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2009

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„FORESTRY IN ACHIEVING MILLENNIUM GOALS"

HELD OF THE 50TH ANNIVERSARY OF FOUNDATION OF INSTITUTE OF LOWLAND FORESTRY AND ENVIRONMENT

Institute of Lowland Forestry and Environment, Novi Sad, Serbia
Center of Excellence

International Union of Forest Research Organizations

INTERNATIONAL SCIENTIFIC CONFERENCE

„FORESTRY IN ACHIEVING MILLENNIUM GOALS“

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AN INTRODUCTION TO THE MEETING

Scope and Objectives

The scope of the Conference is to provide the answers to the most important challenges in forestry science, taking into account the global climate changes causing significant changes in forest ecosystems, changes due to human activities, as well as the occurrence of the new pathogens and pests. Experts in various disciplines in the spheres of forestry, biology, ecology and environment will exchange their experiences, new knowledge, all in the aim of attaining the millennium goals related to forestry.
By celebrating the 50th anniversary of the Institute, the intention of the Conference organizers is to lay a foundation for the closer cooperation among the European institutions dealing with the research in the field of forestry, in the aim of accomplishing the Institute's mission to become the leader in this field in this part of the West Balkan. The identification of the most significant global objectives and challenges will contribute to the definition of the local priorities, which will by all means enable the research in this region of the Balkan to be directed towards the creation of the appropriate platform necessary for the accelerated development of all forestry disciplines.

Specific Objectives

1. Presentation of the latest research results in forestry,
2. Strengthening the relationships and networking of the scientific and research institutions in forestry,
3. Creating the possibility for the scientific and research institutions in the sphere of forestry to be included in the European research activities

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C O N T E N T

THE ASSESSMENT OF WILLOW PLANTS POTENTIAL FOR TREATMENT OF SOILS IN AREAS CONTAMINATED BY RADIONUCLIDE DEPOSITS

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Summary: *The most effective use of agricultural lands contaminated by radionuclide as a result of Chernobyl disaster is both ecological and economical problem. The alternative method for agricultural crops is short crop rotation willow cultivation. It is possible to use biomass of willow as renewable energy source. The goal of our investigation is the development of special technology methods for willow growing enable to combine high level of biomass production and not extra accumulation of radionuclides. The field experiments show that potassium mineral fertilizer is the key factor for radionuclides accumulation monitoring. It is possible to get normative "clean" biomass of willow cultivated on the soils with the level of cesium pollution to10 Ci/km^2.*
Keywords: *Willow, biomass, radionuclide contaminated soils, fertilizer, potassium, agriculture, renewable energy*

INTRODUCTION

Republic of Belarus does not have an adequate potential of its own fossil fuels supplying, and nowadays we use about 5% of Belarus demand of energy at the cost of local renewable recourses. The National State Program was approved in order to increase this input to 25% till 2012. The most perspective resource of renewable energy in Belarus is bioenergy. Belarus has about 5.7 million hectares of arable lands, and 3 million hectares of pastures. Part of lands may be used for biomass production on the base of cultivation of fast-growing crops like willow. The yield of willow biomass crops may achieve 10-15 tons of dried wood or 5-6 toe per hectare. It means the annual energy potential of willow biomass systems in Belarus is 2.5-3 millions toe.

It is possible to use partially radionuclide contaminated soils for willow cultivation. As a result of Chernobyl disaster the area of the same soils in Belarus is about 1.3 million ha, including 0.8 million ha of arable lands.

On April 26th, 1986 there was an explosion at a nuclear power plant at Chernobyl, on the border between Ukraine and Belarus. Many tons of radioactive materials were thrown into the air. Some of these were carried around the world, but 70 per cent of the radioactive substances blew north over the territory of Belarus.

The Chernobyl accident in 1986 was the result of a flawed reactor design that was operated with inadequately trained personnel and without proper regard for safety. The resulting steam explosion and fire released at least five percent of the radioactive reactor core into the atmosphere and downwind. 28 people died within four months from radiation or thermal burns, 19 have subsequently died, and there have been around nine deaths from thyroid cancer apparently due to the accident: total 56 fatalities as of 2004. Hundreds of towns and villages were evacuated, and the entire country has been declared a zone of international ecological disaster [1].

