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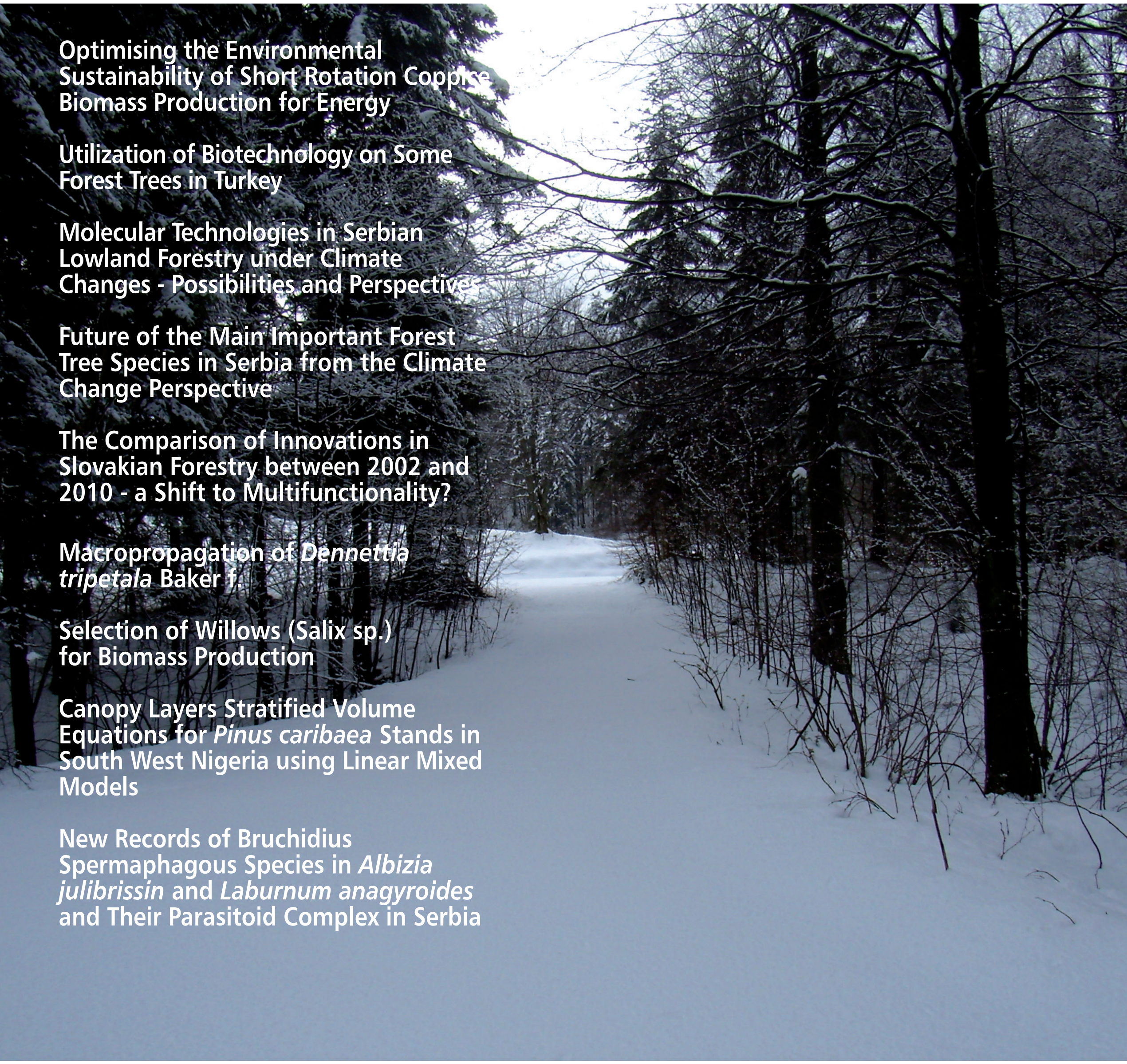

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Review paper

Optimising the Environmental Sustainability of Short Rotation Coppice Biomass Production for Energy

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Abstract

Background and Purpose: Solid biomass from short rotation coppice (SRC) has the potential to significantly contribute to European renewable energy targets and the expected demand for wood for energy, driven mainly by market forces and supported by the targets of national and European energy policies. It is expected that in the near future the number of hectares under SRC will increase in Europe. Besides producing biomass for energy, SRC cultivation can result in various benefits for the environment if it is conducted in a sustainable way. This paper provides with an overview of these environmental benefits.

Discussion and Conclusions: The review of existing literature shows that SRC helps to improve water quality, enhance biodiversity, prevent erosion, reduce chemical inputs (fertilizers, pesticides) and mitigate climate change due to carbon storage. To promote and disseminate environmentally sustainable production of SRC, based on existing literature and own project experience, a set of sustainability recommendations for SRC production is developed. In addition to numerous environmental benefits, sustainable SRC supply chains can bring also economic and social benefits. However, these aspects of sustainability are not addressed in this paper since they are often country specific and often rely on local conditions and policies. The sustainable practices identified in this manuscript should be promoted among relevant stakeholder to stimulate sustainable local SRC production.

Keywords: biodiversity, biomass, bioenergy, short rotation crops, water quality, soil quality

INTRODUCTION

Biomass plays a key role among renewable energy sources in Europe, accounting for almost 70 % of all renewables, and showing steady growth. It is expected that the demand for wood as fuel for energy (heat and electricity) will increase, driven mainly by market forces and supported by the targets of national and European energy policies [1]. Solid biomass from short rotation coppice (SRC) has been identified with high potential to significantly contribute to European renewable energy targets [2]. The term SRC refers to biomass productions systems cultivated for energy purposes using fast-growing tree species with the ability to resprout from the stumps after harvest. Harvest occurs in short intervals (2-6 years). The management practices for SRC such as soil preparation, weed control, planting, fertilisation, harvest, resemble more those of agricultural annual crops than of forestry, despite that the currently used species in commercial SRC plantations in Europe are tree species. As SRC species, willows and poplars have been predominantly used in Europe, since they are fast-growing with good coppice ability that reach high growth rates even under very short harvest intervals. Other tree species such as black locust and eucalyptus have been also considered for larger implementation as SRC systems for energy. To avoid misunderstandings between SRC and the more general term Short Rotation Forestry (SRF), we need to point out that SRF is a broader term describing forest systems for biomass production not only for energy purposes but also for others. SRF uses also fast-growing tree species and having denser spacing and more intensive management than traditional forestry, and trees are typically harvested after 2 to 25 years depending on the desired end-product. In this context SRC represents a more specialised and intense practice of SRF dedicated mainly for energy purposes.

SRC is a perennial crop grown on agricultural land that differs from arable crops in many ways. SRC plantations will remain in place for a number of years (10-25 years), therefore taking the land out of arable rotations. SRC is much taller than other arable crops since trees can reach c. 5-8 m at harvest. Moreover, harvest normally occurs in winter or early spring. Once established, no annual soil cultivation is required, and considerably less agrochemical inputs and fertilizers are applied, although herbicides are used during the establishment phase. When grown, SRC plants are deeper rooted and have a high water consumption compared to conventional crops. As a result of the lower intensity and of less agrochemicals, SRC has a considerably lower carbon footprint compared with food or biofuel production from annual arable food crops [3].

SRC for biomass production used for heat and/or electricity is considered as a very promising system to meet the European targets to increase the amount of renewable energy, and SRC cultivation in larger scale could help meet social and economic targets of other EU policies (e.g. EU Rural Development, CAP reform). This combination of technological and political drivers has stimulated the interest and a rapid large-scale shift from “conventional” agricultural crops to SRC has been predicted [1]. This will have positive and negative implications on a range of environmental issues, which this paper will further analyze. An increase of SRC grown on agricultural land is anticipated in areas neighboring power stations or local producers of heat, using biomass as a fuel. In such areas, SRC might need to be cultivated on a substantial fraction of all available agricultural land to fulfill biomass needs for fuel, being simultaneously economically and energy efficient. This, coupled with the above-mentioned special features of SRC will surely affect the landscape and have potential implications for the local water and soil quality, hydrology, carbon storage in soil, and biodiversity.

The aim of this paper is to refer to and analyze above-mentioned aspects of environmental sustainability of SRC production and to provide with a number of things-to-consider and criteria about SRC practice that will enable to optimize SRC cultivation towards an environmentally sustainable local SRC production. We will refer in detail to the impact of SRC on soil quality, water and biodiversity that is referred in the literature, and based on the existing knowledge we will recommend management measures for optimized sustainable SRC production.

IMPACTS OF SRC ON SOIL QUALITY

The potential effects of SRC on soil quality are usually divided into two large aspects, the first referring to the changes of soil carbon and the second to the changes of the heavy metal concentrations when SRC is cultivated. Mann and Tolbert [4] concluded that soil ecological benefits of SRC can be caused by the following mechanisms that are characteristic for SRC as a crop in arable soils: a) the continuous plant cover intercepts rainfall and decreases erosion potential, b) the increased root development at greater depths stabilizes soil, improves nutrient uptake and reduces leaching losses, and increases organic matter input, c) litter and vegetation intercept surface runoff and enhance infiltration, and d) the cooler soil temperatures decrease the rate of decomposition. These authors suggested that soil ecological benefits of SRC with fast growing tree species were predicted to become detectable already in 3 to 5 years, a period that for a number of other authors is considered rather short. Especially when concerning empirical studies estimating changes in carbon (C) storage in the soil of willow and poplar SRC have provided conflicting results, with most of them reporting increases in C stocks in the topsoil when SRC is cultivated for a number of years [5-8], but also others reporting decreases in C stocks [9-11]. It has been concluded that the site-specific variability in the effects of SRC on the soil C pool is high, that previous studies may not have covered a sufficiently long period to detect significant changes in soil C stocks, and that the fundamental mechanisms responsible for soil organic C accumulation in SRC are not well understood. However, when comparing carbon concentrations in the topsoil between SRC grown for a number of years with the respective concentrations in adjacent to SRC arable fields, increased carbon concentrations are found [12,13]. The same was reported for the carbon concentrations in subsoil, showing the great potential of SRC for storing carbon in agricultural soils compared to current land uses. The amounts of carbon stored seem to be governed by the initial soil properties, and therefore approaches for the selection of most promising sites for carbon sequestration must be developed.

Another soil quality parameter that has been broadly connected to the positive impacts of SRC cultivation is the reduced trace element concentrations in the soil, mainly for cadmium (Cd) [14,15]. Cd entering the food chain from agricultural soils is broadly considered as the most hazardous trace element to human health. The ability of willow trees to take up rather high amounts of Cd in their shoots, which can be removed from the field at harvest has been proposed as a solution to combine biomass production and remediation of moderately contaminated soils [16]. Based on this feature of SRC, it is common to apply sewage sludge to SRC fields. A supply of trace elements occurs, but several studies and calculations of flows in willow SRC stands suggest that uptake is enough to compensate for this. A reduction of Cd even after sludge amendment is highly probable, but questionable for other investigated trace elements [17,18]. Concerning uptake of other than Cd trace elements, such as Cu, Pb, Zn, Cr, Ni, As, several studies have been conducted and showed positive results concerning uptake in the shoots, but most of these results have been based on experiments in pots or under hydroponic conditions and their results cannot be generalized referring to field conditions [19-22]. Due to the ability of especially willow to take up trace elements from the soil, SRC plantations have been used for phytoremediation of soils and waters containing these hazardous elements. The use of SRC as multi-purpose plantations for phytoremediation of contaminated soils (e.g. extraction of Cd, Zn and other heavy metals, and degradation of organic compounds) can be combined when biomass is produced in such sites and improve the soil quality of moderately contaminated arable land, but also of marginal land that can be returned to agricultural production after SRC cultivation for a number of years [23].

IMPACT OF SRC ON WATER

The two issues that are mostly brought up when discussing the SRC impact on water are SRCs impact on water balances and on groundwater quality. The decision behind using willow and poplar as SRC for production of biomass for energy was among other reasons based on their characteristic to be fast-growing producing higher biomass compared to other tree species, especially in central and northern Europe. Increased biomass accumulation has been linked with high water use, especially in warmer climates. Willows in particular are known to grow in places with high water availability such as river banks. Coupled with the fast-growing feature of SRC, fears for high water use and consequent concerns about the effects on local hydrological balances and flow to neighbouring streams/rivers have been expressed in several reports predicting future biomass supply from agriculture [24, 25].

A large number of studies have been performed to estimate the evapotranspiration in SRC fields with the aim to evaluate expected changes in water balances in relation to other land uses. For willows, most of them were primarily conducted in Sweden, since it was there that cultivation of SRC for biomass was initiated and then commercially practiced. Similar research on poplars has been conducted in a range of countries with more temperate climate than Sweden such as in Germany and the UK, where poplar has been considered as more appropriate species than willow grown as SRC, gaining large interest during the last years. From the several estimates for evapotranspiration for poplar and willow, there seem to be variations in the figures reported. For irrigated and fertilised willow SRC grown in clay in south Sweden for four years, Persson and Lindroth [26] simulated seasonal (May-November) evapotranspiration between 360-404 mm. Persson [27] estimated that the average seasonal evapotranspiration (May-October) from six fields in different locations in south Sweden areas was 435 mm, confirming in a way the previous findings. For SRC poplar fields, Bungart and Bungart and Hüttl [28] estimated mean annual transpiration rates between 1996-2002 at the Lusatian mining region in Germany equal to 266 and 241 mm, for two different poplar clones, respectively (Beaupré, *Populus trichocarpa* x *P. deltoides and Androscoggin, P. maximowiczii*). Evapotranspiration was 404 and 373 mm, respectively. Annual evapotranspiration of 351 mm and 360 mm for a 3- and a 9- year old SRC poplar plantation, respectively, located in Neuruppin, Germany, has been calculated by Knur et al. [29]. In the UK, Hall [30] estimated that ca. 600 mm will be used by SRC willow in a clay soil which receives precipitation of 700 mm. According to Hall [30] this corresponds to an annual evapotranspiration of about 500 mm. From the above it can be assumed that there has been vast variations for the estimated evapotranspiration of SRC and that safe predictions of evapotranspiration from an SRC stand cannot be granted.

When comparing the evapotranspiration of SRC with willow and poplar, in most cases it is significantly higher than arable crops and lower than other forest [27, 30, 31]. In contrast to this, Hall et al. [32] indicated that in case of dry summers when there is significant water deficit, the water use of poplar SRC will probably be considerably less than that of coniferous forest and closer to that of grassland. Sensitivity of willow SRC to dry summers have been also reported by Linderson et al. [33], where transpiration rates varied between willow clones and were equal to 100-325 mm. Therefore, the levels of water consumption of SRC in relation to other crops grown in the same area seem to depend on site-specific factors as soil type, precipitation and others, and might vary from case to case, although SRC seem to have higher evapotranspiration than arable crops in most cases. Concerning the impact on local level, modelling exercises conducted by Stephens et al. [31] indicated reductions of 10-15 % of the hydrologically effective rainfall in SRC fields compared to arable crops in the UK. Despite this, the authors claimed that the effect on hydrology to the catchment level would be minimal, after extrapolations based on the model results obtained and the assumption that 2500 ha SRC will be planted in an area of 40 km radius from a biomass-driven power plant. This was due to the fact that the mean reduction in hydrologically effective rainfall for the catchment area would be ca. 0.5 % of the mean annual amount, which would be only a very minor portion, compared to the respective effect of cereals.

SRC is generally considered as a crop that improves the water quality in a certain area due to the management practices that has been mentioned above for this crop [25]. Almost all water quality research has been conducted concerning leaching of nutrients and not determination of chemical compounds as pesticides in the groundwater, since the use of pesticides is limited. Fertilization in willow SRC is recommended and in Northern Ireland the recommendations are 120-150 kg nitrogen (N) per hectare and year. In Sweden the recommendations is ca. 100 kg N per hectare and year, but not after the second year of growth when no fertilisation occurs. In general, SRC fertilisation recommendations can be considered rather moderate compared to respective ones for arable crops [34]. However, the fact that fertilisation to SRC fields cannot be applied every year but usually only every year after harvest due to the nature of the crop (high stems that do not allow the available equipment to apply fertiliser every year) makes the applied amounts of nutrients relatively high. Bergström and Johansson [35] measured very low N concentrations (less than 1 mg/l N) in the groundwater of an intensively fertilised willow SRC field in south Sweden. Measurements of N in the surface groundwater at the same field for a period of eight years with average annual application rates of 112 kg N/ha showed that N concentrations remained below 1 mg/N for the whole period except during the year of establishment [36]. These results came in agreement with Mortensen et al. [37] that measured close to zero N concentrations in drainage water from Danish SRC fields, except for the establishment year. Dimitriou et al. [38] reported that the differences in the nitrogen leaching from commercial willow SRC fields in Sweden compared to adjacent arable fields were of a factor of 20 (lower for SRC), indicating striking differences. Due to this ability of utilizing N in combination with low N leaching to the groundwater, SRC has been used to treat and utilize N-rich wastewaters such as municipal wastewater or landfill leachate, but also solid residues such as sewage sludge (combined with the ability to take up heavy metals as analysed above - [23]). There have been extensive research evaluating leaching of N but also of P from such practices when very high N amounts have been applied trying to optimize the systems (e.g. up to 300 kg N/ha yr), with the results very low leaching from SRC [39-42].

All the above show the potential for using SRC in intensively managed agricultural areas to reduce nutrient leaching either by replacing existing crops or by using SRC as buffer zones between intensively managed arable land and water bodies to reduce surface run off and groundwater leaching.

IMPACT OF SRC ON BIODIVERSITY

Concerning the impact of SRC on biodiversity, comparison between SRC and alternative uses in arable land has been of great importance when considering the reduction of biodiversity in European landscapes. Protection and increase of biodiversity is a political commitment set by the European Union, and therefore it would be of key importance if biodiversity could be increased within the stand and/or in the surroundings when SRC replaces other crops in agricultural areas intensively managed. For such comparisons, it is interesting to consider the reports of Baum S. et al. [43] about the impact of SRC on phytodiversity, where it is reported that there are indications about increased biodiversity in SRC in comparison with other arable crops. Several authors reported an increased number of species in SRC compared to neighbouring arable land [44-46] but in most cases rare or endangered (red-listed) species are not found. The few rare or endangered species occasionally found in SRC plantations are predominantly light demanding pioneer species recorded in the first years of a plantation and disappearing with increasing plantation age. Weih et al. [47] found not a single rare species in 21 young poplar stands grown in Sweden, however, Kroiher et al. [48] recorded a higher number of rare species in SRC plantations in northern Germany, having their main distribution in nutrient-poor habitats. The relatively high occurrence of rare species is probably related to the great tree distances in poplar plantations and the resulting favourable light and temperature conditions. The number of red list species declined with increasing canopy closure of the poplars after two years, implying that the shortening of rotation time probably supports the establishment and survival of endangered species. It has been also mentioned that plantation size and shape seem to be important for biodiversity, with higher species numbers recorded at the edge of a plantation than within it. Baum et al. [43] reported that despite the lack of long-term studies that would enable better understanding on how SRC affects phytodiversity in time and space, there are indications that it would be increased if SRC is planted in areas dominated by agriculture or coniferous forest. The authors also identified areas where SRC establishment might negatively affect phytodiversity, especially habitats of threatened species such as undisturbed peat land, forest wetlands, or areas adjacent to lakes or rivers.

Schulz et al. [49] claim that research studies for animal diversity in SRC has been conducted mostly for birds and ground beetles, and that more research for invertebrates is needed. Vertebrate diversity, equated with species richness, differs considerably in SRC in comparison to arable fields; whereas bird diversity in SRC is higher than in agricultural cropland [50-52] higher diversities of ground beetles have been found in arable fields. Britt et al. [45] found significantly more ground beetle species in arable fields than in poplars on British sites, and the same was observed in North German SRCs than on the neighbouring intensively farmed agricultural crop land [49-52]. For mammals, little research has been conducted, but species observed in SRC plantations in England included 17 mammals [53] suggesting that SRC provided a more attractive habitat for small mammals than arable land, with older coppices being more attractive. The varying results for zoodiversity are explained on the dependency of animal diversity on a number of factors such as the age of the plantation, the tree species/clone, the plantation size, the habitat structures and the location of the plantation (surroundings and other uses). The influence of the surrounding landscapes on the diversity of SRCs and the influence of SRCs on the diversity of the surrounding landscapes need to be considered, and the importance of the decision for locating the plantations is very critical for optimizing the obtained biodiversity. Aspects of deciding about the how and where an establishment of SRC should take place in certain landscapes, and their consequences, are developed in the next chapter.

CRITERIA AND RECOMMENDATIONS FOR SUSTAINABLE SRC PRODUCTION

The criteria to characterize SRC as sustainable can be fulfilled if a number of general recommendations to optimize SRC practice towards sustainable production of SRC can be developed. These have to be based on the obtained research results, combined with practical issues that will enable highest economic profit. In an effort to list all these recommendations for SRC management/practice to increase positive impacts and decrease negative impacts on the environment, the most important recommendations considering biodiversity, soil and water issues are presented below.

Considering the impact on soil:

- SRC could be cultivated in fields with low initial soil organic matter content to increase this content and with this the fertility and carbon storage of the soil.
- SRC should be cultivated especially in areas with a high risk of erosion, e.g. with relief of less than 13% or open landscapes, to lower the loss of fertile topsoil and nutrients by water and wind.
- Application of municipal residues such as sewage sludge for recycling of nutrients to SRC can be encouraged, since SRC can contribute to prevent nutrient losses and can extract heavy metals efficiently.
- SRC should be used to remediate soils with increased Cd concentrations caused e.g. by the long-term use of Cd-containing P-fertilizers or other sources of environmental pollution.
- SRC fields should be established at the same location for at least three cutting cycles to achieve soil quality improvements concerning carbon storage and Cd uptake.
- SRC should be harvested in winter when soil is frozen to avoid soil compaction and corrosion risks due to alkaline inputs from fresh plant material in the boilers.

Considering the impact on water:

- SRC could be cultivated in fields located close to N sources (e.g. animal farms, N vulnerable zones, wastewater treatment plants etc) to decrease N outflow to adjacent water bodies.
- SRC should be cultivated in areas where low groundwater level is anticipated (potentially flooded areas and areas near water bodies which can potentially flood).
- Application of municipal residues such as sewage sludge for recycling of nutrients does not affect water quality, and should therefore be encouraged.
- More frequent harvests lead to a higher average groundwater recharge, and therefore should be encouraged to ameliorate possible negative impact of groundwater recharge reductions.

Considering the reported impact on phytodiversity:

- The establishment of SRCs in areas with high ecological status should be avoided (e.g. areas with protection status for nature conservation, areas with rare species, wetlands, peat bogs, swamps).
- High structural heterogeneity provides habitats for different plant requirements and thus increases diversity. High structural diversity at one SRC location can be achieved by: i) Planting different tree species and clones; ii) Harvesting at different times so that the trees have different rotation ages within one area.
- Edges of SRCs have great species diversity, and planting several smaller plantations instead of one big SRC is advised because smaller plantations have longer edges for their size than larger ones. If that is not possible, planting long rectangular plantations can provide more benefits considering increased phytodiversity.
- An increase in forest ground species can be achieved by reducing the irradiance reaching the ground vegetation. This can be done by long rotation periods, high plant densities and planting willow instead of poplar. Another possibility is aligning planting rows in the east-west direction to reduce radiation reaching ground vegetation by shading the planted crop.

