on their age and cultivation and the climatic conditions.

– When the shoots are coppiced annually, their shoot regeneration ability declines after their sixth year.

– As the stumps get older, the survival rate declines.

– All ten clones showed a markedly fast initial growth of their shoots.

– The increase of the shoot diameter depends on the age of stump, the number of shoots per stump and the amount of precipitation during the growing period. The best thickness growth rates of the shoots of all clones were recorded in the first year.

– Of all 10 clones the best yield for the whole period of study was recorded for *Salix alba* 'IX/4' clone.

– Despite their lower survival rate, shrub clones had biomass yield comparable to that of the deciduous clones.

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ANTLER OF FEMALE RED DEER (*CERVUS ELAPHUS* L. 1758) IN BULGARIA

Gradimir V. Gruychev* and Hristo G. Hristev

Department of Wildlife Management, Faculty of Forestry, University of Forestry, 10 Kliment Ochridski Blvd., 1797 Sofia, Bulgaria. E-mails: gradi.val@gmail.com*; ico681@abv.bg

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Abstract

It was observed an appearance of antler in female Red deer (*Cervus elaphus*). The emergence of right antler was established in early January 2015. At that very time it was at the length of 10 cm. The antler was covered with velvet and was not changed. The hind gave birth to a normally developed female yearling in 2014. The hind was shot in November 2015, and then the antler was 18 cm. The internal organs were normal and an embryo in the uterus was found formed. Craniometrical measurements fell within the normal for Red deer. Probable cause of this case is the occurrence of changes of hormone levels.

Key words: antlers, Cervidae, craniometrical measurements, hind.

The antlers are one of the secondary sexual characteristics in males of all generations of the family Cervidae, except genus *Rangifer*, where both sexes have antlers (Bubenik and Bubenik 1990). Growth of antler antlers in female deer was achieved with other genera of family Cervidae after testosterone impacts (Jaczewski 1976). The antlers that appear as a result of this usually do not undergo the transformation of normal antlers and are covered with velvet constant (Bubenik et al. 1982).

There are numerous descriptions of female deer with antlers and most of them belong to the genera *Capreolus* and *Odocoileus*. The presence of antler antlers in female to genus *Cervus* is quite rare, probably due to multiple pregnancy, and it is rarer compared to the species of genera *Capreolus* and *Odocoileus* (Bubenik 1966, Bubenik et al. 1982, Goss 1983, Bubenik and Bubenik 1990). Cases of hinds with antlers were described in Wapiti (*Cervus canadensis* (Erxleben 1777)), Sika deer (*Cervus nippon* Temminck 1838) and only several cases in Red deer (*Cervus elaphus* L. 1758) (Bubenik and Bubenik 1990).

Antler of a female Red deer with a length of 18 cm grew after transferring from one hunting region to another hunting area (Fig. 1).

The female was caught by an Iskar Hunting ranch (right bank of Iskar Dam) along with 31 Red deer (6 males and 26 females). They were in a quarantine corral in hunting area in Sashtinska Sredna Gora Mountain and stayed there from February to September 2014. Then the herd was released into an acclimation corral with 540 ha in same hunting area. The red deer were not manipulated with pharmaceuticals to stimulated growth of antlers and in acclimatizing of them was used only natural food. The emergence of right antler was established in early January 2015. At that very time it was at the length of 10 cm. The

Fig. 1. Skull of female Red deer (*Cervus elaphus* L.) with the right antler.
Note: Scale bare – 5 cm. Photo: Gradimir Gruychev.

Table 1. Craniometrical measurements of the skull.

Craniometrical measurements	Value, mm
Total length	382.7
Condylobasal length	377.0
Basal length	353.1
Total breadth	159.4
Zygomatic breadth	148.5
Interorbital distance	120.3
Rostrum length	233.0
Nasal length	136.7
Upper tooth row length	107.9
Neurocranium breadth	95.6
Mandible length	317.2
Lower tooth row length	115.9
Diastema length	100.4

Note: Measurements adapted from Lowe and Gardiner (1974) and Peshev et al. (2004).

antler was covered with velvet and was not changed. In 2014, the hind gave birth to a normally developed female yearling. The female deer was shot in November 2015, and the length of antler was 18 cm. It was at age of 10 according to Aydman method (Botev and Ninov 1992). The internal organs were normal in appearance and an embryo in the uterus was found formed. In the skull there are no signs of trauma or old injures that can cause the appearance of the antler. Craniometrical measurements (Table 1) of the skull fall in the average ones, measured for Bulgarian populations (see Botev 1981, Markov 2014).

The appearance of antlers in hind of Red deer is associated with higher testosterone levels (Mattioli 2011). Growth antlers in female Red deer are achieved after continuous testosterone treatment every vvtwo weeks (Li et al. 2003). Increased of hormone levels in female deer in nature is caused by presence of genitals characteristic of males (Haugen and Mustard 1960, Mierau 1972), ovarian atrophy (Bubenik and Bubenik 1990) or tumors in the adrenal cortex (Goss 1983). The described case in this research is the first for Bulgarian Red deer population and probable cause of the antler is the occurrence of changes in hormone levels.

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