A quarter of the country's best farmlands and forests have been poisoned for hundreds of years by cesium 137 and strontium 90. The radionuclide contaminated areas situated mostly in Gomel region and Mogilev region of Belarus. Mogilev region was contaminated as a result of precipitation from radionuclide clouds.

The most effective use of agricultural lands contaminated by radionuclide as a result of Chernobyl disaster is both ecological and economical problem. The most part of contaminated soils are located in Polessie region, a typical rural area close to Chernobyl reactor. The basic radionuclide element on contaminated areas is cesium. The level of cesium conyamination of agricultural lands varied from 5 to 40 to Ci/km.^{2} The optimal system of agriculture in contaminated area is complicated problem, because traditional crops such as grass and cereals may accumulate extra radionuclides.

The effective alternative may be willow production for biomass. The willow does not accumulate a lot of radionuclide, but has big potential for control of erosion process, for treatment of hardly contaminated soils and can be used as wood for bioenergy purpose.

There are some publications concerning cultivation of willow for this goal.

Goor F. and others carried out assessment of the potential of willow SRC plants for energy production in areas contaminated by radionuclide deposits [4] The Polessie district, a typical rural area located close to Chernobyl, was chosen as reference for the development of a GIS-based multidisciplinary approach combining maps of soil resources and Cs-137 deposits, modeling of SRC biomass production and estimations of Cs-137 soil-to-wood transfer. From an agronomic viewpoint, the very dry sandy podzolic soils in the reference area are not suitable for SRC growing because they cannot supply adequate water for SRC during the early summer period. Moreover, on the other soil types, both careful weeding and water supply during the early months after plantation are required, until both leaf cover and root system are sufficiently developed. From the radio-ecological viewpoint, and according to the local legislation, the SRC biomass produced on loamy sand, sandy loam and loamy soils is suitable for firewood. SRC biomass from the highly productive peaty soils (39.4% of the land area of Polessie) may also be used but only if its conversion into heat or electricity is carefully managed.

Gommers A. and others get the same conclusion in experiments with radiocesium uptake by one-year-old willows planted as short rotation coppice [3]. Radiocesium uptake and distribution were measured in a willow (Salix viminalis L. var. Orm) short rotation coppice (SRC) stand. This system allows production of energy from the harvested biomass. Experimental plots were established on two soil types of contrasting texture (loamy versus sandy), and contaminated with 8 X 10(6) Bq Cs-134 m(-1). Concentrations of Cs-134 were measured in wood, litter, roots, and cuttings after 1 yr of growth. At the end of the growing season, only 0.0012% (loamy soil) and 0.0065% (sandy soil) of the initial radiocesium was transferred to the plant biomass (including belowground plant parts). Stem wood contained the lowest concentration of Cs-134 among all plant parts analyzed. Wood radiocesium concentrations were 82 Bq kg(-1) for the loamy soil and 192 Bq kg(-1) for the sandy soil. These values are well below the exemption limit for fuel wood put forward in the Commonwealth of Independent States (CIS) (740 Bq kg(-1)). Even at this high soil contamination level, radiocesium concentrations in wood do not exceed appreciably the naturally occurring K-40 content in the wood (135 Bq kg(-1)).

The potential opportunity of effective willow production on radionuclide polluted areas was confirmed in research of Norway scientists [2]. They compare the accumulation of radionuclide by different types of trees. The results showed that willow accumulate radionuclide not as intensive as birch or alder. The positive fact is the absence of visible morphological disturbing of trees has been grown in the condition of extra radiation.

These publications indirectly confirm that it is possible to get comparatively clean biomass of willow on radionuclide polluted soils. The problem is absence of adequate technology of willow production for polluted soils with high level of radiation. The goal of our investigation is the development of special technology methods for willow growing enable to combine high level of biomass production without extra accumulation of radionuclide.

MATERIALS AND METHODS

A two-year field study (2007-2008) was conducted at Krichev district of Mogilev region in eastern Belarus, close to the Russian border. The radioactive pollution in the region has been conditioned by precipitating from clouds. As a result, local cesium “spots” appeared with the level of cesium pollution from 1 to 40 Ci/km^2. The level of contamination in the place of our experiment varied from 5 to 10 Ci/km^2 and it is expected to be nearly the same for the nearest decade (figure 1).