Considering the impact on zoodiversity:

- Where possible SRCs should be designed with a large edge to interior ratio.
- A mix of varieties and clones should be used.
- Rotational harvesting in mixed age-class blocks should be preferred.

- Huge blocks of SRC should be seperated, e.g. by rides and hedges.
- Where possible, and in case of growing willow, planting of willow hybrids (Salix sp.) with a range of flowering times should be preferred.
- The use of pesticides should be limited if highest zoodiversity is to be achieved.
- A percentage of the SRC area should be reserved for small habitats like strips of grass and stepped wood boundaries.
- There should be no SRCs in high wildlife-value habitats like wetlands, wet meadows, set asides, dry fallows, semi-natural grassland.

The balance between maximum environmental benefits and maximum attained biomass production from SRC is a big challenge that all stakeholders involved in SRC cultivation (farmers, decision-makers, researchers, and others) should deal with. Despite all the above-mentioned expected positive environmental impact of SRC, farmers need to be convinced to cultivate the crop, and this is typically achieved when the economic profit from the cultivation of a new crop such as SRC is equal to or higher than that of other "established" or "conventional" crops. Such issues are dealt with in Köhn [54], Dimitriou et al. [55]. To encourage farmers to grow SRC instead of other crops in order to achieve environmental benefits, decision-makers should be prepared to contribute with direct or indirect incentives to the farmers. For instance, a potential economic compensation to the farmers could be a form of "reward" for those helping to fulfill national and European environmental goals already set and simultaneously keeping agricultural land into production. Such issues concerning the added value of SRC cultivation, when at the same time important environmental goals are achieved, should be one of the drivers for sustainable SRC cultivation, besides or in combination with drivers for producing biomass for energy to achieve renewable energy commitments.

CONCLUSIONS

The extensive review of the existing research on the impact of SRC production on the environment has shown that a number of benefits could be achieved with SRC. If the recommendations developed within this paper are followed, SRC production can show significant improvement of soil and water quality, enhance biodiversity and diversify the landscape. It is important to note that a balance between the potential maximum profit for the farmer and the maximum environmental advantages for the society need to be achieved at a specific area. Before establishing a SRC plantation, all related aspects and obtained research findings need to be considered, in combination with the site-specific features of the available sites towards a sustainable SRC cultivation.

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Review paper

Utilization of Biotechnology on Some Forest Trees in Turkey

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Abstract

Background and Purpose: Raw wood material requirements are increasing with rapid population growth both in Turkey and in the world. In order to supply deficit for closure of forest products, productivity and quality of production should be improved. Basic ways to increase efficiency in forest production involves silvicultural implementations and classical tree breeding studies. Genetic variation can be increased by utilizing the existing diversity. Thus, new combinations can be obtained and we can raise efficiency using some selection strategies. At this point, biotechnological methods are required to meet the genetic material. Studies of forest tree breeding are a slow process due to the size of the genome and the length of the tree life span. Biotechnological applications in forest trees provide many important benefits in terms of time saving and reducing cost when compared to classical breeding studies. Sustainable forestry practices are gaining rapid acceleration via biotechnology and modern sciences practices. In this study, for some forest tree species in Turkey, the evaluated biotechnology methods included; 1- tissue culture and clonal propagation, 2- molecular marker applications, 3- marker assisted selection and breeding, 4-genomic and proteomic studies, 5- genetic modification and genetic engineering applications.

Conclusions: In this study, the works were carried out on forest tree breeding/propagation in Turkey and it was mainly focused on vegetative production techniques with 25 broadleaves and 9 conifer taxa, which were possible to express. Molecular genetic studies were carried out on 12 broad leaves taxa and 9 conifer taxa; genetic transformation studies were conducted on poplar species. Thus, it might be suggested that a combination of biotechnological tools and traditional propagation methods will ensure advantage for the development of forest-tree species.

Keywords: tree breeding, biotechnology, sustainable forestry, tissue culture, genomics studies

INTRODUCTION

In vitro breeding, gene transfer and marker-assisted breeding approaches of biotechnology contributed greatly on genetical improvement of forest trees to the level comparable with sophistication of ordinary genetic improvement applied for agricultural varieties [1]. It is described in FAO documents that forest biotechnology comprises three main field as follows; using molecular genetic markers to get information on genetic futures of populations and genetic base of traits, using advanced breeding technologies to produce consistent high quality planting rootstock at low price and in order to offer new economic future or ecological value, genetic engineering of trees [2]. Due to big size and long growing times of trees, the advance of forest tree breeding is a slow and challenging process. Recently, forest genomics has offered new medium for studying adaptation in trees. The gene technology for forestry has been used to solve the primary environmental issues, to produce cheap renewable energy, to specify relevant genes for the adaption of forest tree. Especially, forest trees' genes having growth future such which is resistant to disorders, herbicide and environmental stresses and wood future such as reducing lignin and increasing cellulose attracts much attention. The usage of modern genotyping technologies that are a highly beneficial medium to analyze effect of dense forestation process, to specify genes that control amazing phenotypes designates allelic assortment for the candidate genes in forest tree populations and measures adaptive allelic assortment for thousands of genes at the same time [3].

Forest biotechnology is related to a wide range of modern procedures practicable for agricultural and forest science and only some part of these procedures are relevant to genetic engineering. Biotechnology term in silviculture comprises all perspectives of propagation of tree and cloning of plant, genotyping of DNA, manipulation and transfer of gene [4]. Conventional breeding depends more intensely on sexual crosses and monitoring trait phenotypes. In order to ensure more precise or a more sweeping results than the results that can be obtained only using phenotypic selection, biotechnology surrounds different procedures that entails one or more laboratory or greenhouse intensive steps. Such procedures may contribute to saving time, reducing expenses or achieving new goals.

Tissue culture, clonal breeding, genetic markers, gene transferring and genomic technologies are extensively used biotechnological practices. The key determinants of this quick development process are promoting powerful and applicable techniques in the biotechnology field and completing genome sequences of certain forest tree forms. Such techniques and studies have contributed exceptionally to the project of propagation of forest trees. As a consequence of the newly found gene regions, gene transfers studies, etc.; creating genetic maps, clone breeding and improving the quality of wood in forest trees were performed. Approximately 45 000 gene sources have contributed greatly to genomic investigation by sequencing poplar tree (*Populus trichocarpa*) genome. Therefore, QTL analysis, genetic modifications and EST sequencing facilitate finding new genes. Additionally, such studies assist in developing specific robust tree forms for specific environmental conditions [5].

Biotechnological procedures having or probably having specific impact on forest tree propagation and the position of the research in Turkey have been presented in the present study. Namely, for some woody taxa in Turkey, the biotechnology methods: 1- tissue culture and clonal propagation, 2- molecular marker applications, 3 - marker assisted selection and breeding, 4 - genomic and proteomic studies, 5 - genetic modification and engineering applications, were evaluated.

BIOTECHNOLOGY PRACTICES FOR FOREST TREE PROPAGATION

Biotechnology can be assessed in three main fields as follows; traditional propagation, molecular genetics, and genetic transformation. Traditional propagation has been used to develop plants for centuries. Over the last two decades, developments in molecular genetics were introduced into the scientific circles and they completed the tools that have been used by traditional breeders. There are two distinct subcategories in molecular genetics. The first one is "Non-controversial technologies", the plant genome is not altered. This category includes; molecular markers used for DNA fingerprinting and MAS (QTL mapping and association genetics); sequence analysis (genomic DNA, cDNA libraries (ESTs) that helps to discover the gene; and *in vitro* breeding such as somatic embryogenesis. The second in this category is "controversial technologies" that includes recombinant DNA and gene transfer practices. Genetic engineering makes it possible to include new genes among the existing, elite genotypes [6].

Biotechnology comprises change and development of genetic capabilities of plants through different tissue culture and genetic engineering practices. Biotechnology is a technology that is widely practiced and which has a big potential to reduce expenses of production and protection through the energy obtained from non-renewable energy resources as well as to increase agricultural productivity [7].

New biologic inventions in previous years have offered scientists many options, especially in silviculture, to acquire this knowledge.

When we focus on forest trees, biotechnology collaborates with various freestanding disciplines such as vegetative breeding, namely; cuttings, organogenesis, somatic embryogenesis, maturation and micro-propagation; molecular genetics, namely, molecular markers, cloned plant genes and quantitative trait loci and genetic transformation namely; somatic hybridization, gene transfer methods, gene transfers, prospects and limitations [8].

Vegetative Propagation (VP)

Vegetative breeding influences forest trees' developments by using available genotypes for the production of new genotypes valuable in terms of trade. Cutting and in-vitro methods are the major vegetative breeding procedures of forest trees; organogenesis and somatic embryogenesis. Based on their purpose and other similar things, the techniques used differ among different species and within species. The field tests have great importance due to probable unsteadiness in in-vitro regenerated plants [8].

Molecular Genetics (MG)

In recent decades, big developments in molecular genetics of plants enabled the use of it for tree breeding. The possible effect of it relies on the usage of molecular markers and the cloning and characterization of genes and their promoters that manage biological processes' improvement and function [8].

Genetic Transformation (GT)

The traditional gene transfer technique was used effectively in hybridization, however, as it is already known, this technique is only applicable for sexually suitable tree species and it takes many years for its implementation. Such drawbacks can be avoided using the new gene transfer method [8].

In Table 1, a framework for current possible applications of biotechnology for certain forest tree in Turkey can be found.

TABLE 1. Potential applications of biotechnology (VP - vegetative propagation, MG - molecular genetics, GT - genetic transformations) on some forest tree species in Turkey

Family	Tree species	Propagation type		
		VP	MG	GT
Pinaceae	*Pinus sylvestris* L. [9-11]		x	
	Pinus nigra L. [9-15]	x	x	
	Pinus brutia Ten. [9, 16-23]	x	x	
	Pinus pinea L. [9, 11]		x	
	Pinus halepensis Mill [17]		x	
	Pinus pinaster Ait [9].		x	
	Picea orientalis L. Link. [24-28]	x	x	
	Picea abies L. Karst [29]	x		
	Abies spp.[30-32]		x	
	Cedrus libani (A. Rich.) [33, 34].		x	

TABLE 1. Potential applications of biotechnology (VP - vegetative propagation, MG - molecular genetics, GT - genetic transformations) on some forest tree species in Turkey - continuation

Family	Tree species	Propagation type		
		VP	MG	GT
Fagaceae	*Fagus sylvatica* L.[35, 36]		x	
	Fagus orientalis Lipsky [35, 37]		x	
	Quercus petraea ((Mattuschka) Lieb.) [38-40]	x	x	
	Quercus robur L. [38-40]	x	x	
	Quercus ithaburensis Decne subsp. [38-40]	x	x	
	Quercus cerris L. [38- 40]	x	x	
	Castanea sativa Mill. [41, 42]	x		
Salicaceae	*Populus nigra* L. [43-45]		x	
	Populus spp. [45-48]		x	x
	Populus tremula L. [45, 49, 50]	x	x	
	Salix alba L. [51, 52]		x	
	Salix excelsa S.G. Gmel. [51, 52]		x	
Tiliaceae	*Tilia platyphyllos* Scop. [53]	x		
	Tilia tomentosa Moench. [38, 54]	x		
	Tilia rubra DC. [54, 55]	x		
	Tilia cordata Mill. [54]	x		
Betulaceae	*Betula pendula* Roth.[29]	x		
	Betula medvediewii Reg. [56]	x		
	Alnus glutinosa (L.) Gaertn. [57-59]	x		
	Corylus colurna L. [10]	x		
Cupressaceae	*Sequoia sempervirens* (Lamb) Endl. [60, 61]	x		
	Juniperus communis L. [62]	x		
	Juniperus foetidissima Wild. [63]	x		
	Juniperus excelsa Bıeb. [63]	x		
Oleaceae	*Fraxinus oxycarpa* Wild. [38]	x		
Fabaceae	*Robinia pesudoacacia* L. [38]	x		
Elaeagnaceae	*Eleagnus angustifolia* L. [38, 64]	x		
Aceraceae	*Acer negundo* L. [38]	x		
Platanaceae	*Platanus orientalis* L. [38]	x		
Altingiaceae	*Liquidamber orientalis* Mill. [38, 65-68]	x	x	
Myrtaceace	*Eucalyptus* spp. [69, 70]	x		
Cornaceae	*Cornus mas* L. [71]	x		
Taxaceae	*Taxus baccata* L. [72]	x		
Ulmaceae	*Ulmus minor* Mill. [10]	x		
Lauraceae	*Laurus nobilis* L. [73-75]	x		
Anacardiaceae	*Pistacia lentiscus var. chia* [76]	x		

DISCUSSION

Due to the big size and long generation periods of trees, until now, the development of forest tree has been a slow and challenging process. In conformity with tree propagation and forestry programs, it is important that forest biotechnology meets the industry requirements for forest products while enabling protection of natural forests mining [2].

It is required to implement comprehensive precipitated - propagation programs to reforest and develop current forest-tree species. It is predicted that a combination of biotechnological tools and traditional propagation methods will ensure advantage for the development of forest-tree species [77].

In addition to biotechnology's economic advantages for silviculture such as the increase in production, cost effectiveness for consumers and modified trees for allowing easy processing or other production values, the advantages of biotechnology contribute to the protection of biodiversity and to the decreasing global warming issue [78].

Using such technologies will allow forest trees to be more tolerant to abiotic and biotic stresses, gene expression for rapid growth and modification in wood structure.

Additionally, the usage of these technologies on research has some benefits such as escalated genetic gain per generation by an improved selection in traditional propagation programs, quick application of genetically developed tree species for plantations and better understanding of the genes that manage commercially crucial futures. Such trees will carry the silviculture to a different position in terms of productivity and quality. Additionally, when trees are modified to grow on arid or saline soils that were not suitable for growing before, the created forests can improve the wood production and at the same time contribute to the watershed protection and sequester carbon for the mitigation of climate change and similar things [4].

CONCLUSIONS

Phenotypic assessments require a lot of time and investments and in turn they don't ensure information on the gene's variation that manages adaptive variations. A vast number of molecular marker technologies is available, however, most of these technologies measure neutral or high conservative genetic variations of limited adaptive value. In order to assess the vast number of adaptive genes and prospective trees for *in-situ* conservations, it is required to develop quick and elucidative identifying methods. In order to study adaptation in trees, genomics offers new mediums. In order to specify DNA sequences and to genotype a vast number of individuals, forest geneticist may use technologies that are automated, highly effective, quick and productive [79].

One of the areas where biotechnological methods were used recently was the implementation of biotechnology in forest trees. Biotechnological techniques were widely used on important subjects such as obtaining resistance to diseases and herbicide of forest trees, elevating tree growth rates and developing resistance to environmental stresses such as drought, salinity, climate change and similar things. Additionally, reducing lignin and increasing cellulose to develop wood, attracts much attention. This issue is discussed through the application processes, positive and negative impacts of transgenic trees on the environment and it was also tried to be procured on the auditing legislations related to the studies [80].

In short, in addition to the advantages of using biotechnology in silviculture such as the increase in production, cost effectiveness for consumers and modified trees for allowing easy processing or some production values, the advantages of biotechnology are contributing to the protection of biodiversity and decreasing global climate changes.

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Review paper

Molecular Technologies in Serbian Lowland Forestry under Climate Changes - Possibilities and Perspectives

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Abstract

Background and Purpose: Vojvodina province, the northern part of the Republic of Serbia, is predominantly lowland agricultural region with over 75% of arable land which in previous years, has been highly impacted by drought. The annual precipitation is lower than 700 mm and it is the limit for the growth and development of natural forest vegetation. Unfortunately, the atmospheric precipitation is still a major source of water for plant biodiversity. Taking these facts into account, it is highly recommended to primarily use the xerothermic tree species, which have a well-developed root system for "classical" afforestation. Some species from *Salicaceae* and *Fagaceae* like poplars, willows, oaks and beeches are surely the best option for afforestation in temperate zones strongly influenced by drought.

Conclusions: In order to develop stress-based genomic information in *Populus* and the rest of woody plant species from Vojvodina, an integrated genetic research needs to be done. The aim of this particular paper is to analyse and summarize data regarding stress-based biotechnology perspectives in Vojvodina and to give recommendations for future forest tree breeding. Drought as a strong negative ecological factor must be carefully considered. In order to achieve sustainability, new forest management plans must consider wide approaches, from molecular to ecosystem level.

Keywords: abiotic stresses, climate changes, drought, transgenic trees, Vojvodina Province

INTRODUCTION - IMPORTANCE OF FORESTRY IN VOJVODINA

In terms of global climate change, abiotic stresses (salinity, drought, temperature etc.) are major causes of loss of natural vegetation, agricultural cultivars and crops. Vegetation mortality in association with a high frequency of drought and an increased temperature has recently been documented on all six vegetated continents. When these observations are combined with forecasts of rising global temperature, declining regional precipitation and more extreme droughts, a scenario emerges in which many vegetation communities could be pushed past their mortality thresholds in coming decades [1].

Abiotic stress causes various morphological, physiological and molecular changes that affect plant growth and productivity. Managing abiotic stress is especially important to the long-term growth of tree species. Forests are particularly sensitive to climate change, because the long life-span of trees does not allow for rapid adaptation to environmental changes [2].

This paper provides an overview of recent trends of lowland forestry research due to drought stress and warm temperatures in forests in Vojvodina, comparing with research trends in Europe. Climate as a driver of drought and directly responsible for tree mortality is also tackled, summarizing scientific understanding for assessing possible relationships between changing climate and forest conditions in lowland forestry of Serbia and worldwide. Note that while climatic events can damage forests in many ways, our emphasis is on environmental-induced physiological stress driven by drought (decreased water availability in soil) and warm temperatures. The ecological effects of increased mortality in forests and the associated consequences for human society remain largely un-assessed.

We conducted a systematic search for published literature of forest stress-based biotechnology, using standard search engines and databases, such as ISI Web of Science, PubMed and Google Scholar. From the extensive set of documents, we used two specific criteria to determine whether the reference was appropriate for setting discussion and recommendations. Criteria for reference inclusion were:

1. usage of modern molecular methods and applicative results in forest tree species research, and
2. documentation of a strong correspondence between increases in mortality/drying/senescence of forest tree species and increased water stress or high temperatures.

ENVIRONMENTAL CHALLENGES AFFECTING FORESTRY IN SERBIA

Europe is getting warmer above the rate of global average. Until 2007, the average annual temperature of the European land area had been 1.2 °C higher than in the preindustrial period, whereas in the combined land and sea area the temperature increased by 1 °C. The projections of annual temperatures, established on the basis of climatic models designed for various climate change scenarios, estimate that the temperature will increase from 1 to 5.5 °C by the end of this century. During winters, the highest warming is expected in the east and north, whereas in summers it is predicted in the south-west and Mediterranean part of Europe [2].

The Republic of Serbia is a landlocked country located in the Balkans and in the Pannonian plain. Climate of Serbia is of temperate continental type. Proximity of the mountain ranges of Alps, Carpathians, Rhodopes, as well as Adriatic Sea and Pannonian plain affect the climate. Average annual air temperature for the area with the altitude of up to 300 m amounts to 11 °C. The areas with the altitudes of 300 to 500 m have average annual temperature of around 10.5 °C, and over 1 000 m of altitude around 6 °C. Precipitation, in lower regions, ranges in the interval from 540 to 820 mm, areas on altitude over 1 000 m receive in average 700 to 1 000 mm, and peaks of some mountains in south-western Serbia up to 1 500 mm. The major part of Serbia has continental precipitation regimen, with a peak in the early summer period, except for the southwest, which receives its highest precipitation in autumn [3]. Climate change scenarios predict increasing mean air temperature between 2.4 and 3.8 °C until 2100. Concerning precipitations, climate projections indicate an increase of precipitation for Serbia of 20 to 30 mm per year for 2001-2030 and a decrease of precipitation of up to 30 mm per year for 2071-2100, compared with 1961-1990 [3].

The domination of a negative trend of the annual air temperature in Serbia ceased in 1982. Since 1983, and particularly since 1987, positive trends have been detected, first in shorter, and later in increasingly longer intervals. The intensity of decline of annual precipitation is 5 % of the regular amount in 50 years. In Serbia, the beginning of the period of the air temperature increase is accompanied by a period of reduced annual precipitation sum [4].

Serbia contains 2.3 million ha of forests which cover approximately 29.1 % of the total area. The dominant growing stock in Serbia are coppice forests with 64.7 % of the total forest area, natural high stands cover 27.5 %, and artificially established stands (with plantations) cover 7.8 %. According to the National Forest Inventory, 49 tree species are identified in Serbia. Broadleaf species (40) dominate over coniferous species (9). The dominant species is beech and its percentage in total volume is 40.5 %, and in volume increment 30.6 %. The most represented coniferous species is spruce. Its percentage accounts for 5.2 % of volume and 6.7 % of volume increment [5].

Another distribution for Serbia's 2.3 million ha of forests means that 77 % is in Central Serbia, 18.6 % in Kosovo and Metohia and 4.4 % in Vojvodina. These forests comprise willow (*Salix fragilis* L., and *Salix alba* L.), poplar (*Populus* spp.), pedunculate oak (*Quercus robur* L.), sessile oak (*Quercus petraea* (Matt.) Liebl.), Austrian oak (*Quercus cerris* L.), Hungarian oak (*Quercus frainetto* Ten.), narrow-leafed ash (*Fraxinus angustifolia* Vahl), alder (*Alnus* spp.), European hornbeam (*Carpinus betulus* L.), beech (*Fagus moesiaca* (Domin, Maly) Czeczott), pine (*Pinus nigra* Arn.), and mixed beech-fir (*Abieti-Fagenion moesiacae*, Jov. 1976), beech-fir-spruce (*Abieti-Piceenion* Br. - Bl. 1939) and high-mountain spruce forests (*Vaccinio-Piceion* Br. - Bl. 1939). Beech is the most frequent species (37 % of forest area), followed by oak (35 %), other deciduous species (20 %) and conifers (8 %, of which pines are the most prevalent species) [5].

The increase of forest cover percentage compared to the reference year 1979 is 5.2 %, which by all means had a positive effect on the state and quality of the environment in general. Negative human impact on forest is visible, especially, through the percentage of coppice forest. Signs of global change impact are pronounced in the cases of Penduncuiate and Sessile oak, due to high percentage of tree declining in almost the entire area [3].

TABLE 1. Climate conditions in Serbia for several extremely dry years [6]

Year	Temperature April - October (°C)	Precipitation April – October (mm)	Temperature Yearly (°C)	Precipitation Yearly (mm)
2012	18.4	367.0	/	/
2011	17.1	300.5	10.8	469.8
2007	17.0	454.6	11.6	775.3
2003	17.1	421.5	10.8	606.3
2000	17.7	262.0	11.9	436.8
1961-1990	15.6	437.1	10.0	690.7

FORESTRY OF VOJVODINA UNDER DROUGHT STRESS

In the Vojvodina region which is predominantly agricultural region with over 75 % of arable land, drought can have considerably negative impact on agricultural production. As a consequence of the insufficiently developed irrigation systems, the atmospheric precipitations are still the major factor in providing water to the soil and crops. In a greater or lesser intensity, drought occurs almost every year and it is the limiting factor of high yields [4, 7].

It was estimated that 13.2 % of the territory of Vojvodina, located mostly in the central and southeast parts, is highly vulnerable to drought. The main reasons for this are the occurrence and long duration of severe droughts. Recurrence times of the severe droughts vary from 12.2 to 15.4 months. Regions with the most frequent severe drought occurrences are the eastern parts of Vojvodina (municipalities: Kikinda, Zrenjanin, Vršac), northern areas around Palić and southwest area around Sremska Mitrovica. Residence times of severe drought are ranging from 1.7 to 2.2 months. Longest durations of severe droughts are in central areas around cities of Novi Sad and Zrenjanin. The most vulnerable regions to drought are central, southeast and southwest parts of Vojvodina [7, 8].

The analysis of climate characteristics of Vojvodina shows that there are conditions for the development of forest vegetation. Taking into account the annual precipitation of 550 to 670 mm, which is somewhat lower than 700 mm, which is the limit for the occurrence of natural forest vegetation, it is recommended to use primarily the xerothermic tree species, which have a well-developed tap root, except in the areas along the natural and artificial watercourses, where the hygrophilous tree species would be the most favourable species for the establishment of plantations and forests [9, 10].

The selection of species for forests establishment depends on the above mentioned climate, hydrological and soil conditions and potential natural vegetation in Vojvodina [9, 10]. The most representative species for the hydromorphic soil type is common oak and highly productive varieties of poplar. The halomorphic soil type, due to unfavourable site conditions, supports very few woody species, of which the author recommends common oak, based on the potential vegetation. Reports from Jovanović [11] on chernozem and meadow black soil, as the most represented soil types in Vojvodina, the optimal species is *Q. robur*, and the recommended species are the clones of *Populus albae* and the xerothermic fruit-tree species. A wider spectre of species in the genera *Populus, Acer, Morus, Tilia, Coryllus, Betula, Hypocastanum, Fraxinus* and *Juglans* is recommended on meadow black soil thanks to the moister soils. For hydromorphic black soils, the above authors recommend hygrophilic species, mainly in the genera *Populus* and *Salix,* while on brown forest soil, the advantage is given to the species *Quercus petrea, Quercus cerris* and *Tilia argentea* for the tree layer and *Acer campestre, Carpinus betulus, Corylus avellana, Sambucus nigra,* and *Cornus sanquinea* for the shrub layer. On other soils, such as arenosol which prevails in the sands of Deliblatska Peščara and Subotička Peščara, the protection and economic forest plantations should mainly consist of xerothermic tree species, of which the best are *Robinia pseudoaccacia, Pinus nigra* and *Populus alba* [10].

BREEDING POTENTIAL OF POPLARS DUE TO DROUGHT STRESS

Trees have evolved various mechanisms which help them to cope with limited water supply. Responses to drought include:

1. reducing the water deficit by developing root systems able to take up water deeper in the soil;
2. minimizing water losses through stomatal closure and producing small leaves;
3. accumulating osmoprotective substances [12, 13].

Poplars are truly multipurpose tree species. They provide a nearly endless list of wood and fiber products, non-wood products, and are grown increasingly in bioenergy plantations for firewood. They have a positive role in the rehabilitation of degraded lands, forest landscape restoration and climate change mitigation [14].

Poplars are potentially the best option to increase biomass production in temperate zones, such as Vojvodina in the Republic of Serbia, but there is a need to identify clear objectives on how to use this material in the forestry wood chain. In the past, growth, disease resistance and adaptation to climatic conditions have been the major drivers for selection and breeding of poplars. Improved material characterization as well as high throughput methodology to support selection and breeding will allow future strategic decisions for afforestation of fast growing species in the Vojvodina province [15]. *Populus nigra*, that establishes naturally within or along the active channel and that is strictly dependent of the morphodynamics of the Danube River, will be exposed to three new threats:

(i) enhancement of the frequency and severity of drought with summer decrease of the water level, which is extremely noticeable in recent years,
(ii) extreme heat waves, especially in summer (temperature is a key factor for survival and development of seedlings) and
(iii) more intense flooding [16].

Populus nigra L. is important species of the European alluvial forests that are protected under Habitats directive 92/43/EEC in entire Europe. This species is often regarded as a good indicator of geomorphological and biological quality of this ecosystem and is an active support of riparian biodiversity. This species is also threatened by anthropogenic disturbances and gene introgression, justifying French and European (EUFORGEN) programme on *in situ* and *ex situ* conservation of its genetic resources [16]. This species is common on the territory of Vojvodina province, having the same importance for domestic agriculture production, forest and environmental management.

Young poplar trees (*Populus deltoides*, *Dvina* and *Populus* x *canadensis*, I-214) were grown under reduced soil water availability. The stress intensity was estimated by measuring soil water content, predawn leaf water potential, leaf relative water content, leaf growth, leaf conductance and maximum photosynthesis, maximum daily shrinkage of the stem. Radial growth was recorded by point dendrometers and the effect of water deficit on differentiating xylem and wood was investigated by high resolution stem growth analysis and anatomical investigation. After 16 days of withholding irrigation, significant differences were recorded between treatments and genotypes in term of leaf RWC (Relative Water Content), total leaves number, total leaf area increase and stem length increase. On the basis of results, the hybrid I-214 has showed a higher susceptibility to water stress than Dvina [17].

Poplar transformed with a gene for pine cytosolic glutamine-synthesise isozyme (GS1; look at NCBI: P52783) was shown to be more tolerant to drought stress than wild-type trees. At all levels of water availability, the transgenic trees had higher photosynthetic assimilation rates and stomatal conductance than the corresponding controls. All GS1-containing lines also showed an irreversible decline in photosystem II (PSII) antennae transfer efficiency after drought and during recovery, but the increased photo-assimilation capacity of the transgenic poplar allowed more resources to be allocated to photo-protective mechanisms [18].

PLANT LIFE SCIENCE RESEARCH AS A BASIS FOR FOREST STRESS-BASED BIOTECHNOLOGY

Responses of plants to stress may be revealed at a whole plant level as an integrated tissue system, while some of the responses occur at the cellular level. For example, closing of stomata results from subtle biochemical and molecular changes in the guard cell itself but this event is ultimately induced by the signalling transduced by other cells like root cells. Sometimes a comparison between cellular response and whole plant response may reveal the level of organization where the adaptation operates. In the contrary, seed germination and seedling growth declines with increasing water stress, while proteolysis in cotyledons during storage mobilization is retarded by water stress. Metabolic changes that occur in plants, particularly mesophytes, in response to water stress have been major targets of research interest while searching for molecular mechanism of stress tolerance. Such studies have been strengthened further with the aid of molecular tools like microarray and differential expression of genes [19].

On molecular level, drought stress also induces reactive oxygen species (hereinafter: ROS) generation as a primary response of plant. Gross level of ROS could facilitate the stress induced damages to most of the cellular components, unless compromised by plant antioxidant system. However, depending on spatial and temporal ROS generation and scavenging, responses can be characterized as toward conferring protection by arousing the protection system or as directly leading to injuries or death. Gradual imposition of drought stress, which is more common in nature, probably triggers ROS generation in the apoplast by plasma membrane-localized NADPH oxidase, where Ca^{2+} plays a role as an upstream as well as downstream messenger forming a positive feedback loop [19]. Effects of drought-induced generation of ROS in poplar and concomitant induction of ROS detoxifying enzymes in leaves have been investigated [20- 22]. Morabito and Guerrier [23] found that roots are the most sensitive organ of poplar to oxidative stress after 12 h drought, but nothing is known about reactions in roots after longer drought events [13].

There is an enormous potential for speeding up tree breeding cycles by the use of genetic modification. Systems have been developed for gene transfer, selection of novel gene-containing shoots and stimulating regeneration for both broadleaved and coniferous trees [24, 25].

Comprehensive analysis of the relationship between the transcriptome of homologous *Arabidopsis* and *Populus* genes facilitates to identify the correlation of the response systems and the roles of the homologues among the species [13].

The plant-specific GRAS/SCL transcription factors play diverse roles in plant development and stress responses. Poplar SCL (SCARECROW-LIKE) gene, PeSCL7, was functionally characterized in *Arabidopsis thaliana*, especially with regard to its role in abiotic stress resistance. Expression analysis in poplar revealed that PeSCL7 was induced by drought and high salt stresses, but was repressed by gibberellic acid (GA) treatment in leaves. Transgenic *Arabidopsis* plants over-expressing PeSCL7 showed enhanced tolerance to drought and salt treatments. These results suggest that PeSCL7 encodes a member of the stress-responsive GRAS/SCL transcription factors that is potentially useful for engineering of drought- and salt-tolerant trees [26].

The expressed small RNAs from leaves and vegetative buds of Populus have been isolated using high throughput pyrosequencing [27]. By the analysis, almost 80 000 small RNAs were identified with 123 novel small RNAs belonging to previously identified miRNA families from other plant species and 48 novel miRNA families that could be Populus-specific [27]. The putative target genes of Populus-specific small RNA were involved in development and resistance to stress. Lu et al. [28, 29] have identified abiotic stress-responsive miRNAs from *P. trichocarpa*, whose expression was altered in response to cold, heat, salt, dehydration and mechanical stresses. Some of these stress-responsive miRNA families are conserved among various plant species, such as *Arabidopsis*, rice and poplars. The other *Populus* or tree-specific miRNAs were also identified and predicted to function in the adaptation to long-term growth and survival from stress conditions in woody plants [30].

FOREST GENETIC AND BREEDING RESEARCH IN SERBIA

Despite the area of forests of Vojvodina province are just 4.4 % of total forest area of Serbia, it still presents huge agricultural potential for Serbia, especially its continental-temperate zone with a unique opportunity for some species to grow (*Salicaceae* species, *Quercus* species etc.).

First studies on examination of molecular markers and adaptive traits of forest tree species in Serbia, in order to accelerate the selection process of poplar and willow, as well as to define critical parameters in the growth process, were designed at the Institute of Lowland Forestry and Environment, University of Novi Sad. Forest breeding programs at the Institute of Lowland Forestry and Environment, University of Novi Sad, have until now been based only on conventional clonal identification system, meaning the combinational research of morphological and phenological traits characterization [31]. Revealing and characterizing genetic background of poplar clones, using molecular markers systems like AFLPs and SSRs, is one of the major contributors to novel taxonomy and breeding perspectives for woody plant species in Vojvodina province. While AFLP markers have been utilized in evaluating hybrids and in parentage assessment in many other species [32-35], the highly polymorphic, consistent and co-dominant markers, such as SSRs, are excellent markers for clone and cultivar identification in poplars so far [36, 37].

To begin dissecting genome information in *Populus,* integrated genetic and gene expression data needs to be done, with phenotypic traits measured in populations of *P. deltoides* and *P. nigra*, which are common for Vojvodina province and some parts of Western Balkans. In recent years, studies of forest genetic resources in Serbia were conducted on numerous tree species. Researches of molecular markers were focused mainly on investigation of genetic diversity in natural populations of European beech [38], Austrian pine and Scots pine [39-42], Serbian spruce [43], *Sorbus* spp. [44] and Sessile oak [45]. Numerous research on investigation of variability of adaptive traits were conducted in the natural populations of Wild cherry [46, 47].

Research in the European beech provenance trials were aimed on analysis of variability of leaf anatomical, physiological, biochemical and morphological traits. Some results have been published to date [48, 49]. Research in the beech provenance trials were also aimed on investigation of environmental conditions in the provenance trials [48].

Various reports of forest management from Srem region of Vojvodina province in the Republic of Serbia showed that some parts of the population of Pendunculate oak are endangered, under the influence of environmental aridity. To determine the genetic basis of senescence caused by drought, the system of molecular analysis needs to be performed in order to make recommendations for developing new strategies to preserve the oak gene pool. Specifically, the aim of these pioneer experiments would be to target candidate genes responsible for senescing and drought stress tolerance of Pendunculate oak from Srem [50-52].

Nonić et al. [53] reported the results of monitoring and collecting information on public opinion, analysis of laws and legislation regarding genetically modified plants and possibilities of scientific research on transgenic tree species in several European countries. The proposed survey included results from 8 countries (Italy, Slovenia, Romania, Bulgaria, Serbia, Croatia, Montenegro and Bosnia and Herzegovina), aiming to contribute to the common future of EU policy in this field. First results indicate that public opinion is divided and provide a good basis for understanding the issue of implementation of stress-based transgenic technology, in order to define clear scientific attitude as a recommendation, both in EU member states and Western Balkans countries.

Poplars Oxidative Stress and Functional Genomic Research in Vojvodina

The effect of different concentrations of three heavy metals ions, Cu^{2+}, Ni^{3+} and Cd^{2+}, on oxidative stress in three poplar clones (PE 19/66, M1 and B229), from two different species, *Populus deltoides* (*Marshall*) and *Populus euramericana (Dode-Guinier*) were analysed. Possible antioxidant capacity of these clones in response to different concentrations of heavy metals ions in substrate was measured in order to find which clone is most appropriate for phytoremediation processes. Results showed variable responses within poplar leaves and roots in response to oxidative stress induced by heavy metals and the most promising clone for phytoremediation of contaminated soils is B229 clone, while M1 and PE 19/66 showed variable antioxidant response [54]. Another study on oxidative stress profiles of three mentioned poplar clones shoots [55], while being treated by the same heavy metal stress treatment, showed that the most acceptable phytoremediation response to the pollution in soil showed clone M1. PE 19/66 clone in both studies resulted as not being suitable for possible phytoremediation application of contaminated soils by heavy metals.

Also, another study included also poplar clones PE 19/66, B229 and clone Panonia (*Populus x euramericana*) regarding induced oxidative stress in leaves after treatments with heavy metals, herbicides, diesel fuel, as well as a mixture of heavy metals and diesel fuel in the experimental field. Biochemical responses to the induced stress were estimated by using *in vitro* tests for determination of radical scavenger capacity and total antioxidant activity, DPPH and FRAP test. Obtained results showed great correlation between antioxidant and scavenger activities of poplar clones extracts which shown increased both activities under the applied treatments. Treatment of combined stress inducers (heavy metals and diesel fuel) showed synergistic effect upon all clones for both determined tests comparing to separately applied stressors. Clone B-229 showed indication of higher tolerance to applied stressors compared to other clones [56]. These oxidative stress screening tests showed the possibility of poplars genotypes for afforestation of contaminated areas and soils in Serbia. Climate changes are causing various abiotic and biotic stresses in environment, also changing the life cycle of various contaminants.

The study of Štajner et al. [57] was designed to examine and compare antioxidant and free-radical scavenging activities of leaves of six different melliferous plant species (*Populus alba, Robinia pseudoacacia, Sophora japonica*, *Euodia hupehensis*, *Tilia* sp., *Fraxinus* sp.) from Serbia in order to evaluate their drought-induced oxidative stress tolerance. An experiment was conducted during June, July and August. In this study, they reported the results concerning proline accumulation, soluble protein content, quantities of malonyldialdehyde, total anti-oxidant capacity determined by FRAP method and scavenger activity determined by DPPH method. According to the results, all melliferous plant species were subjected to drought, leading to oxidative stress during July when soil humidity decreased. During July, proline content and MDA quantity increased and soluble proteins decreased in all investigated species. High and permanent antioxidant activity during the whole investigated period was observed in *P. alba*, but insufficient to protect its leaves from oxidative injury during the period of drought in July.

On ILFE, functional genomic approach was applied, to have insight into the oxidative stress responses of Serbian poplar clones with different genetic background. The objective was to identify genes with altered transcript accumulation during salt stress and to characterize their expression [58]. Genetic transformation gives the possibility to achieve various applied goals in plant stress biotechnology. Recent publications related to salt and drought-inducible poplar GRAS protein SCL7 showed that this gene is potentially useful for engineering drought and salt tolerance in trees, thus directing our attention to a GRAS/SCL transcription factor (TF) as a candidate gene of interest. After DNA sequence polymorphisms were described within Serbian species by their SNPs patterns and the number of polymorphic sites was revealed, one step forward was made toward genetic transformation of the same poplar clones for GRAS/SCL. For this study,two different agronomically important clones, (*P. deltoides* and *P. x euramericana*) were sampled. PCR (either with genomic or cDNA) with primers for Gateway clonning from PtGRAS/SCL7 TF exon part was successfully done and fragments of *P. deltoides* and *P. x euramericana* were obtained and ligated using BP clonase into pDONR vector. This is the preliminary result toward improvement in tolerance to abiotic stresses in poplar species [59].

While many potential benefits can be envisioned and have been described for genetic engineering in forest trees, there are also risks and they must be thoroughly evaluated. These risks can be placed into two general categories:

1. risks to plantations of transgenic trees;
2. risks associated with the migration of transgenes (i.e., gene flow) into neighbouring environments.

The second type of risk entails transgenes migrating and either reducing or increasing the fitness of trees in the recipient populations. Transgenes are packed in genotypes that combine to produce phenotypes that the environment acts on. Furthermore, the potential for these genes to migrate into the same or related species by gene flow (i.e., pollen or seed movement) must also be evaluated [60, 61].

An alternative to minimize gene flow is the use of sterile genetically modified trees. Many efforts have been put in facilitating research and understanding genes involved in the flowering process of trees [62, 63]. The use of transgenic trees in the forestry sector has very different objectives than those used for crops. They are mainly based in improving wood quality, so as to diminish the pressure on the land. The genes involved in these processes are quite specific and are mainly present in trees; therefore its possible escape to the environment does not have a major risk [64].

FUTURE OF FORESTRY IN VOJVODINA

An in-depth understanding of the physiological stress responses and the molecular events in woody plants, which are some of the major components of the global ecosystem and biomass resources, will always be required. The findings reviewed here would contribute to understanding of the possible need of the introduction of stress-based biotechnologies to control stress responses of forest tree species in Vojvodina province, Republic of Serbia. Developing the stress-tolerant woody plants models and practices would require further understanding of various aspects of the molecular responses under the stress conditions. The main objectives for future sustainable forest policy of Vojvodina Province are to increase the area and vitality of the forests (through afforestation, reclamation and cultivation), to create the technical base for all types of forestry work, to maximize financial returns [65] and to start develop transgenic technologies for research purposes with the goal to cope with extreme drought waves on this territory.

Whichever aspects of transgenic technologies advance most rapidly in the future, environmental risk assessment should always be carried out, on a case-by-case basis, until a sufficient body of knowledge on the anticipated benefits and the possible risks of this exciting technology are established [66]. Possible introducing new sterile transgenic plant tree species into arid area may be seen as an important way forward. This genetic strategy of developing transgenic species of forest plant species will enable sustainable forest ecosystems that will allow continued production of necessary oxygen and accelerate the elimination of harmful carbon dioxide emissions that cause the greenhouse effect and thus affect global warming and imbalance the level of the entire plant layer on the planet Earth. Drought is still causing negative environmental and economic consequences in all of Serbia, especially in the Vojvodina province and decision makers need to improve planning of mitigation practices and future sustainable forestry management on molecular level, especially for one of the most important woody plant species for that particular Serbian region: poplar, willow, oak and beech species [48].

Construction of gene banks responsible for different aspects of resistance of all tree species to a variety of abiotic stresses should become an imperative for sustainable agroforestry that can respond to various environmental challenges which is facing today and will face in the future. The greatest challenge facing humanity in the field of controlled and responsible production of transgenic trees is the ethical approach and a clear intention to help and develop the planet in the upcoming climatic challenges.

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Future of the Main Important Forest Tree Species in Serbia from the Climate Change Perspective

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Abstract

Background and Purpose: Climate change is possibly the biggest 21st century challenge for the European forestry. Serbia is also under pressure, since the regions of South Europe and Mediterranean are expected to suffer the most. Main purpose of this study was to predict how distribution of several tree species in Serbia may change in the future.

Materials and Methods: Our study integrates climate change scenarios for the region of Serbia together with the current distribution of forest tree species. Evaluation was performed using forest aridity index which takes into account mean temperatures and sums of precipitation of the critical months during the growing season. Distribution data of the nine most abundant tree species in Serbia (European beech, Turkey oak, Sessile oak, Hungarian oak, Pedunculate oak, Norway spruce, Silver fir, Black and Scots pine) were taken from the National Forest Inventory.

Results: Significant change of bioclimatic niches is expected for the majority of the studied tree species. The most endangered will be Pedunculate oak due to the extreme change of its habitats, while drought prone species (like pines and Hungarian oak) will be less endangered. Sessile oak, Turkey oak, Silver fir, Norway spruce and European beech will be out of their 20th century bioclimatic niches before the end of 21st century according to A2 scenario.

Conclusion: Our results suggest that some of the most important tree species in Serbia (Sessile oak, Turkey oak, Silver fir, Norway spruce and European beech) will be endangered by the end of 21st century. General adaption options and specific measurements for forestry sector have to be made for the region of southeast Europe due to the expected extreme change in climate.

Keywords: climate change impact, adaptation, forest management, bioclimatic niche

INTRODUCTION

Change of climate conditions which are observed in past thirty years as well as climate change predictions for future, present a great challenge for forestry [1].

Globally, world's forests influence climate through different ways and their mu-tual interaction is complex [2], although, interaction between forests and climate on the regional and local level is complex, climate as a global phenomenon has a stronger impact on regional forests then *vice versa*. Negative impacts of climate change on forests are already observed globally and in Europe [3-6]. Also, in Serbia change of realized bioclimatic niches were observed [7-10].

Climate change projections from the year 2000 predict a change of temperature in the range from 2 up to about 6 degrees according to different CO_2 scenarios by the end of 21st century [11, 12].

By the year 2009 most of the CO_2 emission scenarios from the 2000 were exceeded [13], which pointed to quite an uncertain future for the upcoming generations, as well as for forestry. According to this perspective current forest management has to be re-considered in the following years with the aim of adapting to changed climate conditions.

Main purpose of this study was to predict how the distribution of nine tree species in Serbia (European beech, Turkey oak, Sessile oak, Hungarian oak, Pedunculate oak, Norway spruce, Silver fir, Black and Scots pine) may change in the future.

MATERIALS AND METHODS

Führer et al. [14] proposed a new forest aridity index (FAI) originally designed for yield assessment that is based on the relationships between precipitations and mean monthly temperatures and proved that growth of various species is closely correlated with that index according to a formula:

$$FAI = \frac{100 * \frac{TVII+TVIII}{2}}{PV+PVI+2*PVII+PVIII}$$

where *TVII, TVIII* represent mean temperature in July and August and *PV, PVI, PVII* and *PVIII* the sum of precipitation for May, June, July and August, respectively.

We used that relationship, the climate data and the National Forest Inventory [15] to predict future distribution of forests in Serbia. Climate data for the reference period 1961-1990 was obtained from the Republic Hydro meteorological Service of Serbia (resolution 90×90 m) while the unbiased climate data for A2 scenario was provided from the regional climate model [16] for the periods 2011-2040, 2041-2070 and 2071-2100 (resolution 25×25 km, [17]). According to A2 scenario the temperature in Serbia will rise for about 3.8 degrees by the end of century.

Maps of FAI were created for the periods 1961-1990 (observed climate), 2011-2040 A2, 2041-2070 A2 and 2071-2100 A2 with ArcGIS software.