The soils of experimental plot were sandy and sandy loams with single grain structure. It was excluded from agricultural practice after Chernobyl disaster. Available water capacity was moderate to high.

The goal of our investigation is the development of special technology methods for willow growing enable to combine high level of biomass production without extra accumulation of radionuclide by means of different level of fertilizer application. The practical experiment included 2 blocks with different types of soils and some variants:

1. Control (C).
2. Variant $N_{30}P_{60}K_{90}$ (V-2)
3. Variant K_{30} (V-3)

4. Variant K_{60} (V-4)
5. Variant K_{90} (V-5)
6. Variant N_{30} (V-6)
7. Variant N_{60} (V-7)

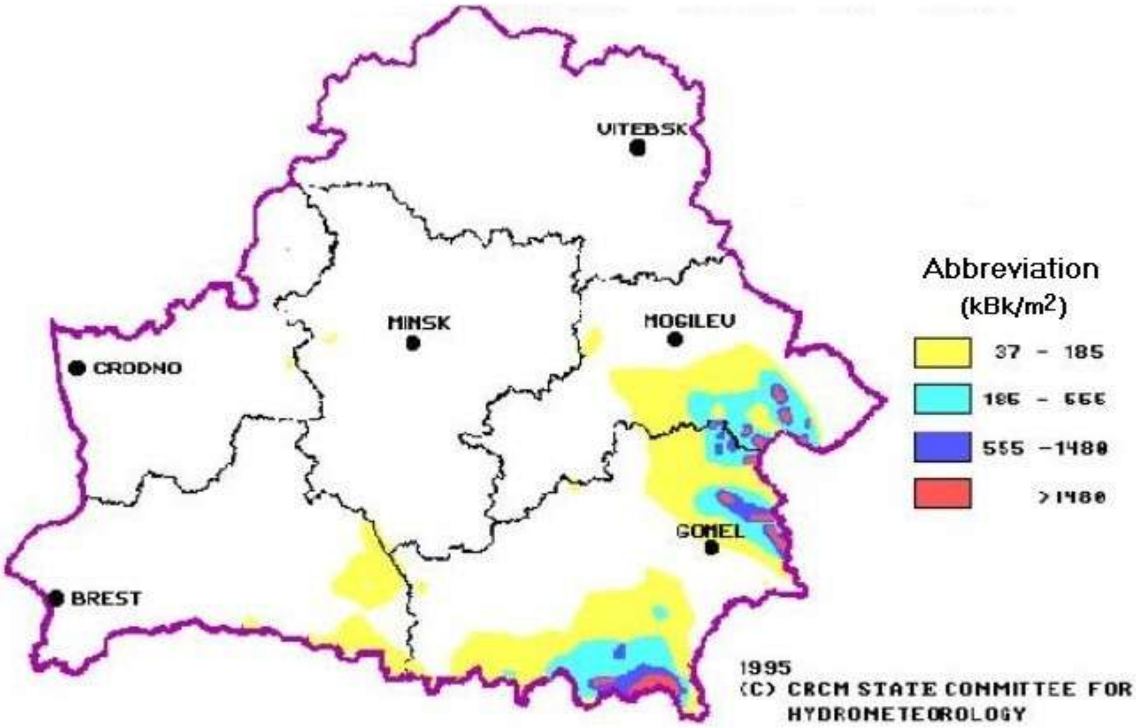

Figure 1. The prognosis contamination of the area of Republic of Belarus with cesium – 137 for the year 2016. (kBq/m^2)

The different rates of potassium fertilizer were used as it is chemical analog of cesium. In accordance with our hypothesis the additional application of potassium enables to decrease the level of accumulation cesium in willow biomass.

The different rates of nitrogen fertilizer in experiment should let us find optimal balance of the element. From one side the rates of nitrogen should be optimal for willow growing but not so high for extra weed development from another side.

The experimental design was randomized by complete blocks of four treatment replicated four or five times. Each elementary plot was 7 m long by 7.2 m wide (50m ^{2}) and contained 4 double rows of plants.