Graphs of FAI index values for current forest tree species were obtained with SAGA GIS and Statistica 8.0 software.

RESULTS

Calculations of FAI showed that change in bioclimatic conditions in Serbia may be considerably large (Figure 1), which could, in a long run, have an impact on the current forestry and agriculture practices.

Results suggest that at the end of the century, bioclimatic conditions will be much more arid on the whole territory of Serbia. FAI values below 10 which were present across Serbia in the reference period 1961-1990, will rise to above 15 in some parts of country by the end of the century. The bioclimatic conditions in most of the Vojvodina region in the period 2011-2040 will be equivalent to the conditions in most arid parts in North Banat.

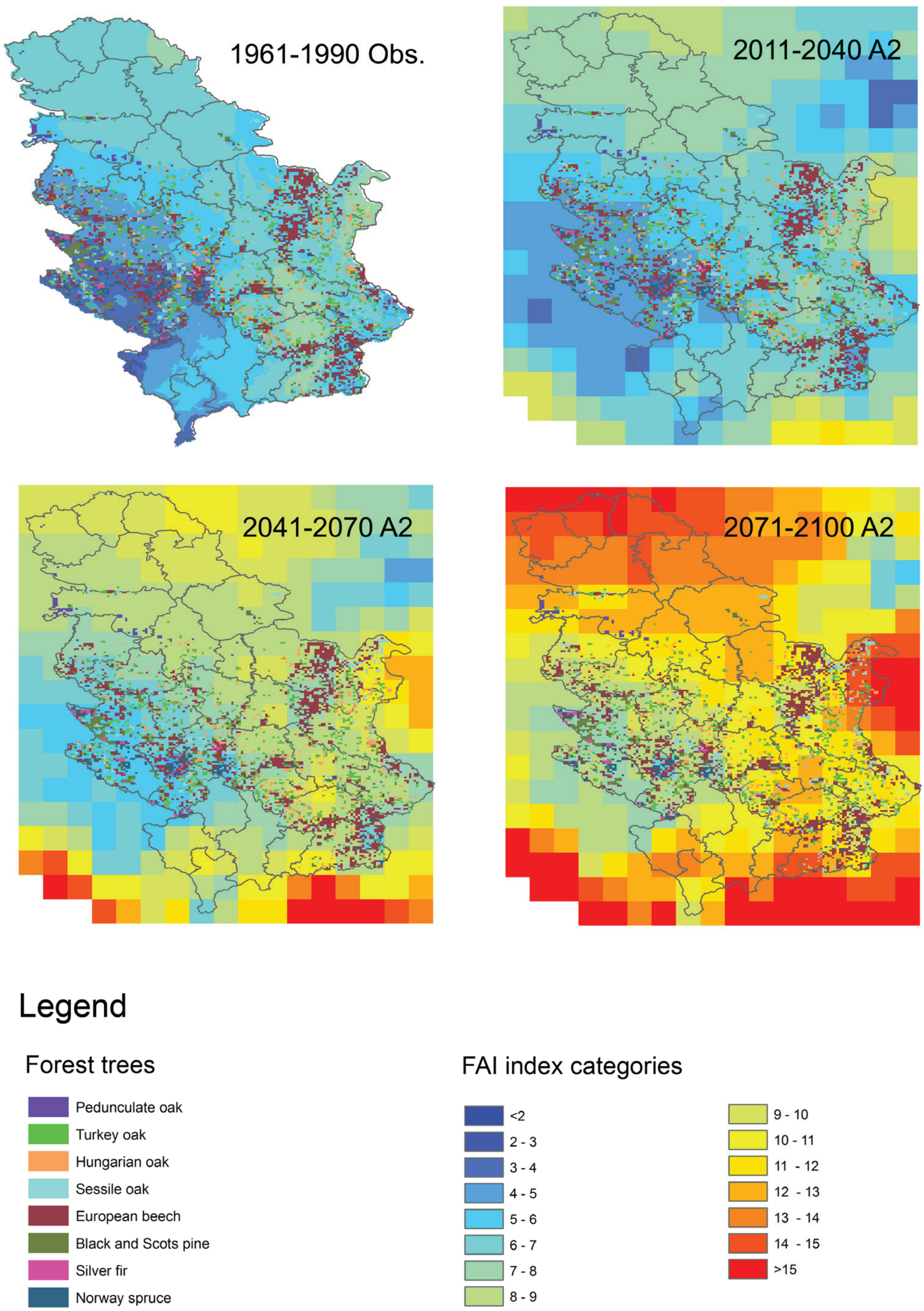

FIGURE 1. Maps of Forest Aridity Index (FAI) for four climatic periods (1961-1990 – observed climate, 2011-2040, 2041-2070 and 2071-2100 - predicted climate according to A2 scenario) and indicated distribution of eight forest tree species in Serbia

Distribution changes have been evaluated for nine species, where Black and Scots pine are allocated in one category as it is recorded in the National Forest Inventory (Table 1).

Distributions of oaks (Table 1, Figures 2, 3, 4 and 5) in the reference period 1961-1990 were within 5.5 and 7.7 of FAI values. In the period 2011-2040 the Hungarian oak will change distribution slightly, while the Pedunculate oak will show a more pronounced change. For future periods, the change of distribution for all oak species will become more drastic.

Beech forests were also taken into consideration (Table 1, Figure 6). According to A2 scenario, the near future will not be too different from the 1961-1990 period, but changes may occur when the climate becomes more arid.

Conifer species (Table 1, Figures 7, 8 and 9) also showed variations in their future distribution as did the broadleaved species. Black pine and Scots pine, as well as other species that survive in dry habitats, will suffer the lowest change in comparison to current habitat conditions. The fact that their distribution in the 20th century partially overlaps with the end of their 21st century distribution seem encouraging for forest managers.

Change of Silver fir distribution will be greater than the change of Norway spruce (Table 1, Figures 8 and 9). The main reason for that is that the niche of spruce is wider in the referent period compared to the niche of fir, which is a result of its better adaptability to drought conditions.

TABLE 1. Mean, maximum and minimum FAI values of current forest distribution in four climatic periods for nine forest tree species

		1961-1990 Obs.	2011-2040 A2	2041-2070 A2	2071-2100 A2
Pedunculate oak	Mean	5.8	6.7	8.2	11.9
	Minimum	4.8	5.1	7.1	9.3
	Maximum	6.9	8.0	10.0	15.7
Turkey oak	Mean	5.7	6.2	8.0	11.0
	Minimum	4.3	5.3	6.8	9.4
	Maximum	7.0	7.6	10.3	13.8
Hungarian oak	Mean	5.9	6.0	8.2	10.9
	Minimum	3.6	4.3	5.6	7.7
	Maximum	7.7	8.6	12.0	16.0
Sessile oak	Mean	5.5	5.9	7.8	10.5
	Minimum	3.3	4.3	5.6	7.7
	Maximum	7.6	8.3	10.8	15.1
European beech	Mean	5.6	6.0	8.0	10.7
	Minimum	3.5	4.3	5.6	7.8
	Maximum	7.6	8.0	10.7	14.7
Black and Scots pine	Mean	4.7	5.2	6.9	9.5
	Minimum	3.1	4.2	5.5	7.6
	Maximum	7.7	8.1	11.5	15.8
Silver fir	Mean	3.6	4.5	6.0	8.3
	Minimum	2.9	4.2	5.5	7.6
	Maximum	6.1	6.4	8.9	11.9
Norway spruce	Mean	4.0	4.8	6.4	8.8
	Minimum	2.8	4.1	5.5	7.6
	Maximum	7.4	6.9	9.4	12.7

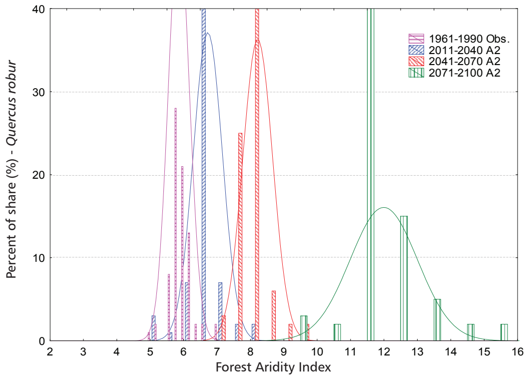

FIGURE 2. Distribution of Pedunculate oak across FAI categories in four climate periods

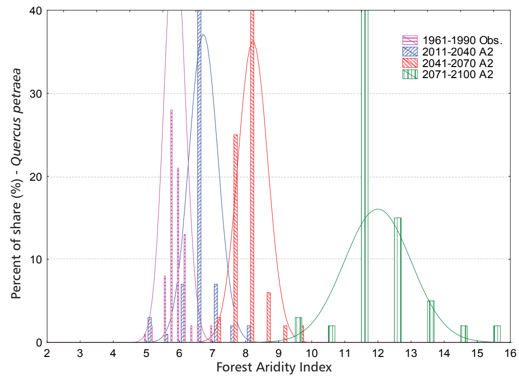

FIGURE 3. Distribution of Sessile oak across FAI categories in four climate periods

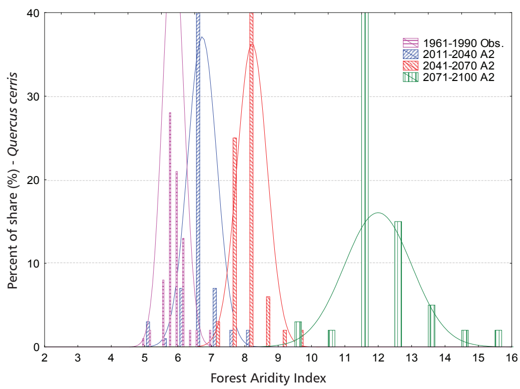

FIGURE 4. Distribution of Turkey oak across FAI categories in four climate periods

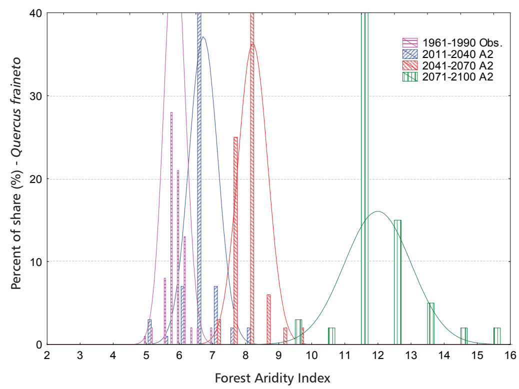

FIGURE 5. Distribution of Hungarian oak across FAI categories in four climate periods

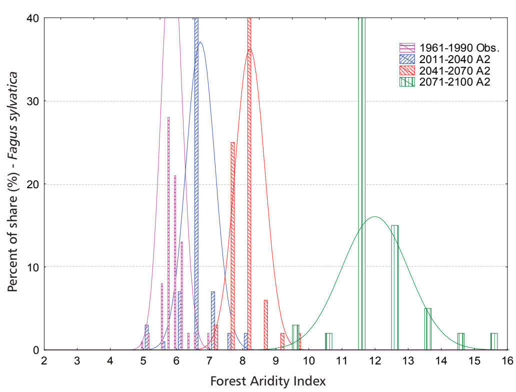

FIGURE 6. Distribution of European beech across FAI categories in four climate periods

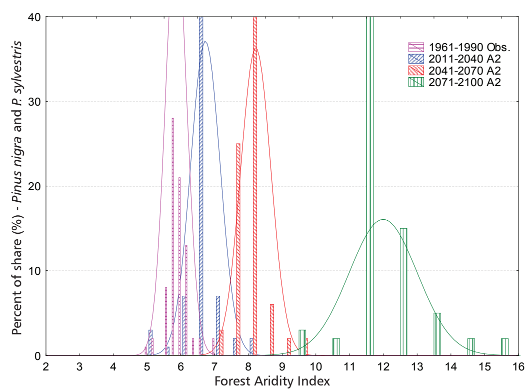

FIGURE 7. Distribution of Black and Scots pines across FAI categories in four climate periods

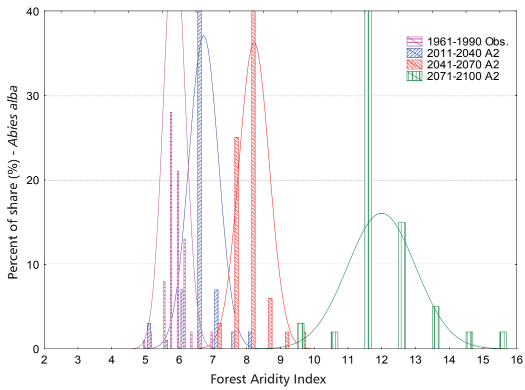

FIGURE 8. Distribution of Silver fir across FAI categories in four climate periods

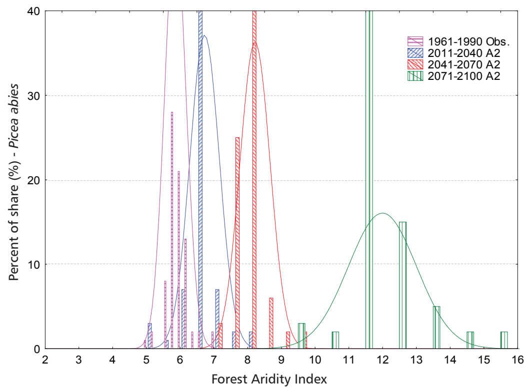

FIGURE 9. Distribution of Norway spruce across FAI categories in four climate periods

DISCUSSION

Similar studies have been carried out in the region. Czúcz et al. [18] found that, according to various climate change scenarios, a decrease of European beech forests in Hungary by the year 2050 can be very high due to the reduction of their bioclimatic niches which are predicted to be between 56 and 99 %. Móricz et al. [18] found that by the middle of century only about 35 % of the current beech and 75 % of the sessile oak stands will stay above their current potential distribution limit in the same country.

Stojanović et al. [9] found that by the end of century about 90 % of the European beech in Serbia will be outside of their realized bioclimatic niche and that about half of the stands will be exposed to mass mortality.

Advantage of this approach in comparison to using EQ is that FAI focuses on the climate condition during the growing season.

The change in the seasonal distribution of precipitation is expected, so it can be concluded that FAI can be more sensitive than EQ for this kind of analysis.

It is expected that Pedunculate oak suffers the most from the climate change since it is dependent of groundwater which is experiencing a general decline in Serbia in past years. Additionally, it is situated in lowlands which are the most prone to aridification and temperature increase according to climate change projections [20].

According to this methodological approach, besides Pedunculate oak, Sessile oak, Turkey oak, Silver fir, Norway spruce and European beech will also totally exit their 20th century distribution before the end of the 21st century.

It can be assumed that species, such as Black and Scots pine and Hungarian oak will be the least affected by the climate change. Although some of their current stands will be found in extremely harsh climate conditions in future. It is important to stress that Black pine is more resistant to drought conditions, but these two pine species were evaluated together since they are joined in the National Forest Inventory database.

This study didn't consider the possibility of moving the analyzed species to higher altitudes.

In order to adapt the European forestry to climate change, Lindner et al. [21] emphasized some of the most important measurements which could successfully be applied in the case of Serbia:

1. Additional research
2. Selection of tree species, provenances and genotypes which are more tolerant to expected changed conditions
3. Change of tending and thinning regimes
4. Reduction of biotic and abiotic disturbance risks.

CONCLUSION

The most vulnerable tree species to the change of climate among the nine species with the highest abundance in Serbia is Pedunculate oak. Current distribution of species such as the Sessile oak, Turkey oak, Silver fir, Norway spruce and the European beech are under considerable risk due to the climate change, while the Black pine and Hungarian oak are the most resistant in this group.

Our methodological approach, as well as climate projections, suggests that general adaption options and specific measurements for forestry sector have to be made for the region of southeast Europe in the future.

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The Comparison of Innovations in Slovakian Forestry between 2002 and 2010 - a Shift to Multifunctionality?

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Abstract

Background and Purpose: Innovations play an important role in multifunctional forestry due to changing demands for forest goods and services. The multifunctionality can be a result of a joint provision of several outputs from individual forest holding or specialized forest activities. The goal of this paper is to make a comparative analysis from data obtained in two surveys in 2002 and 2010 related to innovations in forest enterprises. The main focus was on ownership, size, and management strategy of forest.

Materials and Methods: The paper presents the results of two surveys on innovation in the years 2002 and 2010 with more than 250 representatives - owners/managers of Slovak forest enterprises. The questionnaire in 2002 was sent out by regular mail in the random sample of 1072 forest owners and managers. The response rate was 25 % (in total 279 respondents). The response rate in 2010 was 37 %, and the number of valid responses was 254. The willingness of forest managers to implement innovation was evaluated in the context of different sizes of forest holdings, management strategies and property conditions. Log linear statistics models (Pearson Chi-square) were applied using the software Statistica for data analysis and Microsoft Excel to present the results.

Results: This paper presents the development of innovations in order to reach the multifunctionality on the case of Slovakian forestry. Product innovations have doubled from 17 % in 2002 to 34 % in 2010. Large-sized holdings are significantly ($p < 0.01$) more engaged in innovation and are offering new products. The highest overall innovation activity is revealed in the state-owned enterprises, intermediary in the municipal forests, and the lowest in the private holdings. Forest managers who realized the benefits of innovation were "profit increasing" oriented. Forest managers who did not realize the benefits of implementing innovations preferred conservative management goals - the capital maintenance. The innovative behaviour of managers depends significantly on forest managers' goals and their strategy ($p < 0.05$). The comparison between the two periods shows that innovation activity has increased from technological innovation to products and services. Wood still remains the main product of forest holdings, but compared to 2002, the importance of bio energy becomes visible.

Conclusions: Results show that innovation activity has increased during the time. There was a shift towards innovation from 2002 to 2010 which is visible in the successful innovation cases.

Keywords: forestry, non-wood forest products, forest managers, innovations

INTRODUCTION

Innovation is vital to economic growth and development. Through innovation, new products are introduced into the market, new production processes are developed and introduced, and organizational changes are made [1].

Innovations in this paper is understood according to the definition in Oslo Manual as "[…] the implementation of a new or significantly improved product (goods or service), or process, a new marketing method, or a new organizational method in business practices, workplace organization or external relations" [2]. Applied to forestry, it includes those changes within forest holdings, which are either new to the firm or new to the market. Innovations that are new to the firm are innovations that can be well established in the market already, nevertheless, they are newly introduced in the portfolio of products of a certain firm [3, 4].

Innovation and innovation policy in forestry was investigated by different projects on national, regional and EU level. Rametsteiner et al. [1] summarized the collected results from sectoral network approach used in the European Forest Institute Project Centre INNOFORCE. Currently, the regional cross-disciplinary approach in innovation research is supported by the Central-East European Regional Office of the European Forest Institute (EFICEEC). COST Action E51 on "Innovation and Development Policies for Forest Sector" from 2006 to 2010 contributed significantly to the study of innovation policies and processes in the forest sector in Europe. Innovation plays an important role in multi-functional forestry due to changing of demands for forest goods and services [5].

Multifunctionality is internationally discussed by international organizations, such as the Food and Agriculture Organization [6, 7], the Organization for Economic Co-operation and Development [2, 8] and the European Union [9]. Within the European Union, the term multifunctionality is discussed against the background of changing frame conditions for agricultural and forestry production. This differentiation of usage demands and perceptions coincides with a different understanding of agriculture and forestry being responsible for the management of rural area and thus pre-setting an aspect of multifunctional land use [10]. As a result, forestry is put less into the context of the production of timber (commodity-outputs), but rather into the context of non-commodity-outputs (ecosystems functions and services). On-going changes in environmental and socio-economic conditions are affecting the balance of desired ecosystem services and functions provided by all European forests. Timber is still a dominant product, but the multifunctionality requires development of other services or new forests' products. .

Achieving multifunctionality of European forests will depend on new ecosystem services and products, as well as on socio-economic, ecological and ownership conditions. In order to achieve the multifunctionality via new non-wood forest products and services, forest owners and managers have to implement innovations in their forest holdings.

Multifunctional forestry goals are presented in the National Forest Program of the Slovak Republic [11]. The basic information about Slovak forestry are provided in Table 1, which captures data about the area and ownership of Slovak forestry [12].

TABLE 1. Slovak forestry in 2010 (source [12])

Forest land area (ha)	Public forests (ha)	Private forests (ha)	Not restituted forest land (ha)	No. of subjects that manage forests	Average tenure (ha)
1 938 904	974 181	785 632	179 091	7 051	8.93

In the forest sector policy, as in other sectoral policy discussions, innovation is specifically discussed in the context of improving the competitiveness of sustainable forestry vis-à-vis other sectors of the economy and vis-à-vis the forest sectors of other countries [3, 4, 13-16].

Forestry is an important source of income for forest owners and for employees in rural areas. The future of the people, who make a living in rural areas from forestry, will considerably depend on how individuals and institutions react in view of the changes, how forest owners and managers obtain new knowledge and put it into practice in forestry, and how institutions, especially forest administration, extension services, fo-rest research or other institutions best deal with emerging changes. The restructuring of forestry and the development of wood prices tend to have a negative impact on em-ployment. To compensate for the negative impacts, product and service innovations based on the multifunctional use of forest and the efficient use of the growing stock of wood can provide new opportunities for rural employment [17].

The main goal of this paper is to make a comparative analysis from the data obtained in two surveys conducted in 2002 and 2010 related to innovations in forest enterprises. The main focus was on the ownership, size, and management strategy of the forest.

MATERIAL AND METHODS

The data collection was based on a questionnaire using closed and open questions, which were focused on the following areas: innovation activity and behaviour, fostering and impeding factors to innovation, successfulness of innovation, product mix and market expectation of forest owners and managers for the future. We conducted surveys in 2002 and 2008 using the questionnaire of the project INNOFORCE from 2002 [5] on the Survey on innovations and entrepreneurship of forest holdings. The questionnaire in 2010 was slightly adapted on current conditions and this allowed the comparison with the results from INNOFORCE surveys and current situation. The minimum requirement for an innovation in the survey was that the product, process, marketing me-thod or organizational method had to be new to the forest enterprise and that it has been implemented in the market during the last 3 years. The main features of the innovative entrepreneurship towards multifunctional forestry management included an autono-mous activity of forest holdings, creativity, target-orientation, initiative, novel approaches in nonstandard situations, ability to make decisions in uncertain situations, and the wi-llingness to take risk.

Respondents were chosen randomly from the national forest holdings database, maintained by the National Forest Centre. The 26 regional enterprises of the State enterprise "Forests SR" and non-state forest holdings represented the basic population of the survey. Their owners and managers who are responsible for the management and product or process-related decisions, were the target information sources. Questionnaires were sent by regular mail and by email to the state and non-state forest holdings during the years 2002 and 2010 respectively. The questionnaire in 2002 was sent out by regular mail in the random sample of 1072 forest owners and managers. The response rate was 25 % (in total 279 respondents). The response rate in 2010 was 37 %, and the number of valid responses was 254, of which 76 were from public holdings and 178 from private holdings (including community and church forests). A summary is presented in Table 2. The questionnaire was broadly focused on innovation, but for the purposes of this study we chose only the evaluation of innovation activity described by the willingness of forest managers to innovate. Data was analysed by descriptive statistic methods. Standard contingency method was used to analyse innovation behaviour in dependence on various variables. Loglinear statistics models (Pearson Chi-square) were applied using the software

TABLE 2. Structure of respondents according to forest ownership

	Total sent	Received	State	Municipal	Communal	Private	Church
2002	1072	279	20	21	210	24	4
2010	693	254	21	55	164	14	0

Statistica for data analysis and Microsoft Excel for the presentation of results. The following research questions were formulated:

1. Are there any differences in innovations from 2002 to 2010?
2. How does holding's size influence innovations?
3. How does ownership influence innovation in forest enterprises?
4. Which are the appropriate management strategies to implement innovations?

RESULTS AND DISCUSSION

Progress in Innovation Activity

The types of innovations, which were successful, can be divided into three categories: products, services and technological or organizational innovations (T-O-I). Technological and organizational innovations hold the biggest share on successful innovations in 2002 (56 %) compared with 38 % in 2010. Higher intensity of technological innovations in 2002 could be explained by the continuing transition to the market economy, where technological innovations are undertaken continuously as new technological means or principles become available. As the transformation process in Slovakia continues, innovations become more product-oriented. Product innovations have doubled from 17 % in 2002 to 34 % in 2010.

In Table 3 we can see the number of forest holdings that implemented one or more successful innovations during the period of 3 years before the survey. The innovation activity has significantly increased in 2010 ($p < 0.01$).

Forest Holdings Lager then 500 ha Innovate More

Table 4 provides the overview of small and large forest holdings according to their innovation activity. The results are related to the second research question, and they show a difference between smaller and larger forest holdings in innovation activity. Small forest holdings (manage less than 500 ha of forest land) prefer round wood to other products. Some of their managers stated that they do not offer any products, because they manage the forest for self-consumption (17 managers in 2002 and 21 managers in 2010). Large-sized holdings are significantly ($p < 0.01$) more engaged in innovation and in offering new products and services.

TABLE 3. Impact of time on the willingness to innovate

Innovation	No	Yes	Total
Survey 2002	183	86	269
Survey 2010	145	114	259
Total	328	200	528
Pearson Chi-square	**Chi-sqr**	**df**	**p**
	110.2159	4	0.000000

TABLE 4. Impact of forest holding size on innovation activity*

Innovation	No	Yes	Total
Small forest holdings	247	67	314
Large forest holdings	81	133	214
Total	328	200	528
Pearson Chi-square	**Chi-sqr**	**df**	**p**
	14.15794	4	0.006808

* presented data represent results from joined dataset (2002 and 2010 survey)

Nowadays, other wood products as Christmas trees and seedlings, game, non-wood products and rental services have got a bigger share in the forest product mix. Recreation and tourism gained the most important role (Figure 1).

The positive shift towards non-wood product offer is a result of adopted strategic documents (such as National Forest Program, Rural Development Program) that emphasize the principle of sustainable forest management and the importance of the forestry sector in rural development. Supporting measures (financial, informational) for diversifications exist for all types of forest holdings at a national level, but they are partly funded from EU resources.

Public Forest Holdings Innovate Easily

The type of ownership appeared to be important for the innovation activity of forest holdings. The ownership structure in Slovakia is rather complicated due to restitution processes in the past decades. We can identify the following categories of forest holdings: private (individual or corporate owned), community (shared ownership by individual persons), church, municipal and state. The state and municipal forests are evaluated as public forests according to the Forest Europe classification [18]. The empirical observations show that the highest overall innovation activity is revealed in the state-owned enterprises, intermediate in the municipal forests, and lowest in the

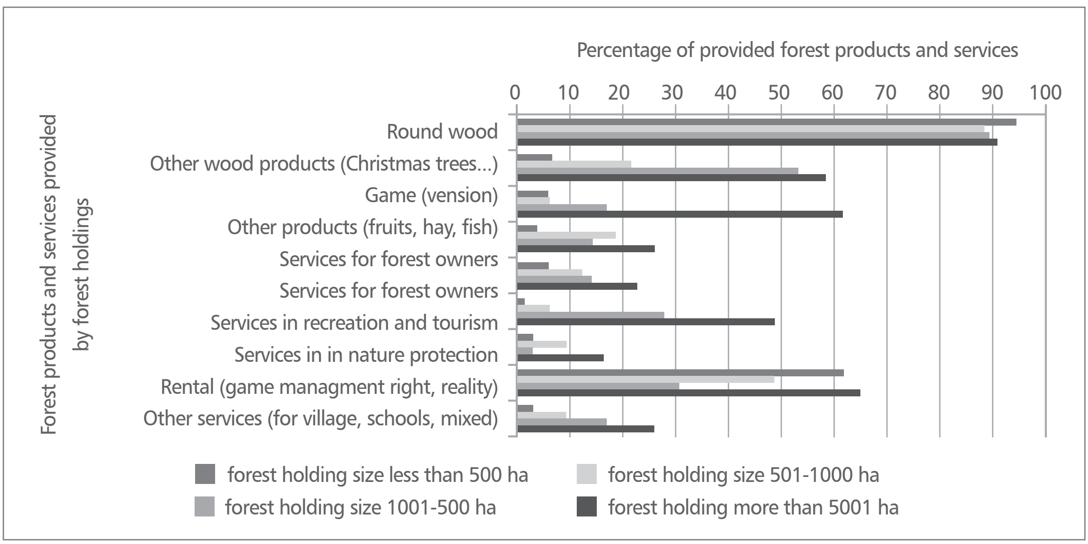

FIGURE 1. The product mix in Slovakia 2010

TABLE 5. Impact of forest ownership structure on innovation activity*

Innovation	No	Yes	Total
Public holdings	41	95	136
Private holdings	287	105	392
Total	328	200	528
Pearson Chi-square	**Chi-sqr**	**df**	**p**
	9.738639	4	0.045068

* presented data represent results from joined dataset (2002 and 2010 survey)

private holdings (owned by land associations and individuals) (Table 5). This can be explained by the lack of disposable financial resources for the non-state forest owners [19].

In the second survey, 67 % of state and municipal forest managers reported the implementation of one or more innovations. This proportion is significantly higher than random ($p < 0.05$). Forest managers were also asked to name the most successful innovation. The responses showed that the innovations concerning biomass for energy purposes (e.g. wood chips supply) have higher success than other products. This is only a small step away from timber production, but very positive in accordance with the aspirations on the utilization of alternative energy resources in the EU. Slovak forest owners and managers have also recognized this opportunity.

The Innovation Towards Multifunctionality Needs Appropriate Management Strategies

There is a logic relation between innovation activities and management goals. Increasing of profit and productivity can't be achieved without an increased effort oriented to improve, modernize and rationalize forest production. The management goal is therefore an important motivating factor for innovative behaviour. Forest managers were asked about their goals for forest management: 1. increasing profit, 2. maintaining capital and 3. abandoning of forest management.

In 2002, in non-state forests the goal of maintaining the capital dominated, namely in municipal forests (62 %) and in shared forest holdings (52 %). The situation was the same in 2010, the majority of forest managers (52 %) stated that they managed the forest with the goal to maintain capital. However, the share of mangers that managed state forests with the goal to increase profit decreased from 59 % to 26 %.

A more detailed analysis was focused on the means how forest managers want to achieve these goals:

1. specialization to one product production (timber),
2. offer of various products,
3. more intensive evaluation of products and/or services,
4. leaving out and/or decreasing the extent of works (within the framework of forest management plan),
5. rationalization, costs reduction,
6. co-operation in associations, mechanization centres,
7. by buying additional forests,
8. sale of (non-state only) forest,
9. maintenance of up-to-now way of work,
10. other means (e.g. improvement of forest roads).

Surveys showed that there are two major strategies according to the means how to achieve selected management goals. Conservative strategy is focused on "business as usual" (9), specialization on timber production (1), decreasing extent of works within the framework of forest management plan (4) and reduction of timber production costs (5).

Modern strategy is described by diversification (2), improved marketing of non-wood products and services (3), cooperation with non-wood products and services introduction (6) and expansion of property for new products and services (7). However, the share of innovation in forest holdings in combination with both management strategies seems to be the most successful. Innovation activity in relation to management strategy is presented in Table 6. Only a few small forest owners or managers responded that their actual aim was to sell property or abandon forest management (2 % in 2002 and 3 % in 2010).

Forest managers who realized the benefits of innovation were "profit increasing" oriented. Forest managers who did not realize the benefits of innovation preferred conservative management goals - the capital maintenance. According to the results it could be summarized that the willingness to implement innovation is in relation with the chosen management strategy (Table 7). The innovative behaviour of managers depends significantly on forest managers' goals and their strategy ($p < 0.05$).

The essential changes in the Central and Eastern European countries in the nineties of the past century resulted in the introduction of technological and organizational innovations into forest management. At present, the most implemented types of innovations are new products and services. This fact can be explained by the accession of many Central and Eastern European countries to the European Union in

TABLE 6. Management strategy and number of implemented innovations

Survey	Strategy	Innovations		Category of innovation			Relative proportion of innovation		
		no	yes	Products	Services	Tech. Org.	Products	Services	Tech. Org.
2002	n.a.	10	0	0	0	0	-	-	-
	Conservative	86	20	12	10	23	26.7	22.2	51.1
	Modern	27	11	10	8	10	35.7	28.6	35.7
	Combination	59	54	61	55	77	31.6	28.5	39.9
2009	n.a.	6	1	1	2	1	25.0	50.0	25.0
	Conservative	84	26	10	14	28	19.2	26.9	53.8
	Modern	22	27	33	18	25	43.4	23.7	32.9
	Combination	32	59	64	54	64	35.2	29.7	35.2

n.a. stands for forest managers that did not choose any of the management strategy

TABLE 7. Impact of management strategy on innovation activity*

Innovation	No	Yes	Total
n.a.	17	2	19
Conservative strategy	171	47	218
Modern strategy	50	39	89
Combination of strategies	92	114	206
Total	330	202	532
Pearson Chi-square	**Chi-sqr**	**df**	**p**
	17.43784	8	0.025859

* presented data represent results from joined dataset (2002 and 2010 survey)
n.a. stands for forest managers that did not choose any of the management strategy

2004 which is linked with the possibility to use the financial means from the European funds for diversification of forestry activities. The interests of forest owners and the whole society were reoriented on the offer expansion of new services and new products in relation to ecosystem services [20].

Pudivítrová and Jarský [21] reported no increase in the share of innovative behaviour of foresters in the Czech Republic in comparison with the situation 10 years ago, although they found significant changes in the structure of implemented innovations towards new products.

Slee [22] pointed out that when we are talking about multifunctional forestry, we need to think about scale and temporal issues. Locally demanded functions may differ from national demands and current preferences do not need to indicate preferences in the long run. This progress is visible by the market expectations of forest owners in 2002, when, besides wood, drinking water was also considered as the main gain that forests could provide. Changes are visible and are in line with the implementation of European policy targets in the energy policy. In 2010, beside the above-mentioned goods, recreation, environmental services and carbon sequestration were also prioritized. The climate change policy and the supporting measures for biomass use as an alternative energy resource on the global level and in Europe, gained a significant role of bio energy services in forestry practice. Our research supports these findings. According to the survey in Slovakia, it is assumed that an increase of importance of drinking water and bio energy will be visible in the future (Figure 1).

According to Nastase et al. [14] the evidence from Romania show that the innovation in rural areas is about "doing traditional activities in a new way", and all innovations are strongly linked with social processes such as creation of networks, strengthening of local identities, and creation and transfer of knowledge.

CONCLUSIONS

Results show that the innovation activity has increased during the observed timeframe. This correlates with the forest policy which aims to support multifunctional forestry by implementing new services and products. Small forest holdings prefer round wood to other products. Large-sized holdings are more engaged in innovation and in offering new products. Type of ownership appeared to be an important factor for the innovation activity of forest holdings. State and municipal forest enterprises are more inclined to innovation than private and communal ones.

Overall it can be concluded, that there was a shift towards innovation from 2002 to 2010 which is visible in the successful innovation cases. Forest managers are innovative in accordance with the strategic objective 4 of the National Forest Programme of the Slovak Republic: Increasing long-term competitiveness and priorities strive for increased competitiveness and economic viability of multifunctional forestry. They offer more non-wood forest products, support the use of forest biomass to produce energy and cooperate more in order to maintain and foster the ecosystem services.

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Original scientific paper

Macropropagation of *Dennettia tripetala* Baker f.

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Abstract

Background and Purpose: *Dennettia tripetala* Baker f. is a small fruit-producing tree, which both animals and humans depend upon for their survival. Consequently, there is a great pressure on the seed, which is the only alternative for its inexpensive propagation. Hence, the species has been rendered threatened in the forest. Based on this fact, this study examined the macropropagation of *Dennettia tripetala* in order to domesticate and ensure its continuous availability for human utilization.

Materials and Methods: The propagules (cuttings) used for this study were collected on five year old mother trees in Oloruntele, Ondo State while the experiment was conducted at the Department of Forest Resources Management, University of Ibadan, Nigeria. Cuttings were prepared using two factors; indole butyric acid (IBA) concentrations (0 ppm, 1000 ppm, 2000 ppm and 3000 ppm) and nodal positions (upper, middle and base), set under a high humidity propagator and watered twice a day. The experimental design used was a completely randomized design and the replication was done twice. Data were collected on percentage rooting, number of root per cutting, ramets' height growth, ramets' collar diameter and ramets' leaf number. Statistical analysis was done using ANOVA.

Results and Conclusions: The optimum rooting was observed in 2000 ppm IBA treated cuttings (95.50 %) and upper nodal cuttings (72.39 %). ANOVA test shows that hormone concentrations and nodal positions significantly affected the rooting of *D. tripetala.* The study also showed that hormone concentrations and nodal positions do not significantly ($p > 0.05$) determine the ramet's height growth of the species, being that the ramet that had the highest height was 0 ppm (6.11 cm) and middle nodal position (5.96 cm). From the findings, 2000 ppm IBA hormone and upper node have been discovered to be the ideal hormone concentration and nodal position at which five year old *D. tripetala* cuttings can be best rooted and survive.

Keywords: IBA hormone, hormone concentration, nodal position, stem cutting, *Dennettia tripetala* Baker f.

INTRODUCTION

Dennettia tripetala Baker f. belongs to the family Annonaceae. It is a tropical small tree that abounds throughout the rainforest zone of Africa. It is found in the tropical rainforest region of Nigeria and sometimes in Savana areas [1]. This tree flourishes at the onset of rain, from April to June [2].

The fruits of the tree are edible and have a peppery and spicy taste. It serves as mild stimulant and may also serve as a source of some vitamins. The leaves are used to treat mild fever in combination with mango leaves [3]. Pharmacologically, the oil extracted from the fruit of *D. tripetala* is used in the manufacture of mouth wash [3]. Some of the fruit extracts have been shown to be active as antifungal agents against *Candida* sp., *Crytococeus* sp., *Geotrichum* sp., *Rhizopus stolonifer*, *Aspergillus* sp. and *Fusarium* sp. [4]. The fruit also contains an essential oil, which has been used as an effective preservative for stored grains such as cowpea and maize without negatively affecting their viability [5].

Macropropagation involves the use of cuttings in producing clones of large quantity from the ortet for the purpose of conservation [6]. There is dearth of information on the propagation of *D. tripetala*. However, a lot has been done about the vegetative propagation of tropical African tree species, using simple and inexpensive technologies [6-14]. For instance, Atangana et al. [7] reported that the rooting of most of our indigenous trees occurs between 6-12 weeks of propagation. However, their study only focused on the domestication of *Allanblackia floribunda*. Also, different authors have worked on the propagation of *D. tripetala* by means of seeds [15-17]. However, its macropropagation is very significant as there is too much pressure on the seed of the species, which is the only alternative for achieving inexpensive domestication. According to Osaigbovo et al. [18], *D. tripetala* has inconsistent fruiting, poor seed germination and slow seedling growth. Within the short period available, maximal number of seeds must be collected before the competition, such as game, cattle and humans.

In macropropagation, various factors have been reported to influence the rooting of cuttings in tree crops out of which hormone concentrations and nodal position are the most important [7]. Tree species vary considerably in the optimal application of hormone and there is also much intraspecific variation [19]. This form of variation is not well understood in most of our indigenous tree species. Tchoundjeu and Leaky [8] reported that the best concentration of auxin IBA for the rooting of African mahogany was found to be 1000 ppm. They equally discovered that a greater percentage of cuttings from basal nodes have rooted compared to those from apical nodes. Therefore, nodal position is a significant factor to be considered when carrying out any meaningful vegetative propagation. It is also very important in macropropagation because it determines to a large extent the rooting ability of plant cutting. For some plants, upper node may be the most successful position while some other plants are easily domesticated when the older parts (middle and base) of the cuttings are propagated. Considering the diversity of use of *D. tripetala*, together with its threatened conservation status in the forest, this study investigated the possibility of domesticating the species from cuttings as its source of germplasm.

MATERIALS AND METHODS

The propagules (i.e. cuttings) used for this study were collected on five year old mother trees in Oloruntele, Ondo State, while the experiment was conducted at the Department of Forest Resources Management, Universit y of Ibadan, Nigeria. Oloruntele is a village in Ileoluji-Okeigbo Local government, Ondo state. It lies in the dry lowland rainforest belt [20]. The climate is characterized by two seasons i.e. wet and dry seasons. The dry season occurs between November and March while the wet season is usually between April and September.

University of Ibadan is located north of Ibadan along Oyo road at approximate latitude 7°26'42.7308"N and longitude 3°53'57.1560"E.

It is located at an altitude of 277 m above the sea level [11]. The climate is the West Africa monsoon with dry and wet seasons. The dry season is usually from November through March and is characterized by dry cold wind of harmattan [11]. The wet season usually lasts from April to October with occasional strong winds and thunderstorms [11].

The cuttings were collected at the upper, middle and basal position on the mother tree species. The leaves of the cuttings were reduced to half of the original sizes and then treated with IBA hormone, prepared into 4 different concentrations (0 ppm, 1000 ppm, 2000 ppm and 3000 ppm) using quick deep method [11, 21-22]. The cuttings were set in seed plastic trays containing sterilized river sand. Each of the treatment contained 12 cuttings. Replication was made twice making an aggregate of 288 cuttings. The experiment was arranged in a completely randomized design (CRD) under a high humidity propagator. Watering of the set cuttings was carried out twice a day using a knapsack sprayer. The cuttings were monitored for a period of twelve weeks. During this period, a number of rooted cutting, number of sprouted cutting, number of survived cutting, number of root per cutting was obtained by visual counting. In addition, length of root per cutting was measured using a meter rule. From these results, percentage rooting, percentage sprouting, and percentage survival were estimated. After twelve weeks of monitoring the cuttings under a high humidity propagator, rooted cuttings were transplanted into polythene pots filled with top soil and assessed every day for another twelve weeks. Hence, measurement of height of ramets was accomplished with a meter rule, while collar diameter was measured with vernier caliper and number of leaf was achieved with visual counting.

One-way ANOVA was used to test differences between mean values of measured characteristics between the treatments. Mean values were separated using Fisher's Least's Significance Difference (LSD) test, with significance level of $p < 0.05$ ($\alpha = 0.05$).

RESULTS

All the hormone treatments had increased sprouted cuttings right from the first measurement period (2 weeks after planting) to the fifth measurement (10 weeks after planting) and stabilized at sixth measurement period (12 weeks after planting) (Figure 1). At first measurement (2 weeks after planting), none of the cuttings treated with 3000 ppm IBA and those without treatment sprouted (Figure 1), whereas about 4.86 % and 1.38 % of cuttings treated with 2000 ppm and 1000 ppm IBA, respectively, have sprouted. At the end of the experiment, 2000 ppm IBA treated cuttings have also produced the highest sprouting percentage (89.44 %) while 3000 ppm IBA treated cuttings had the lowest sprouting percentage (33.19 %). Hormone concentration (HC) had significant effect ($p < 0.05$) on the sprouted cuttings of *D. tripetala* (Table 1).

Regarding the cuttings' nodal positions, there was a gradual increment in the percentage sprouted cuttings from first to fifth measurement period, after which there was a period of stabilization in the sprouted cuttings (Figure 1). Results show that cuttings taken from the upper nodal position had the best sprouting percentage right from the first period of measurement to the end of the experiment (Figure 1). The final measurement results (Figure 1) revealed that 70.52 % upper nodal cuttings sprouted, while only 58.64 % and 53.22 % of the middle and basal cuttings correspondingly were able to sprout. Similar to the results obtained from HC, the percentage sprouted cuttings of *D. tripetala* from different NP were significantly different ($p < 0.05$) (Table 1).

Considering the effect of HC on rooting of *D. tripetala*, 2000 ppm IBA treated cuttings rooted best (95.50 %), this was closely followed by 1000 ppm (72.22 %), 0 ppm (51.25 %) while the 3000 ppm IBA treated cuttings ended up having the least rooting percentage (Table 1). There was significant difference ($p < 0.05$) in percentage rooted cuttings among the four levels of HC. Effect of the nodal position (NP) was significant on the rooted cuttings. Upper nodal cuttings (72.39 %) rooted more than middle (62.81 %) and basal (54.58 %) nodal cuttings.

Root length significantly increased with an increase in hormone concentration (Table 1). In other words, the root length of 3000 ppm IBA treated cuttings (6.31 cm) were more than those of 2000 ppm (5.79 cm), 1000 ppm (4.21 cm) and 0 ppm (2.90 cm). With respect to the NP, upper nodal cuttings had the highest root length (5.70 cm), followed by the middle (4.91 cm) and the base (3.80 cm). Significant difference (p < 0.05) was also observed in root length among the nodal positions.