The willow clones (Salex viminalis) were planted at the end of April. At the end of first growing season willow plants were coppiced.

RESULTS

The percent of survived willow plants varied from 88 to 92 for different variants. It was not noticed any differences for surviving so as for rates of growing in the first month for willow planted on sandy and sandy loam soil. But the weather conditions in future were not optimal for willow growth (hot and dry May and June). It was not typical for Belarus and it was not any rains in this period at all. As a result, the majority of willow plants growing on light sandy soil perished (figure 2). The further investigations in this site were not fulfilled.

Table 1. The average height and diameter of willow plants for variants (31 July)

Parameters	Variants						
	C	V-2	V-3	V-4	V-5	V-6	V-7
Height, cm	105	122	103	104	107	109	110
Diameter, mm.	8,8	9,8	8,6	11,2	9,8	10,2	11

Figure 2. The state of willow plants on the site with sandy soil (25 May)

The willow plant on the site with sandy loam soil survived but rates of their growth were not high. The active growth of willow began in July after intensive rains fall in the region. At the end of July it was noticed considerable differences between variants (table 1) and willow plants state was quite normal (figure 3).

Figure 3. The state of willow plants on the site with sandy loam soil (31 July)

The most optimal parameters were obtained for willow plants of the second variant with complex application of fertilizer ($N_{30}P_{60}K_{90}$). The variants with additional application of nitrogen had better parameters to compare control, but were close to each other. It is obviously that high rates of nitrogen fertilizer application do not enable any progress of willow plants growing, but stimulate active grows of weed, especially at the beginning of vegetation.

The results of accumulation of cesium in willow biomass confirmed that it mostly depends on potassium application (figure 4).

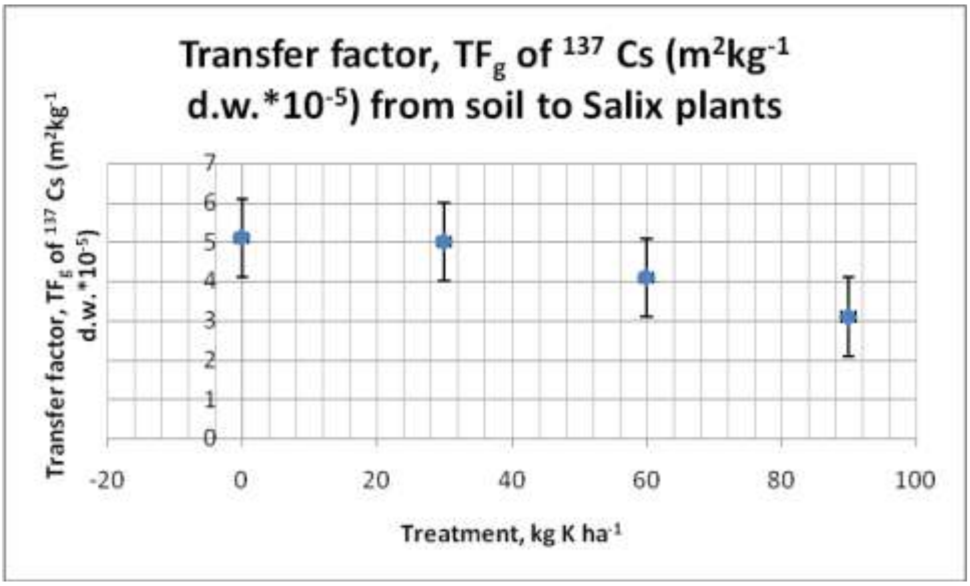

Figure 4. The dependence of cesium – 137 accumulations in willow biomass from the rates of potassium fertilizer application

The results showed that increasing of the rates of potassium application from 30 to 90 kg per hectare enable to decrease cesium accumulation in biomass in several times.

The results of first year of experiments let us formulate some conclusions, but it is necessary also to clear up some issues

1. What are the best rates and systems of fertilizer application for stimulation of willow production?
2. What rates of potassium is optimal from environmental and economical point of view for willow production. It was not demonstrated that rates exceeding 90 kg per hectare could not decrease further accumulation of cesium in willow biomass.
3. Does cesium accumulation in biomass depend on the species of willow?