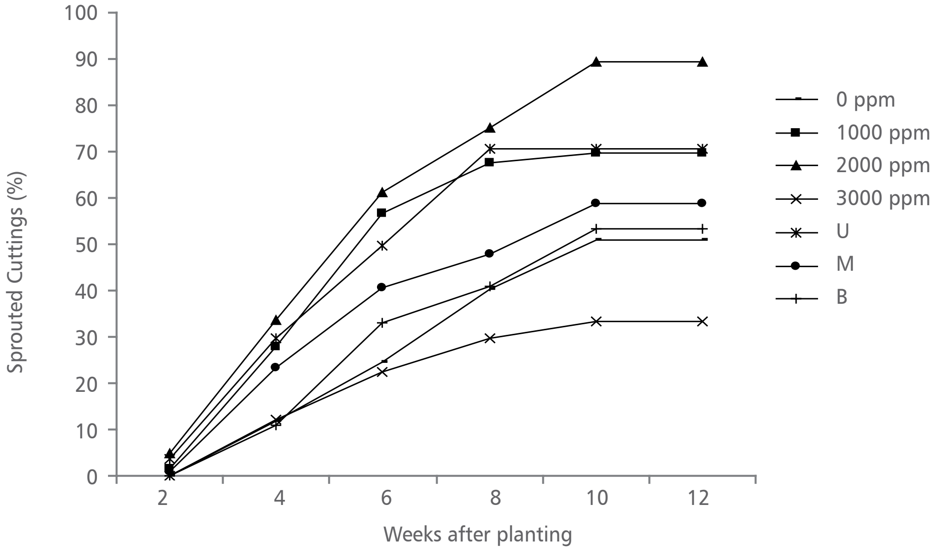

FIGURE 1. Sprouting of *D. tripetala* cuttings using different IBA hormone concentrations and nodal positions (U = upper node, M = middle node and B = basal node)

TABLE 1. Effect of hormone and nodal positions on rooting, sprouting, survival and growth of *D. tripetala*

Hormone concentration	**SC** (%)	**RC** (%)	**S** (%)	**RL** (cm)	**RH** (cm)	**RCD** (cm)
0 ppm	50.83b	51.25b	69.79a	2.90a	6.11	0.704
1000 ppm	69.72c	72.22c	80.38b	4.21b	5.76	0.612
2000 ppm	89.44d	95.50d	84.85b	5.79c	5.82	0.586
3000 ppm	33.19a	37.08a	84.72b	6.31c	5.79	0.687
p-value	0.000*	0.000*	0.002*	0.000*	0.439ns	0.055ns
Nodal position						
U	70.52c	72.39c	65.52a	5.70c	5.93	0.608a
M	58.64b	62.81b	79.50b	4.91b	5.96	0.610a
B	53.22a	54.58a	94.79c	3.80a	5.72	0.724b
p-value	0.000*	0.000*	0.000*	0.000*	0.472ns	0.012*

Means with similar alphabet within the same column of any set of treatments are not significantly different at p = 0.05
SC - sprouted cuttings; RC - rooted cuttings; S - survival; RL - root length; RH - ramet height; RCD - ramet collar diameter; U - upper; M - middle; B - base
* - significant (p < 0.05); ns - not significant (p > 0.05)

Result shows that most (84.85 %) of the rooted cuttings treated with 2000 ppm IBA finally survived during the period of the study (Table 1). In the same vein, approximately 85 % of the rooted 3000 ppm IBA treated cuttings survived. In the case of 1000 ppm and 0 ppm IBA treated cuttings, 80.38 % and 69.79 % of the rooted ones survived, respectively. There was significant difference ($p < 0.05$) in the survived cuttings from the different hormone concentration. Cuttings from the basal positions survived more (94.79 %) than the ones collected at the middle (79.50 %) and the upper nodes (65.52 %). Nodal position significantly affected the percentage survived cuttings ($p < 0.05$).

Figure 2 shows that number of root produced in the species increased as the concentration of hormone increased. Cuttings treated with 0 ppm, 1000 ppm and 2000 ppm had approximately 3 roots each on the average while the 3000 ppm IBA treated cuttings produced an average root number of about 4. Root number did not significantly depend on the concentration of the hormone applied ($p > 0.05$) (Table 2). Conversely, root number significantly depends on the nodal position of the cuttings ($p < 0.05$) (Table 2), with the basal cuttings having the highest average root number (4.38), followed by middle cuttings (3.66) and upper cuttings (1.72) (Figure 2).

Furthermore, slight increments in height and collar diameter of *D. tripetala* ramets were recorded during the weeks of measurement throughout the study period (Figure 3 and Figure 4). Hormone concentration did not

TABLE 2. Effect of hormone concentration and nodal position on root and leaf production of *D. tripetala*

Variable	Chi-square value	df	p-value
N.P. VS R.N.	17.45	4	0.002*
H.C. VS R.N.	8	6	0.238ns
N.P. VS N.L.	25.9	8	0.001*
H.C. VS N.L.	20.35	12	0.061ns

NP - nodal positions; HC - hormone concentrations; NR - number of root; NL - number of leaf
* - significant ($p < 0.05$); ns - not significant ($p > 0.05$)

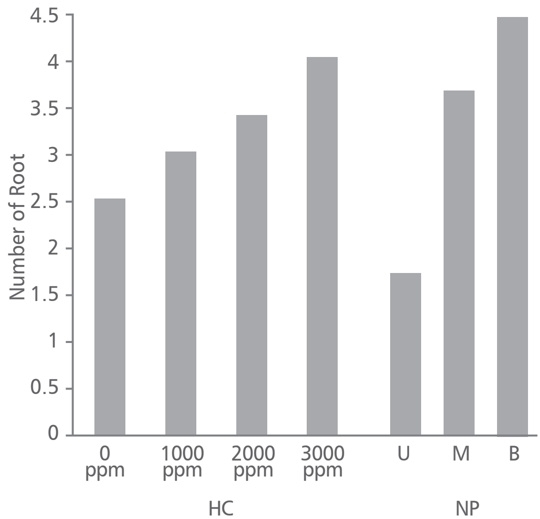

FIGURE 2. Number of root produced in *D. tripetala* cuttings using different IBA hormone concentrations (HC) and nodal positions (NP) (U = upper node, M = middle node and B = basal node)

have significant effect, ($p > 0.05$) (Table 1) on the ramets' height. At the initial stage of measurement (i.e. week 2), ramets that received the IBA treatment of 3000 ppm had the highest height growth rate of 2.01 cm (Figure 3). But at week 4, the highest growth (2.83 cm) was observed in cuttings with no hormone treatment, while at week 6, it was found in 2000 ppm ramets (Figure 3). At the 12^{th} week, 0 ppm produced ramets that had the highest growth (6.11 cm) (Figure 3). However, the effect of nodal positions was not significant ($p > 0.05$) (Table 1) on the height growth of the species. At the first measurement period, the average height of ramets from the basal node was 2.45 cm, middle node (2.02 cm) and upper node (1.21 cm) (Figure 3). In the 12^{th} week after transplanting, middle nodal ramets had the highest height (5.96 cm), they are followed by upper nodal ramets (5.93 cm) and the base nodal ramets (5.72 cm) (Figure 3).

The result obtained from the effect of hormone concentration on ramet collar diameter followed the same pattern as the ramet height, in that that there was no significant difference ($p > 0.05$) among the collar diameter of different hormone treatments (Table 1). Also, the effect of nodal positions was significant ($p < 0.05$) on the collar diameter, with ramets from the basal nodes having the highest collar diameter size (0.72 cm) at the final measurement and they were closely followed by ramets from the middle (0.61 cm) and upper nodes (0.60 cm) (Table 1 and Figure 4).

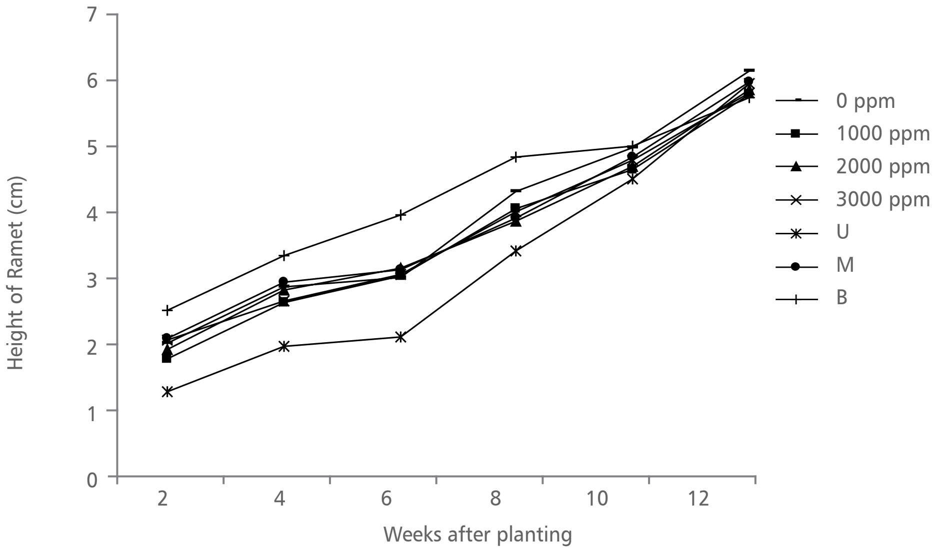

FIGURE 3. Height growth of *D. tripetala*'s Ramets using different IBA hormonne concentrations and nodal positions (U= upper node, M = middle node and B = basal node)

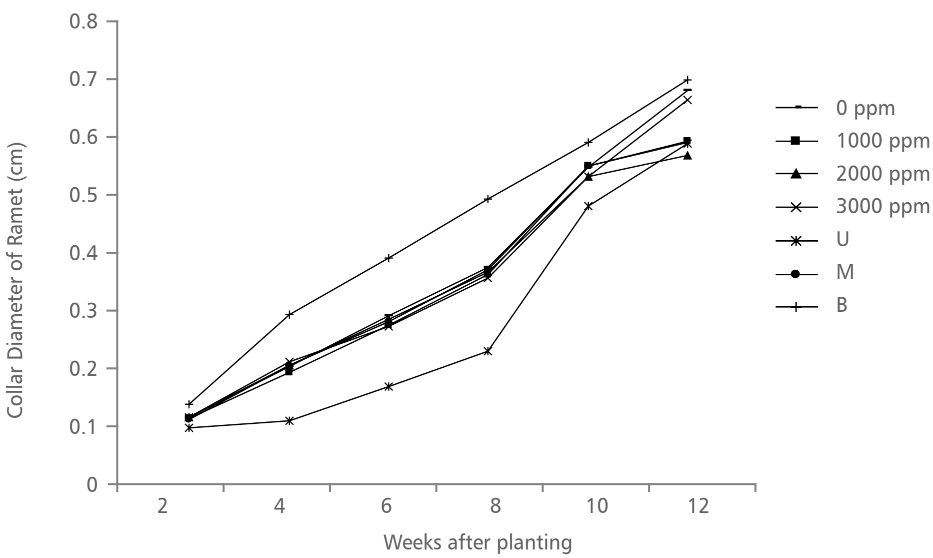

FIGURE 4. *D. tripetala* ramets collar diameter increament using different IBA hormone concentrations and nodal positions (U= upper node, M = middle node and B = basal node)

Results of leaf production in *D. tripetala* are shown in Figure 5 and Table 2. Ramets obtained from cuttings treated with 3000 ppm IBA produced an average number of 3 leaves while ramets from the remaining treatments (i.e. 0 ppm, 1000 ppm and 2000 ppm) only had about 2 leaves on them. Leaf production did not significantly ($p > 0.05$) depend on hormone concentration (Table 2). Ramets from the basal nodes had the highest leaf production, with about 3 leaves while the ramets from upper nodes had the least number of leaves produced (2). Significant difference ($p < 0.05$) was observed in this leaf number among the nodal positions (Table 2).

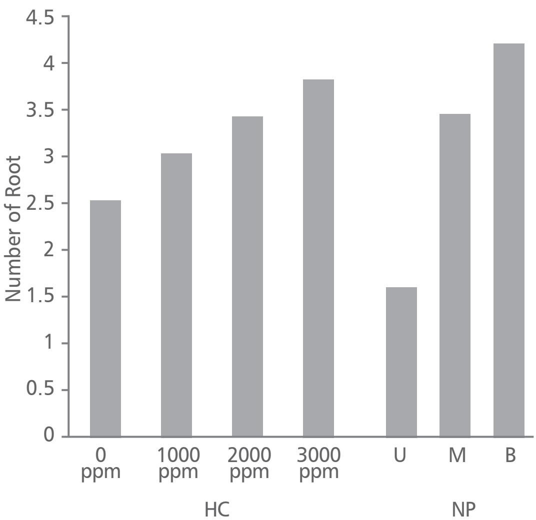

FIGURE 5. *D. tripetala* ramets leaf production using different IBA hormone concentration (HC) and nodal positions (NP) (U= upper node, M = middle node and B = basal node)

DISCUSSION

From the presented results it can be concluded that the best level of exogenous HC needed to obtain an optimum sprouting of *D. tripetala* is 2000 ppm. The decrease in the number of sprouted cutting as the HC is increased above 2000 ppm may however be attributed to the fact that there is a toxic level of auxins with which *D. tripetala* as well as other indigenous medicinal trees will not be able to undergo physiological process that will result with root production [8]. Therefore, any further increase in the HC above this level eventually inhibits the number of sprouted cuttings from the species.

Similar results obtained from this study have been reported by Lee and Bilderback [23], who emphasized that there was a significant increase in the number of sprouted cuttings of *Heptacodium jasminoides* Airy Shaw, as the IBA hormone concentration increased from 0 ppm to 7500 ppm. They concluded that an additional increase in the hormone concentration above 7500 ppm resulted in reduction of the number of sprouted cuttings. In *Ginkgo biloba* L. IBA at 4000 ppm increased the percentage of sprouting in stem cuttings, but higher concentration decreased it [24]. Study by Saffari and Saffari [13] showed that an increase in IBA concentration above 4000 ppm resulted in a decreased percentage of sprouting of *Dodoneae viscosa* L. cuttings.

The higher number of sprouted cuttings observed from the upper NP than that from middle and basal nodes may be due to closeness of the upper nodes cuttings to the meristematic part of the plants, where the endogenous hormones are naturally produced and therefore aided the cell differentiation that resulted in the sprouting of the cuttings. According to Tchoundjeu and Leaky [8], auxins are basipetally translocated in plant's stems, and are largely responsible for the polarity of shoot.

The 95.5 % rooted cuttings recorded in this study, which was more than some of the previous works of other authors such as Abdullah et al. [25] is a significant success. The results from the present study imply that higher concentrations of IBA (3000 ppm) decreased rooting percentages and caused desiccation of cuttings of *D. tripetala.* This is possibly due to a phytotoxic effect of this hormone in tandem with high concentrations of endogenous auxins in cuttings of *D. tripetala.* The application of exogenous auxins may have led to supra-optimal concentrations in plant tissues, but with negative effects on rooting. It has also been suggested that the optimum concentration of auxins is favorable, while supra-optimum auxin levels are toxic to the root regeneration [26]. When IBA is applied in high concentrations to cuttings with high concentration of endogenous hormone, it has an herbicidal effect [27].

Although auxins have been successfully used to promote rooting of hardwood cuttings [28-29], the rooting of dipterocarp cuttings without auxins have been previously reported [30]. Most of the upper node cuttings used for this study were juvenile. Juvenility may be an important factor in the rooting potential of dipterocarp cuttings [31]. Juvenile tissues of woody plants tend to have higher levels of endogenous auxins and are less differentiated (and therefore more prone to re-differentiation) [27].

Findings from this study indicated that survival of *D. tripetala* is closely related to its rooting system, which is also dependent on the concentration of the exogenous hormone applied. Although, it was observed that too high concentration of exogenous hormone is detrimental to the rooting of the species, but those that were able to root ended up surviving. The results corroborate Atangana et al. [7], who reported that application of exogenous hormone significantly increased the percent survival and rooting of *Caesalpinia bonduc* (L.) Roxb.

The higher percentage of survival and number of roots from the basal section indicated that the basal stem part cutting might support a more optimal rooting and survival compared to cuttings from medial and apical sections. The carbohydrate content and related hormones predictably gave significant influences on these conditions. In the basal part, such phyto-hormone and carbohydrate as a source of energy was sufficient for optimal root formation after the bud dormancy breaking. These more favorable conditions might accelerate cell division and further differentiation for root and shoot initiation and formation [32].

The results from the early growth stage of this study have shown that *D. tripetala* is a slow growing species. It also implies that exogenous hormone applied at cuttings preparation stage reduces with time and therefore has negligible effects on the species growth once it has reached the ramet stage. According to Carey [10], more height growth was observed in *Salvia nemorosa* L. without exogenous hormone than those that were treated 4 weeks after transplanting. It has also been reported by Boyle [33] that, as plants grow older, the activities of the auxins in it tend to be diverse. When talking about the effects of the nodal position on the diameter growth, the difference is insignificant, if the assessment period is extended to more than the twelve weeks after transplanting. Therefore, more of the auxins are being utilized for other physiological process such as branching than in height and diameter growth. For instance, authors [34-35] have reported that Cytokinins induce branching in many plants.

CONCLUSIONS

The study discovered that 2000 ppm IBA is the optimum concentration at which five year old *D. tripetala* cuttings can be best sprouted, rooted and survive. Also, upper node was seen as the nodal position with the highest mass propagation potential. In addition if ramet's growth of the species is to be considered, hormone concentration is not a significant factor.

Utilization of single node cuttings is therefore beneficial for mass propagation of *D. tripetala,* in that that it will reduce pressure and competition on the fruit, which is the most important part used by both humans and wildlife. It is also generally more cost effective compared to the micropropagation, which is another alternative for clonal production [27].

The significant differences observed in the factors considered in macropropagation of *D. tripetala* can therefore be tapped and used for any other indigenous fruit and medicinal forest tree species.

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Original scientific paper

Selection of Willows (*Salix* sp.) for Biomass Production

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Abstract

Background and Purpose: Willows compared with other species are the most suitable for biomass production in short rotations because of their very abundant growth during the first years. Nowadays, in Croatia, a large number of selected and registered willow clones are available. The main objective of the research should be to find genotypes which, with minimum nutrients, will produce the maximum quantity of biomass.

Material and Methods: Clonal test of the arborescent willows include the autochthonous White Willow (*Salix alba*), interracial hybrids of the autochthonous White Willow and the English 'cricket' Willow (*S. alba* var. *calva*), interspecies hybrids (*S. matsudana* × *S. alba*), as well as multispecies hybrids of willows. Average production of dry biomass ($DM \cdot ha^{-1} \cdot a^{-1}$) per hectare was estimated in regard to the clone, survival, spacing and the number of shoots per stump.

Results: The highest biomass production as well as the best adaptedness and phenotypic stability on testing site was shown by clones ('V 374', 'V 461', 'V 578' from 15.2 - 25.0 $t \cdot DM \cdot ha^{-1} \cdot a^{-1}$) originated from backcross hybrid *S. matsudana* × (*S. matsudana* × *S. alba*) and by one *S. alba* clone ('V 95', 23.1 - 25.7 $t \cdot DM \cdot ha^{-1} \cdot a^{-1}$). These clones are now at the stage of registration and these results indicate significant potential for further breeding aimed at biomass production in short rotations.

Conclusions: Willow clones showed high biomass production on marginal sites and dry biomass could be considerably increased with the application of intensive silvicultural and agro technical measures. No nutrition or pest control measures were applied (a practice otherwise widely used in intensive cultivation system), while weed vegetation was regulated only at the earliest age.

Keywords: *Salix* clones, short rotation forestry, marginal lands, Croatia

INTRODUCTION

Biomass for energy purposes could be produced by fast growing tree species such as willows, poplars, alder, birch, black locust and others. Such a manner of biomass production is known as Short Rotation Forestry. In previous studies in Croatia, arborescent willow clones have shown greatest potential for biomass production in short rotations. Therefore, testing of arborescent willows continued aiming at identification of clones with greatest production potential, particularly on the so called marginal sites i.e. abandoned agricultural soils and/or sites that are not suitable for growing more valuable tree species (Table 1). According to the results of the field and laboratory research, the genetic differentiation of tested clones with respect to biomass production in fresh and dry matter has been determined [1, 2].

The strategic interest of every country is to increase the level of its energy independence. The only path towards achieving this goal is to test the possibilities of using alternative energy sources. Among alternative energy sources, the so-called renewable energy sources and their advantages in terms of environment protection and sustainable development attract particular attention. By signing certain agreements (e.g. the Kyoto Protocol), the majority of European countries have committed themselves to taking concrete steps in increasing the share of renewable energy sources of the total energy balance.

Biomass is a renewable energy source with the highest potential in Croatia. The national BIOEN program [3, 4] was initiated with the aim of promoting the use of bioenergy for energy purposes. Both Europe and Croatia are faced with the problems of agricultural production of low profitability on marginal and abandoned soils. More recently, such production has additionally been aggravated by adverse climatic changes, soil and water pollution, lack of energy and depopulation in areas with dominant extensive agricultural

TABLE 1. Tested willow clones for biomass production in short rotation forestry (SRF)

Taxon	Clone
Salix alba	'107/65/1', '107/65/6', '107/65/7', '107/65/9', 'V 158', 'B 44', 'B 72', 'B 84', '73/64/8', 'V 161', 'V 160', 'V 111', 'V 83', 'V 95'
S. alba var. *calva* × *S. alba*	'V 052', 'V 0240'
(*S. alba* × *S. alba* var. *vit*.) × *S. alba*	'V 093'
Salix ×*savensis* (*S. alba* × *S. fragilis* × *S. caprea*)	'V 221'
S. matsudana × *S. alba*	'V 277', 'V 278', 'V 279'
S. matsudana × [(*S. m.* f. *tortuosa* × *S. alba*) × (*S. m.* f. *tortuosa* × *S. alba*)]	'V 369'
S. matsudana × (*S. matsudana* × *S. alba)*	'V 373', 'V 374', 'V 375', 'V 458', 'V 459', 'V 460', 'V 461'
(S. matsudana × *S. alba)* × *unknown*	'V 462', 'V 571', 'V 572'
S. matsudana × *unknown*	'V 573', 'V 574', 'V 575', 'V 576', 'V 577', 'V 578', 'V 580'

production. Short Rotation Forestry (SRF) and biomass production follow world trends and aim to put renewable energy sources to better use without generating additional quantities of CO_2, otherwise largely present in fossil fuels.

Biomass of forest tree species can also be produced by intensive cultivation of fast-growing species in SRF. The basic function is the production of biomass as a renewable and ecologically acceptable energy source. However, these species can also be an alternative "agro" culture (in poorer sites), or they can act as agricultural soil diversifiers, as well as provide the possibility of ecologically more acceptable methods of waste water and phytoremediation. In addition, they sequester increased quantities of atmospheric carbon dioxide [5, 6].

In recent research, clones of arborescent willows manifested the highest biomass production potential in short rotations of up to ten years on marginal (clay type of soil, reed habitat), but particularly on optimal soils [1, 7-13]. Tests of different arborescent willow clones continued with the purpose of identifying those with the highest biomass production potential, quality and calorific value, especially on so-called marginal soils [14-17].

MATERIALS AND METHODS

A field trial with ten White Willow (*Salix alba* L.) clones and hybrids with Chinese Willows (*Salix matsudana* Koidz.) was established with cuttings in the area of Valpovo Forest Office, in the eastern part of Croatia (FT1) on a more optimal site within the nursery. At ramets age at 2/3 and 2/5 years, breast diameters and heights were measured, survival and the number of shoots per tree stump was identified.

Tests were established according to the randomized block system design in four repetitions. Each clone is represented with at least 30 ramets planted in 1.3 × 0.8 m spacing. The offshoots were cut, biomass was measured and evaluated on two-year-old sprouts, and the survival of the tested clones was determined. In the year of test establishment, as well as after the first cutting, weed vegetation was mechanically regulated.

All two-year-old shoots from the same stump were cut in each repetition and their weight in fresh condition was measured. Mass samples of 0.5 kg were collected randomly from the cut shoots, separately for each clone. The samples were dried at 105 ^{o}C until constant mass was achieved. The ratio between fresh and dried matter sample was used to determine the average shares of moisture in the wood of each clone. These were then used to assess dry biomass of cut shoots ($t \cdot DM \cdot ha^{-1} \cdot a^{-1}$). Breast diameters and dry biomass of cut shoots were equalized by means of a non-linear regression method. Then, using the obtained regression models (for each clone separately) and the previously measured breast diameters, dry biomass of each particular shoot in the tests was assessed. The production of clone biomass per hectare was assessed in relation to the value of dry biomass of mean shoot, survival, distance between the ramets and the average number of shoots per root.

The data obtained from the measurements were processed by means of descriptive statistical analysis to determine the average, minimal, and maximal values, as well as standard deviations for the investigated properties. All statistical analysis and analysis of variance were performed using the software package Microsoft Office Excel.

Non-linear regression analysis was used to assess dry biomass of particular shoots according to the following equation [18]:

$$W = a + bD_{1.3}^{c};$$

where:

W - dry biomass of shoots

$D_{1.3}$ - breast diameter of shoots

a, b, c - parameters assessed on the basis of the shoot sample cut in one repetition.

Rank correlation was calculated by formula:

$$r_s = 1 - \frac{6 \sum_{i=1}^{n} D_i^2}{n(n^2-1)}$$

RESULTS AND DISCUSSION

Figure 1 and Table 2 shows total biomass production of the investigated clones of arborescent willows in the Valpovo test in first and second successive two-year rotations. In the first years of research (at age 2/3 years), the mean biomass production amounted to 19.1 t·DM·ha^{-1}·a^{-1}, with values ranging from 10.4 (clone 'V 052') to 25.7 t·DM·ha^{-1}·a^{-1} (clone 'V 95'). In the following rotation (age 2/5 years), the mean production was 20.6 t·DM·ha^{-1}·a^{-1} and ranged from 15.2 (clone 'V 461') to 25.0 t·DM·ha^{-1}·a^{-1} (clone 'V 578').

Value of rank correlation for two successive rotation period (2/3 and 2/5 years) was negative (r = -1.08) indicating that there is no clonal phenotypic stability of the biomass production. It also confirms that the production of each clone after the first and second rotation period differs significantly. Four clones which are in the process of DUS testing ('V 374', 'V 461', 'V 95', 'V 578') demonstrated the highest biomass productivity and consistency in the production during the tested of two rotation periods.

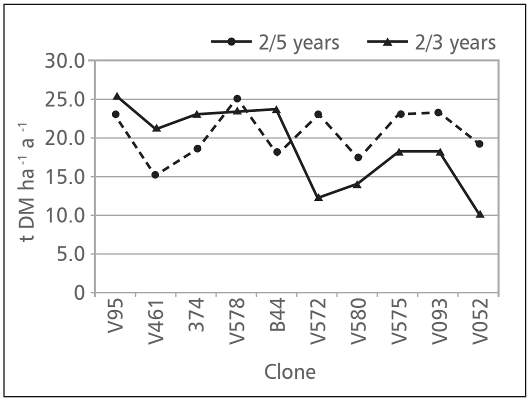

FIGURE 1. Biomass production of the studied willow clones in Valpovo test in two successive rotations period (2/3 and 2/5 years)

Analysis of variance showed that biomass production in both rotation periods (age 2/3 and 2/5 years) didn't show significant genotypic

TABLE 2. Average biomass production of tested willow clones (Valpovo test)

No.	Clone	Botanical name	Average dry biomass (t·DM·ha^{-1}·a^{-1}) 2/3 years	2/5 years
1	**'B 44'**	*Salix alba*	23.8	18.2
2	**'V 093'**	(*S. alba* × *S alba* var. *vitellina*) × *S. alba*	18.5	23.5
3	**'V 052'**	*S. alba* var. *calva* × *S. alba*	10.4	19.3
4	**'V 374'**	*S. matsudana* × (*S. matsudana* × *S. alba*)	22.9	18.8
5	**'V 461'**	*S. matsudana* × (*S. matsudana* × *S. alba*)	21.1	15.2
6	**'V 95'**	*Salix alba*	25.7	23.1
7	**'V 580'**	*S. matsudana* × unknown	14.3	17.4
8	**'V 572'**	(*S. matsudana* × *S. alba*) × unknown	12.2	23.0
9	**'V 578'**	*S. matsudana* × unknown	23.3	25.0
10	**'V 575'**	*S. matsudana* × unknown	18.3	23.0
		Average	**19.1**	**20.6**

differences in biomass production (F = 1.93, F = 1.94). Greater clonal differentiation can be expected at older plantation ages (e.g. at the third or fourth rotation). According to earlier investigations biomass production of all the investigated willow clones shows a different trend in proportion with the test age, except in few clones, in which, after the initial increase, a decrease in the production was recorded between the second and the third rotation [9, 12, 13].

Among all the studied clones, the clones 'V 374', 'V 461', 'V 95', 'V 578' are singled out in terms of their excellent adaptedness to the habitat and to developmental conditions. In addition, they display above-average values of dry biomass of the mean shoot, or total biomass production, as well as sprouting ability. In these experiments and in two rotations, the best phenotypic stability and steady production were manifested by clones, 'V 374' and 'V 461', which originated from backcross hybrids *S. matsudana* × *(S. matsudana* × *S .alba*), 'V 578' (*S. matsudana* × unknown) and by White Willow clone 'V 95' (*S. alba*). With their average production in two-year rotations varying between 15.2 and 25.7 t·DM·ha^{-1}·a^{-1}, they manifested considerable potential for dry wood biomass production on heavy clay type of soils and marginal lands. These clones, with regard to the partial application of agro technical measures could achieved exceptional production and phenotypically represent highly unstable clones of high productivity with specific adaptability to optimal sites. Highly productive pioneer species are grown as monoculture plantations in short rotations. Short duration rotation coppice (SRC) system with willow and short duration rotation single stem (SRSS) systems with poplar species on very suitable soils in Eastern Europe at the age of 5 years results with the productivity of 13.8 to 18.1 t·ha^{-1}·DM for willow and poplars and from 17.7 - 21.8 t·ha^{-1}·DM for *Miscanthus* [19].

CONCLUSIONS

Short rotation coppices with clones of arborescent willows were established on so-called marginal sites, i.e. on abandoned agricultural soils or soils not suitable for the cultivation of more valuable forest tree species. No nutrition or pest control measures were applied (a practice otherwise widely used in intensive cultivation system), while weed vegetation was regulated only at the earliest age. Despite this, the results showed relatively high biomass production of the studied clones. Average dry biomass production of the studied clones at age 2/3 years was 19.1 and in the second two-year rotation at the age 2/5 years it was 20.6 DM·ha^{-1}·a^{-1}. The highest production was manifested by the clones that taxonomically belong to different crossing combinations of Chinese and White Willow and perspective and phenotypically more unstable clones showed average production of more than 20.0 t·DM·ha^{-1}·a^{-1}.

From the results obtained it can be seen that the possibilities of biomass production with the selected arborescent willow clones in Croatia are optimal with regard to the stand production potential and selected genotype assortment. The biomass production can be improved considerably by fertilization which, however, can be avoided by proper selection of clones with low nutrient requirements. In order to increase biomass production, silvicultural measures should be applied so as to reduce the number of shoots to one to two per stump, while production on marginal sites could be considerably increased with the application of intensive silvicultural and agro technical measures.

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Original scientific paper

Canopy Layers Stratified Volume Equations for *Pinus caribaea* Stands in South West Nigeria using Linear Mixed Models

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Abstract

Background and Purpose: Efficient forest stand management requires reliable estimates of growing stock. The reliability of stem volume estimates depends on the range and extent of available sample data. The potentials of canopy layers stratification in pure plantations as a means of improving the accuracy of stem volume equations have not been fully explored. Linear Mixed Model (LMM) approach is a statistical technique capable of yielding a more efficient prediction under clustered data structure. This study investigates the existence and potentials of canopy stratifications for improving the reliability of stem volume prediction equations under pure plantations using linear mixed model approach.

Materials and Methods: *Pinus caribaea* Morelet plantations in Oluwa Forest Reserve, Ondo State, Nigeria were investigated. Individual tree growth variables, including diameters, heights and crown measurements were obtained in 2010 on twenty-five 0.04 ha plots representing five different stands planted between 1979 and 1991. Visual assessment of the trees within each plot was also done to classify them into four canopy strata (i.e. dominant, co-dominant, intermediate and suppressed). Linear mixed model approach was used to analyze the tree growth data using SAS Proc Mixed. Two variants of volume equations; simple linear and exponential were investigated.

Results: Results show that simple linear mixed model consistently give better fit criteria (e.g. AIC) of 135.8, 18.9, -214.7 and -174.6 under dominant, co-dominant, intermediate and suppressed canopy layers, respectively. The covariance parameter estimate for dominant canopy (0.2219) is about 370 as large as that of suppressed (0.0006). This implies that canopy layers not only influence stem volume prediction but also reduce within-stand variance as well.

Conclusion: Simple linear mixed model produced better fitting criteria in terms of lower values of Akaike's and Bayesian Information Criteria. Canopy stratification in pure stands of *Pinus caribaea* showed great potentials in improving the predictive ability of volume equations.

Keywords: stem volume equation, *Pinus caribaea* Morelet, dominant, co-dominant, intermediate, suppressed, linear mixed model

INTRODUCTION

Sustainable forest management requires reliable estimates of growing stock. Such information guides forest managers in timber evaluation as well as in the allocation of forest areas for harvest. For timber production, an estimate of growing stock is often expressed in terms of timber volume, which can be estimated from easily measurable tree dimensions. The most common procedure is to use volume equations based on relationships between stem volume and tree growth variables such as diameter and height. The reliability of volume estimates depends on the range and extent of the available sample data. In Nigeria, considerable work has been done on the development of volume equations for planted forests [1-5]. However, the effects of canopy layers on volume equations have not been fully investigated.

Classifications of individual trees into crown classes on the basis of their relative dominance in a stand have been made for many years. Four classes (i.e. dominant, co-dominant, intermediate and suppressed) are commonly recognized in forest ecology [6, 7]. Crown classification has been useful for predicting mortality, assessing tree vigour and rating tree resistance to certain diseases. However, for most pure plantations, the potentials of canopy layers for improving the accuracy of volume equations have not been fully explored. It has been established that when competition occurs between trees in even-aged stands or even-aged groups the trees begin to differentiate into crown classes.

A number of modeling techniques have been used for modeling stem volumes ranging from stand levels to individual tree approach. An important area that has been consistently overlooked is the fact that stem volume data are generally taken from trees growing in plots, located in different stands and of course under different canopy layers. This hierarchical structure usually results in a lack of independence between observations. This consequently results in biased estimates for the confidence interval of parameters if ordinary least squares regression techniques are used. To deal with this problem, mixed model approaches have been introduced.

Linear mixed model approach is a statistical technique generating improvements in parameter estimation. The approach has been used in many fields of study for nearly more than twenty years. However, in forestry, studies using mixed effects models are relatively recent. Lappi and Bailey [8] described the use of non-linear mixed effects growth curve based on Richards's model. The model was fitted to predict dominant and co-dominant tree height, both at plot and individual tree levels. Gregoire et al. [9] studied linear mixed effects modeling of the covariance among repeated measurements with random plot effects. Zhang and Borders [10] used the mixed effects modeling method to estimate tree compartment biomass for intensively managed loblolly pine stands in USA. Fehrmann et al. [11] used mixed effects modeling to establish single-tree biomass equations for Norway spruce and Scots pine. Furthermore, application of linear mixed models was investigated in many other studies [e.g. 12-18]. Mixed effects models estimate both fixed and random parameters simultaneously for the same model. This results in consistent estimates of the fixed parameters and their standard errors. Furthermore, the inclusion of random parameters captures more variation among and within individuals or subjects.

Linear mixed models are capable of predicting random plot or tree effects unlike ordinary regression models. Hence, they are capable of yielding a more efficient prediction. Linear mixed models also allow for the explicit separation of the between and within canopy layers relationships and thus have the potentials for a correct specification of the model. The objectives of this study were to: (i) develop volume equations for *Pinus caribaea*

Morelet (Pine) in south west Nigeria based on linear mixed modeling approach; (ii) determine and account for variance-covariance structure within individual trees; and (iii) evaluate the predictability of mixed model based on the calibration.

MATERIALS AND METHODS

Field Data

Field data were obtained from Pine stands in Oluwa Forest Reserve, Ondo state, Nigeria. The reserve lies between latitudes 6° 35' and 7° 00' North and longitudes 4° 25' and 4° 55' East. The Pine stands in Oluwa Forest Reserve were established around two local communities namely, Epe-makinde (comprising of 1989, 1990 and 1991 stands) and Omotosho along Lagos-Ore road (comprising of 1979 and 1980 stands). Figure 1 shows the map of Oluwa Forest Reserve with the study locations.

The stands were planted with initial spacing of 2 m x 3 m. There was no record of thinning in the stands. Majority of the soils in the Forest Reserve are representative of soils in the Ondo Association. These comprise of well drained, mature, red stony and gravely soils in the upper parts of the sequence, grading into the hill wash overlying original parent material or hardpan layers in the valley bottom [19]. The texture of the topsoil in the Reserve is sandy loam, which gradually becomes heavier as soil depth increases. The sub soils consist largely of clay with gravel occurring at 30-60 cm depth. According to Orwa et al. [20], Pine grows best in frost-free areas up to about 700 m altitude in more fertile sites with good subsoil drainage and annual rainfall of

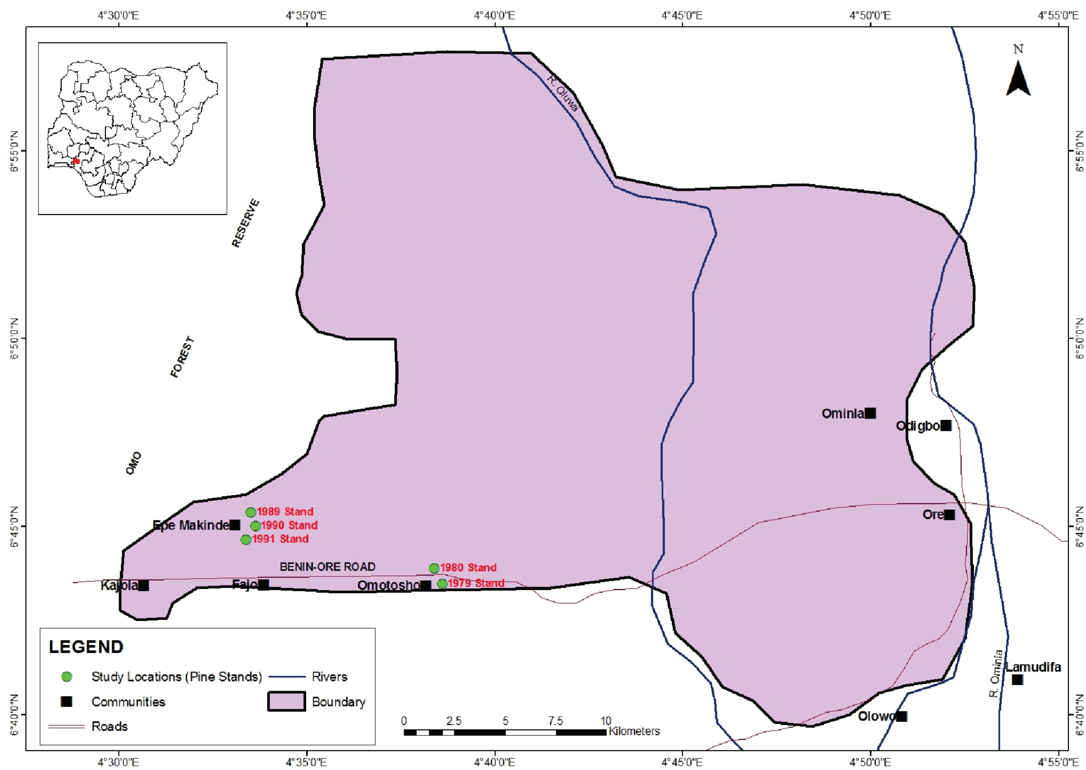

FIGURE 1. Map of Oluwa Forest Reserve showing the study locations

2000-3000 mm. Soil requirements for Pine is usually loams or sandy loams. In some cases high amount of gravel and generally well drained soils have proved suitable. The soil ph is usually between 5.0 and 5.5. The species is rated as moderately fire resistant. It tolerates salt winds and hence may be planted near coast.

Data were collected from Pine stands established in 1979, 1980, 1989, 1990, and 1991. Five temporary sample plots of size 20 m x 20 m were randomly laid in each of the stand ages. Within each plot, tree growth variables, including diameter at breast height, base, middle and top (merchantable limit), total and merchantable heights, crown length and diameter were obtained in 2010. In addition, visual assessment of the trees within each plot was also done to classify them into four canopy layers (i.e. dominant, co-dominant, intermediate and suppressed). Individual stem volume was computed using the Newton's formula. This is given as

$$v = \frac{mht}{6}[A_b + 4A_m + A_t] = \frac{\pi(mht)}{24}[d_b^2 + 4*d_m^2 + d_t^2]$$

where v is stem volume; mht is merchan-table height; A_b, A_m and A_t are cross sectional areas at the base, middle and top; d_b, d_m and d_t are diameters at the base, middle and top. Table 1 gives the summary statistics of the measured and derived variables at individual tree level.

Linear Mixed Model Specification

A mixed model is constructed by incorporating a random component, denoted by z_u, into the conventional formula of a linear model given by $y = X\beta + \varepsilon$. Using a matrix notation, a linear mixed model (LMM) can be written as follows:

$$Y = X\beta + Zu + \varepsilon$$

where Y is the vector of measurement of the study variable, Xβ is the fixed part of the model (similar to the standard linear models) such that X denotes the (n x p) observation or design matrix and β denotes the unknown (p x 1) vector of fixed intercept and slope effects of the model. Zu+ε is the random part, where u is a (q x 1) vector of random intercept and slope effects with an assumed q-dimensional normal distribution with zero expectation and (q x q) covariance matrix denoted by G and Z is the (n x q) design matrix of the random effects. Note that the structure of the covariance matrix G is not specified. The residuals ε can be correlated and the possibly non-diagonal covariance

TABLE 1. Summary statistics of the measured and derived variables of Pine at individual tree level

Variable	Valid N	Mean	Min	Max	SD	SE	CV
age	461	24.01	19.00	31.00	5.14	0.24	0.2141
dbh	461	24.50	7.48	48.13	7.82	0.36	0.3193
tht	461	18.18	5.10	28.75	4.65	0.22	0.2557
mht	461	12.89	2.95	24.40	4.02	0.19	0.3116
cd	461	3.55	1.10	6.85	1.17	0.05	0.3295
cl	461	6.07	1.89	83.10	4.04	0.19	0.6650
d_b	461	28.36	9.10	52.25	8.49	0.40	0.2995
d_m	461	17.08	5.00	39.00	6.51	0.30	0.3809
d_t	461	10.90	3.00	26.00	4.15	0.19	0.3810
v	461	0.4458	0.0110	2.1417	0.3927	0.0183	0.8809

matrix of the residuals is denoted by R. A key assumption for linear mixed model is that u and ε are normally distributed such that

$$E\begin{bmatrix} u \\ \varepsilon \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \end{bmatrix}$$

$$Var\begin{bmatrix} u \\ \varepsilon \end{bmatrix} = v = \begin{bmatrix} G & 0 \\ 0 & R \end{bmatrix}$$

where G is the variance – covariance matrix of U and R is the variance –covariance matrix ε. The variance of the response variable y is $V = ZGZ^T + R$ and can be estimated by setting up the random – effects design matrix Z and by specifying covariance structure for G and R. If R is specified as a diagonal matrix, LMM only considers the random effects such as blocks which can be reflected by G. Thus, the LMM with diagonal R and a specific G is normally called as the LMM block model.

For the purpose of model prediction, it is required not only to estimate the parameters of the fixed and covariance, but also to estimate the random effects. The solutions for β and u in LMM are called the best linear unbiased predictor (BLUP). In the LMM the fixed-effect parameters, β and the covariance parameters, θ (i.e. θ_G and θ_R for the G and R, matrices, respectively) are estimated. Note that by definition, random effects are random variables. Assume that the q random effects in the u_i vector follow a multivariate normal distribution, with mean of vector 0 and a variance-covariance matrix denoted by D. Usually, the maximum likelihood (ML) and restricted maximum likelihood (REML) estimation methods are commonly used to estimate these parameters. In general, ML estimation is a method of obtaining estimates of unknown parameters by optimizing a likelihood function [21]. The REML is an alternative way of estimating the covariance parameters in θ. REML is sometimes called residual maximum likelihood estimation. It is often preferred to ML estimation, because it produces unbiased estimates of covariance parameters by taking into account the loss of degree of freedom that results from estimating the fixed effects in β.

Model Assessment

Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC) are commonly used for model selection and comparison [22]. The AIC may be calculated based on the ML or REML log-likelihood, l (β, θ), of a fitted model as follows:

$$AIC = -2 \log L + 2(p + k + 1)$$

where L is likelihood, p is the number of fixed effect terms and k is the number of random effect terms. The candidate model with the lowest AIC is selected as the best model. The BIC may be calculated as follows:

$$BIC = -2xl(\hat{\beta},\hat{\theta}) + px\ln(n)$$

The BIC applies a greater penalty for models with parameters than does the AIC, because the number of parameters being estimated is multiplied by the natural logarithm of n, where, n is the total number of observations used in estimation of the model. It has been suggested that no one information criterion stands apart as the best criterion to be used when selecting LMM and that more work still needs to be done in understanding the role that information criteria play in the selection of LMM [23].

Furthermore, likelihood ratio test (LRT) has been used extensively as a tool for testing the significance of random effects in LMM. To test the significance of one random effect, it assumes the effect has zero variance in the null hypothesis. Thus, the test statistic of LRT is defined as:

$$-2\ln\lambda_N = -2\ln\left[\frac{L_{REML}(\hat{\theta}_{REML,0})}{L_{REML}(\hat{\theta}_{REML,1})}\right]$$

where $\hat{\theta}_{REML,0}$ and $\hat{\theta}_{REML,1}$ are restricted maximum likelihood estimates under the null-hypothesis and under the alternative hypothesis respectively [24]. The statistic $-2\ln\lambda_N$ follows a χ^2 distribution with de-grees of freedom equal to the difference of the number of parameters of random effects in the model under the null and alternative hypotheses.

Model Implementation

In this study, a close investigation of the scatter plot of the stem volume (v) against diameter at breast height (dbh) revealed approximately a curvilinear shape. Therefore, two functional forms of tree v – dbh relationship (i.e. simple linear and exponential models) were investigated under the four canopy layers using linear mixed model approach. The model forms are as follows:

$$v = \beta_0 + \beta_1 dbh + \varepsilon$$

$$\ln v = \ln\beta_0 + \beta_1 dbh + \varepsilon$$

where ln is natural logarithm, β_0 and β_1 are regression coefficients to be estimated and ε is the model error term. Model residuals are defined as the difference between the observed and the predicted stem volume.

RESULTS

Model Fitting

Table 2 indicates that simple linear mixed models consistently had significant improvement over the exponential models under the four canopy layers. Both AIC and BIC are consistently lower in simple linear mixed models across the canopy layers. Table 2 also displays the covariance parameter estimates. The covariance estimate for dominant canopy layer (0.2219) under simple linear mixed model is about 370 times larger than that of the suppressed canopy (0.0006). This implies that canopy layers not only have significant influence on stem volume prediction but also reduce their within-stand variances as well. The covariance parameter estimates for the different canopy layers under the exponential model do not show any definite order. This further confirms the non suitability of the equations.