To solve these problems the second experimental site was set up in 2008 close to the first site.

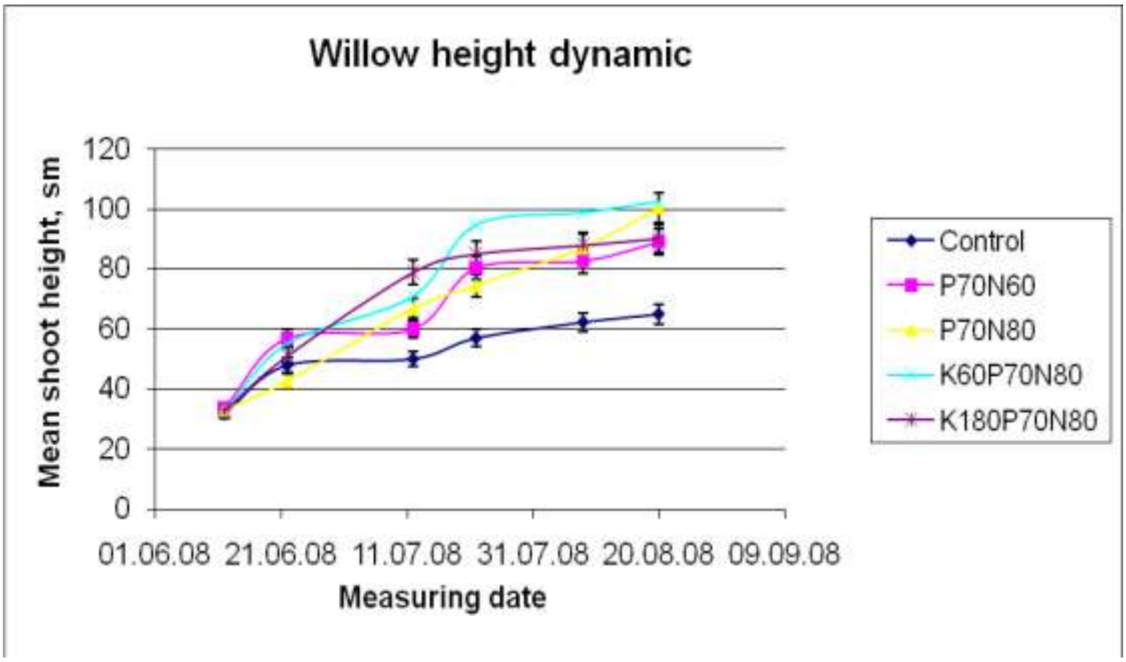

Figure 5. The results of willow height in dynamics (2008)

The practical experiment included the following variants.

1. Control (C). Salix viminalis.
2. Variant (V-2). Salix dasyclados.

3. Variant N_{60} P_{70} (V-3)
4. Variant N_{60} P_{70} K_{60} (V-4)
5. Variant N_{60} P_{70} K_{120} (V-5)
6. Variant N_{60} P_{70} K_{180} (V-6)
7. Variant N_{80} P_{70} (V-7)
8. Variant N_{80} P_{70} K_{60} (V-8)
9. Variant N_{80} P_{70} K_{120} (V-9)
10. Variant N_{80} P_{70} K_{180} (V-10)

The willow species Salix dasyclados was used only for one variant to compare rates of cesium accumulation in biomass to control variant.

The first results of field investigation in 2008 evidenced that rates of willow growth was not stimulated by extra application of nitrogen (figure 5)

The more intensive growth of willow took place in variants with complex application of fertilizer. Additional rates of potassium did not stimulate height of plants. A little difference between variants with rates of nitrogen application was not confirmed by means of statistic methods. These results correspond to the data of the previous year.

The results of cesium accumulation in willow biomass will be calculated after harvesting.