Model Coefficients of Fixed Effects

The estimates of coefficients of fixed effects and standard error (SE) of estimates of the models across the crown layers are

TABLE 2. Model fitting statistics under the two modeling approaches across the crown layers

Model	AIC	BIC	-2Res log likelihood	Covariance Parameter Estimate
Simple Linear				
Dominant	135.8	138.4	133.8	0.2219
Co-dominant	18.9	22.0	16.9	0.0628
Intermediate	-214.7	-211.6	-216.7	0.0136
Suppressed	-174.6	-172.9	-176.6	0.0006
Exponential				
Dominant	172.1	174.7	170.1	0.3225
Co-dominant	299.2	302.3	297.2	0.3296
Intermediate	250.3	253.3	248.3	0.2901
Suppressed	74.1	75.8	72.1	0.3387

TABLE 3. Estimates of model coefficients and standard errors (SE) of the fixed effects

Model	β_0	SE (β_0)	β_1	SE (β_1)
Simple Linear				
Dominant	0.921	0.0473	0.469	0.0335
Co-dominant	0.471	0.0192	0.250	0.0135
Intermediate	0.219	0.0094	0.116	0.0066
Suppressed	0.041	0.0037	0.024	0.0026
Exponential				
Dominant	-0.221	0.0571	0.565	0.0404
Co-dominant	-0.896	0.0439	0.572	0.0310
Intermediate	-1.658	0.0434	0.537	0.0307
Suppressed	-3.366	0.0909	0.575	0.0643

presented in Table 3. Both the simple linear and exponential models produce significant main effects ($p<0.0001$). The standard error values generally are low. However, under the simple linear mixed models the standard error values are lower and also show a decreasing order from dominant canopy to suppressed canopy layer. This implies better fit.

DISCUSSION

The results obtained in this study have demonstrated that LMM has the capacity to improve volume equations under different canopy layers. The simple linear model approach produced better models judging from the lower values of AIC and BIC in contrast with the exponential models. This agrees with past studies [e.g. 25]. However, it is noteworthy that LMM with better fitting does not necessarily produce better model prediction [26].

Crown layer stratification has demonstrated the potentials to improve stem volume – DBH relationship. This is evident from the consistent lower standard error values in the models across the canopy layers. This finding confirms the assertions from previous studies [e.g. 27, 28] of the existence and influence of canopy layers. The use of linear mixed models was designed to deal with the spatial heterogeneity in the data. Thus it produced more reliable estimates of fixed effects and smaller intra block variances of residuals (indicating the reduction of spatial heterogeneity in model residuals).

One area that obviously requires further study is the aspect of evaluating the influence of different covariance structure specification on the LMM with canopy stratification.

CONCLUSIONS

In this study, two variants of canopy layers stratified volume equations were investigated using linear mixed model approach (i.e. simple linear and exponential models) for *Pinus caribaea* stands in Oluwa Forest Reserve. The results indicated that, in general, simple linear mixed models produced better fit judging from the significantly reduced information criteria. This is an indication that LMM has the capacity to address effectively, spatial heterogeneity often encountered in forest growth data. The study also demonstrated the potentials of canopy layer stratification in improving volume prediction. Future work is required to focus on investigating the influence of different covariance structure specifications on the LMM performance. Furthermore, recent studies suggest that better model fitting does not necessarily imply better prediction. This also requires further study to ascertain this claim.

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Preliminary communication

New Records of *Bruchidius* Spermaphagous Species in *Albizia julibrissin* and *Laburnum anagyroides* and Their Parasitoid Complex in Serbia

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Abstract

Background and Purpose: *Bruchidius villosus* feeding in seed of *Laburnum anagyroides,* and *Bruchidius terrenus* seed pest of *Albizia julibrissin* are first recorded and completely new seed-beetle to Serbian Bruchinae fauna. This Chrysomelids which were found in Republic of Serbia during intensive studies from 2012 to 2014 are likely related to a mostly Paleotropical group, including also members of genera *Bruchidius*, *Megabruchidius* and *Acanthoscellides*. These seed-beetles develop in pods of these two woody legumes, widely grown ornamental trees and shrubs. Several recent reports reveal that this species are well established in France, Hungary, and Bulgaria.

Materials and Methods: Bruchine and their legume hosts were observed by extensive field sampling throughout Serbia over three years and by rearing the beetles from the samples in the laboratory. Bruchines and the parasitoids were mass-reared in climate controled rooms under conditions close to those of their area of origin: 12:12 L:D, 3-23 °C and ≤80 % RH (depends of host plant ongoing phenology or experimental needs-proof of weevil monophagous feeding preferences). For the purpose of analyzing the observed phenomena (its intensity and relevance), some of the standard methods of statistical analysis and conclusion have been used.

Results: Levels of seeds infestation still in the pods were high and comparable to other studies. Bruchine beetle infestation in the dehiscent fruits of host plants may be greater after the seeds and pods drop to the ground, as bivoltine generation occurs, but this has yet to be tested. Hypotheses on the geographic origin of this new species are also discussed. The effect of native parasitoids occurrence could potentially be interesting, given that their appearance suggest their specialization on the *Bruchidius* beetle species which is a common seed-predator on the leguminous seeds.

Conclusions: The establishment of this new species is investigated using both morphological data and idioecological analyses. For this purpose, a methodology was developed to assess weevil field densities in a natural environment. However, this needs to be more carefully tested with a larger sample size and experiments. Significant levels of infested seed, leads to the conclusion that these seed parasites could be an important reducing factor of generative reproducing host plant potential.

Keywords: Bruchine, legumes, weevil, parasitoids, pods, seed predation, Serbia

INTRODUCTION

The Old World genus *Bruchidius* Schilsky, 1905 (Coleoptera: Chrysomelidae: Bruchinae), comprises of about 300 species of seed beetles, widespread in the Old World [1]. Some species were introduced with seeds or soil in territories outside their native areal [1, 2]. For European countries 80 species of the genus were recorded [3]. *Bruchidius* is the most heterogeneous genus within Bruchinae [4]. Recent investigations of it reveal several phylogenetic groups that are associated with host-plant taxonomic groups [5]. Nevertheless, the genus is not divided into smaller genera, because some species are intermediate concerning morphological characters and bionomic [4]. Most *Bruchidius* species are reported to feed in the larval stage on the seeds of legumes (Fabaceae), Bruchine chrysomelids are economically important pests of agricultural and stored productsworldwide. *Albizia julibrissin* Durazz (Fabaceae: Faboideae: Genisteae) and *Laburnum anagyroides* L. (Leguminosae, Genisteae) both are planted as ornamental trees in Serbia. We studied the seed beetles *Bruchidius terrenus* (Sharp, 1886) and *Bruchidius villosus* (Fabricius, 1792), which infest the seeds of *A. julibrissin* and *L. anagyroides*, respectively, and their parasitoids, as native entomofauna in progressive adaptation process. Some species are pests due to the losses caused to economically important plants.

Host Plant characteristics

Laburnum anagyroides Golden chain, or Scotch broom is a densely branched medium shrub (1 - 2 m in its native area), with attractive yellow flowers (16 - 25 mm) that bloom between February and July, depending on the locality. This plant mainly grows on acidic soils, in scrubland accompanying oak, beech and pine woodlands up to 2000 m. It is found in clearings on deep, fresh soil in a large part of western Asia and in Europe across to the Canary Islands, including the entire Iberian Peninsula with the exception of provinces with predominantly limestone soils. It has been accidentally introduced to North and South America, Australia, Hawaii and New Zealand as an ornamental plant [6-8]. Scotch broom is dispersed by seeds that fall roughly 2 m from the parent plant and may be dispersed more widely by other dispersive factors and agents such as ants [9]. Its ballistic seed dispersal mechanism permits a high-potential establishment of new individuals despite its low seed density [10].

Persian silk tree, pink silk tree - *Albizia jullibrisin* (Durazz., 1772. non sensu Baker, 1876) (Fabales: Fabaceae) are native to Turkey and planted as ornamental trees. It is a popular ornamental tree planted singly, in groups or lining roads throughout Serbia. Also known as "Mimosa", *A. julibrissin* origin is from Middle and Eastern Asia. In the last three centuries it was introduced as an ornamental tree in many countries in Europe, North America and Asia due to the beauty of its flowers, fern-like leaves and umbrella-like canopy [11].

MATERIAL AND METHODS

L. anagyroides and *A. jullibrisin* seed pods were collected at the many localities in 2012, 2013 and 2014. Pods were held in the laboratory in plastic boxes and transparent bags until the emergence of adult beetles and parasitoids. Each sampled population of pods and/or seeds was put in a bag connected to a clear bottle or tube, following Fursov [12], and kept under semi-natural room conditions - as it is in the regions where collections were made. Emerging weevils and its parasitoids trapped in the bottles were collected daily for a month and weekly later on until no more adults emerged. Weevil's adults were identified by external morphological traits. The insect specimens and part of plant specimens were deposited at the Institute of Forestry, Belgrade and Faculty of Forestry, Belgrade. We extensively reviewed the published work for weevils associated with studied host plants and their geographical distribution to study host ranges of the beetles. Ripe pods of *A. julibrissin* were collected from several isolated trees in October 2013 and from the following several localities in municipality of Ruma, Deteline and Kudos city parts. Additional material was collected in April 2014 from Ruma (Vojvodina). The material was stored in plastic boxes in laboratory conditions. In the periods June - October 2013 and May-June 2014 emer-gence of adult seed beetles was observed. The level of damage caused by the larvae was established in Northern Serbian population. For this purpose, 300 pods of Mimosa were collected; seeds were extracted and observed for emergence holes. Whole seeds were dissected for estimating if they are infested or not. Bruchids were identified after Borowiec [1], Hoebeke et al. [3] and Morimoto [5] and deposited in the author's collection.

Bruchine beetle specimens had emerged from the seeds of legume host plants (Table 1), and mean level of infested seed explored for the localities in Serbia are present in Tables 1 and 2. There are also data about insect stocks cultures bionomic and life cycle developments collect by seed dissection (100 seed per locality). In the laboratory, weevils and the primary parasitoids were mass-reared in climate controled rooms under con-ditions close to those of their area of origin: 12 : 12 L : D , 3 - 23 °C and ≤ 80 % RH (depends on host plant ongoing phenology or experimental needs-proof of weevil monopha-gous or else feeding preferences). Percentages of total pre-dispersal and post-dispersal (in reinfested material), makes the-se insects serious host plant suppression candi-dates. Their bionomic were monitored by conti-nuous infested seed collecting, its disection and observed also in correlation with host specificity ranged from monophagy (at least ecological monophagy) to possible oligophagy. On the basis of presence/absence data we tested the null hypothesis assuming that plant taxa and seed consuming weevil species form congruent phyletic relations at the species level [7, 13]. For this purpose, pods of *L. anagyroides* were collected in April 2012 and 2014, than in 2013 during May and August and in April 2014; 300 seeds were also extracted and observed for emergence holes during spring, April, May and June in 2014.

TABLE 1. Host plant and seed beetles native range, sampling localities and sampling date in Serbia

	Host plant	Seed beetle	Sampling localities and sampling date
	Albizia julibrissin	*Bruchidius terrenus*	Ruma 1. Kudos (August 2012; June - Octobar 2013); 2. Deteline (August 2013; April 2014)
Native range	Southwestern and Eastern Asia	Eastern Asia	
	Laburnum anagyroides	*Bruchidius villosus*	1. Belgrade - Bezanijska kosa (April 2014) 2. Belgrade, Cukarica (May 2012; May, August 2013) 3. Novi Sad, City Fair (April 2014)
Native range	Mountains of Southern Europe from France to the Balkan Peninsula	Native European range	

RESULTS

Insect stocks

Bruchidius terrenus (Sharp, 1886) is native to eastern Palaearctic region, where it is considered as an important pest of *A. julibrissin* [11]. Morimoto [5] reported *Robinia pseudoacacia* (L.) and *Acacia confuse* Merr. as hosts of *B. terrenus* too. Recently, *B. terrenus* was recorded as adventive species in seven southeastern states of USA [3, 11]. The same authors gave a key for identification of North American *Bruchidius* species, as well as diagnosis, re-description and seasonal history of *B. terrenus*. In this paper, *B. terrenus* is firstly recorded to the Serbian fauna. The level of damage on seeds of host-plant *A. julibrissin* was investigated in laboratory conditions (Figure 1).

FIGURE 1. *Bruchidius terrenus* in *Albizia jullibrisin*, damaged pods, seeds and adult insect (Orig.)

Bruchidius villosus (Fabricius, 1792) is first recorded and completely new to Serbian fauna as specialist feeding in seeds of *L. anagyroides*. We use an indirect method to address the issue of the existence of seed beetles in this popular ornamental and decorative important leguminous woody species in Republic of Serbia. The value of the system lies in the accuracy of host affiliations. Indication of (Fabaceae host plant vs. Bruchine seed predator) – *pairs existence,* scientifically examined and geographically established, all in numerous previous research, was a lead for insect detection, so their further ecology investigation. Bruchine and their legume hosts were observed by extensive field sampling throughout Serbia over three years and by rearing the beetles from the samples in the laboratory. Golden chainas host plants was the subject of pod material sampling [14.]. Our findings were recognized as imagoes and larvae, as seed predators – *B. villosus* and their reared parasitoid complex.

Seasonal History and Habits

The seasonal history and habits of *B. terrenus* are based mainly on observations by the original collector, supplemented by reference to the biology of *B. villosus* [8].Overwintered, sexually immature adults most likely emerge in late spring and disperse to mimosa where they attain sexual maturity by feeding on pollen. In 2013 adults were first found in June while surveying for the beetle. The latest observation of an adult in the field was mid-August 2013. Mimosa trees flower in Serbia from May through August and the fruits (pods) mature from September to November. Oviposition begins when green pods are forming, probably in early July. Pods ripen from late Aug to Nov and begin to disintegrate soon after but remain on the trees into winter. Female's lay eggs individually (in clusters) on the young pods and cement them in place. Eggs probably hatch in 1-2 weeks, the larvae emerging from the underside of the egg and tunneling into the developing pod. A neonate larva burrows through the pod wall into a soft green seed. Unhatched eggs were still observed on the outside of pods in late July 2013. By mid-August, late instars were found in seeds. During an examination of several trees samples in September 2013, found seeds heavily infested (>80 % examined), whereas seeds of these same trees in Sep 2012 were only slightly infested (<5 % examined). Pupation occurs within a seed inside the closed pod. The pupation period probably takes from 10 to 20 days. New generation adults chew through the seed coat and then chew through the pod coat to escape. Adults emerged indoors from early to late Sep from infested pods collected in Ruma (first author's personal observation). Adult emergence holes were observed on old seed pods collected in early Sep 2013. New adults, after emergence, probably feed on pollen in the fall if flowers are still available. Adults probably overwinter near host trees in plant litter. Based on collecting in 2012 and 2013, adults are found on the host from early to late June to mid-Septembar, so *B. terrenus* appears to be univoltine in Serbia.

The adults of the predispersal *B. villosus* emerge from seeds of *L. anagiroides* and probably other overwintering sites such as the surface of immature pods. Larvae enter through the pod wall and feed on developing seeds. By mid-August, larvae complete development and pupate, and adults emerge from open pods at the end of August or overwinter within seed of closed pods on the plant. *B. vilosus* appears to be univoltine, also, in Serbia.

TABLE 2. Host plant seed infestation cause's and results in percentage (%) with total number of reared insects for two months laboratory observation period

Seed infestation causes and results in percentage (%)							
(a) host plant - ***Albizia julibrissin***							
Seed beetle - *Bruchidius terrenus*	**01.08. - 01. 10.** (2012, 2013) **-** new generation appearance **01.04. - 31.06.** (2013, 2014) **-** overwintered specimens generation	**TSE**	**TIS**	**EH**	**A**	**PA**	**PP**
	Total	300	265	246	207	39	
			88%	82%	69%	13%	15%
(b) host plant - ***Laburnum anagyroides***							
Seed beetle - *Bruchidius villosus*	**01.08. - 01. 10.** (2012, 2013) - new generation appearance **01.04. - 31.06.** (2013, 2014) - overwintered specimens generation	**TSE**	**TIS**	**EH**	**A**	**PA**	**PP**
	Total	300	69	55	43	12	
			23 %	18 %	14 %	4 %	17 %
	Belgrade, Bežanijska kosa	100	34	34	29	5	
			34 %	34 %	29 %	5 %	15 %
	Belgrade, Cukarica	100	24	15	11	4	
			24 %	15 %	11 %	4 %	17 %
	Novi Sad, City	100	11	6	3	3	
			11 %	6 %	3%	3%	27 %

Table notes and legend:

(a) Emergence of the seed beetle *Bruchidius terrenus* from seeds of *Albizia julibrissin* with emergence of beetle parasitoids (Eupelmid and Braconid) from *B. terrenus*.

(b) Emergence of the seed beetle *Bruchidius villosus* from seeds of *Laburnum anagyroides* with emergence of its parasitoids (Pteromalid,Eupelmid, Eulophid and Braconid).

TSE - total seeds examined; TIS - total infested seeds and percentage of infested seeds; EH - number of seeds with emergence holes; A - number of adults; PA - Number of parasitoids (pteromalid, eupelmid, eulophid); PP - Number of parasitoids (PA) divided with total infested seeds (TIS) (in percentage)

DISCUSSION

The dispersal methods used by host plants lead some predispersive predator insects such as Bruchinae to develop a clear specificity in their host plants. In the case of Fabaceae, there is an insect–plant synchronization of biological cycles in which the end of the larval growth stage coincides with the pod dehiscence, the point when the seeds are ejected some distance from the plant, and with them, the mature larvae or recently formed imagoes (as in the case of the *Bruchidius* genera, respectively) [15-17]. Their occupation of the seed until its ejection at maturity explains the strategies used by insects to consume resources and at the same time avoid interspecific competition with other predispersive predator insects growing in certain parts of the seed [16, 18].

L. anagyroides L. (Leguminosae, Genisteae) is a broom species of European origin introduced both accidentally and as an ornamental plant to Australia, New Zealand and America, where it is classified as a noxious invasive species. One of its main seed pests is *B. villosus*, a weevil with a Palearctic distribution and which has been introduced to United States and New Zealand as a biological control agent [19]. Factors influencing the insect's choice of oviposition location are crucial for the plant's reproductive success [20]. We examined *Bruchidius* seed predation on the two legume host plants, in the Republic of Serbia, during climate change environmentally close to wide European Mediterranean region conditions. Future experimental and observational studies are needed to clarify the ecology of host utilization and parasitoid accumulation process of effective bio control of the invading legumes that has become a pantropic species (Table 3).

Hoebeke et al. (2009) [3] reported about 90 % of seeds of some *A. julibrissin* trees in USA were infested with *B. terrenus*. We also, found seeds of this plant species heavily infested with *B. terrenus* – 88 % (Table 1), an Asian seed specialist of *A. julibrissin* that occurs widely in the eastern Palearctic Region, North America, Bulgaria [3, 21]. According to Stojanova [22] the presence of its host plant, an appropriate climate, and the absence of natural enemies are conditions favorable for fast and successful invasion by *B. terrenus* in new territories outside its native range. All this is confirmed by our results opening some new research, such as native parasitoid fauna adaptation to introduced weevil [23]. Nature of this should be tested intensively with a goal of new idioecological relation recognition, biodiversity comparable studies, with special

TABLE 3. Parasitoid complex as potential biological threatening agents for seed pests

Insect	Biology and host preference of pod pests
***Eupelmu* spp.** and ***Anastatus* spp.** (Hymenoptera: Chalcidoidea: Eupelmidae)	Ectoparasi toids of ***B. terrenus*** and ***B .villosus*** larvae
Pteromalid wasps (Hymenoptera: Chalcidoidea: Pteromalidae)	Ectoparasitoids of ***B. terrenus*** and ***B. villosus*** larvae
***Tetrastichus* spp.** (Hymenoptera: Chalcidoidea: Eulophidae)	Encompass (here) ***B. terrenus*** and ***B. villosus*** parasitoids of the first and second order, so it is needed to proceed to the research in order to determine their status – hyper-parasitism
Braconidwasps (Hymenoptera: Braconidae)	Reared specimens as ***B. terrenus*** and ***B. villosus*** larvae parasitoids. Investigation needs to be continued with a goal of getting more specimens, data, status confirmation and species determination

emphasis on new formed intro or interspecies mutualism linkages on Serbian territory.

B. terrenus might have been similarly introduced to Serbia with mimosa nursery stock. This seed predator might become a pest of mimosa in landscape plantings and could even be considered a beneficial addition to our fauna by those who regard mimosa as an invasive species and, therefore, an undesirable plant [24].

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- papers written in the language other than English (e.g. German, Russian, Croatian, Serbian, etc.) should be translated into English.
4. MARJANOVIĆ H, OSTROGOVIĆ MZ, ALBERTI G, BALENOVIĆ I, PALADINIĆ E, INDIR K, PERESSOTTI A, VULETIĆ D 2011 Carbon Dynamics in younger Stands of Pedunculate Oak during two Vegetation Periods (*in Croatian with English summary*). *Sumar list* 134 (11-12): 59-73

- papers in journals with DOI number:
5. VULETIĆ D, POTOČIĆ N, KRAJTER S, SELETKOVIĆ I, FÜRST C, MAKESCHIN F, GALIĆ Z, LORZ C, at al. 2009 How Socio-Economic Conditions Influence Forest Policy Development in Central and South-East Europe. *Environ Manage* 46 (6): 931-940. DOI: http://dx.doi.org/10.1007/s00267-010-9566-3

- Early View papers (published online, waiting to be published in print version):
6. MATOŠEVIĆ D 2013 Box Tree Moth (*Cydalima perspectalis*, Lepidoptera; Crambidae), New Invasive Insect Pest in Croatia. *South-East Eur For* 4 (2): (early view). URL: http://www.seefor.eu/36-vol4-no2-matosevic.html (20 December 2013)

- or (if doi number is available):
7. ANČIĆ M, PERNAR R, BAJIĆ M, SELETKOVIĆ A, KOLIĆ J 2013 Detecting mistletoe infestation on Silver fir using hyperspectral images. *iForest* (early view). DOI: http://dx.doi.org/10.3832/ifor1035-006

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11. OSTROGOVIĆ MZ 2013 Carbon stocks and carbon balance of an even-aged Pedunculate Oak (*Quercus robur* L.) forest in Kupa river basin. PhD thesis, University of Zagreb, Faculty of Forestry, Zagreb, Croatia, 130 p
12. MARJANOVIĆ H 2005 Applicability of forest growth simulator as an additional tool in forest management in Croatia (*in Croatian with English summary*). MSc thesis, University of Zagreb, Faculty of Forestry, Zagreb, Croatia, 110 p

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14. CROATIAN FORESTS LTD 2013 Forests in Croatia. URL: http://portal.hrsume.hr/index.php/en/forests/general/forests-in-croatia (14 December 2013)

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16. FAO 2012 State of the World's Forests 2012. Food and Agriculture Organization of the United Nations, Rome, Italy, 47 p. URL: http://www.fao.org/docrep/016/i3010e/i3010e.pdf (12 December 2013)

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18. THE MINISTRY OF AGRICULTURE, FORESTRY AND WATER MANAGEMENT 2006 Regulation on forest management (*in Croatian*). *Official Gazette* 111/06, Zagreb, Croatia. URL: http://narodne-novine.nn.hr/clanci/sluzbeni/128205.html (10 December 2013)

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