CONCLUSION

1. The first results of our field experiments showed that it is possible to get normatively “clean” biomass of willow on the site with the level of cesium-137 contamination to 10 Ci/km^2. The level of cesium in biomass varied from 15 (with high rates of potassium application) to 50 (control plants) Bq/kg. In Republic of Belarus the permitted level for firewood is 740 Bq/kg. The opportunity of biomass production on the soil of different types and with higher level of radiation may be modulated after additional experimental results. It is about 0.8 million hectares of radionuclide polluted arable lands in Belarus and part of them could possible be used for willow biomass production. The mostly polluted soils have been excluded from agricultural practice but they may be reclaimed now or in the nearest future by means of willow cultivation.
2. It is obviously that potassium mineral fertilizer is the key factor for radionuclide accumulation controlling. The optimal rates of potassium should be calculated with respect to radiation level in the soil and from economical point of view.
3. The optimal system of cultivation for willow is application of complex of potassium, nitrogen and phosphorus fertilizer. The optimal rates of nitrogen and phosphorus should not be higher than 40-60 kg per hectare. The highest rates do not enable the adequate acceleration of willow plants growth. The high rates of nitrogen fertilizer stimulate weed development and as a rule lead to additional investment for weed controlling.
4. It is a lot of sandy soils in the areas contaminated by radionuclides in Belarus. But it is risky to use them for willow production. The dry and hot beginning of vegetation season stimulated by global climate change may cause willow plants perishing.

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POSSIBILITY OF SEED USAGE OF COMMON ASH (*Fraxinus excelsior* L.) IN BOSNIA AND HERZEGOVINA ON THE BASIS OF ITS GENETIC STRUCTURE

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Abstract: *In this study we analyzed the genetic variability of the populations of Common ash in Bosnia and Herzegovina, with the aim to identify and differentiate between the autochthonous populations. We analyzed 10 equally distributed populations in Bosnia and Herzegovina, with a total 232 analyzed trees with usage of six highly polymorphous micro-satellite loci. Thus we detected high polymorphism in the populations, and the analysis of inbreeding points at its considerable presence in the studied populations. In this study we obtained a very low value of differentiation between the populations, with* F_{ST} *= 0,023 value of the entire genetic diversity.*
Based on the results of the study we can plan the activities in production of the reproduction material, and conservation of Common ash by methods in situ and ex situ.
Key words: *common ash (Fraxinus excelsior L.), microsatellite, variability, differentiation*

INTRODUCTION

Common ash (*Fraxinus excelsior* L.) is a densely distributed species in Europe and in Bosnia and Herzegovina. It usually grows in smaller groups or as separate trees. It also grows as a constituent part of ecologically differentiated forest communities, which are represented throughout Bosnia and Herzegovina, except for the southern and south-western part of the country, as it does not stand the well drought. It predominantly grows on mesophytic stands, and can be most commonly found in karst valleys and by forest creeks, and in Herzegovina on northern expositions, and higher altitudes on slightly better quality types of soils. The best known habitat is the area of central Bosnia, where they form a specific phytoceneses with mountain ash, known as Illyrian forests of maple and ash, *Aceri-Fraxinetum Illyricum*, Horv. 1938. Even though not numerous as species, its economic significance is rather high due to its extremely valued and quality timber.

All changes that occur as a result of human conscious or unconscious activities in forests or forest communities reflect this valued species as well. Therefore, due to its quality timber this species has been considerably declined from forests. Due to such conditions there is a growing need to protect the gene fund of this species, as well as to preserve its seeds and planting material of this valuable species.

The only possibility for protection of this valued species, in the altered economic and ecological circumstances, lies in application of most contemporary methods of molecular-genetic identification of the populations and based on the results in establishing the archives *in situ and* ex *situ*, as well as to provide for genetically quality reproduction material.

The aim of this study is to point at the possibility of applying the results of genetic differentiation between the populations of common ash, obtained through the analysis of micro satellite markers. These results therefore have great significance in the forest economics, and primarily in the activities at common ash breeding, and control of the origin of the seeds and planting material which is significant for the process of artificial foresting of degraded populations of common ash, as well as for the activities related to founding of gene banks and archives by *in situ* and *ex situ* methods.

Before we say anything we need to clarify the term, what exactly is genetic differentiation between populations?

GENETIC DIFFERENTIATION BETWEEN POPULATIONS

Based on the estimate of the extent of genetic differences between the populations included into this study, we may come to a conclusion that these differences are perhaps the consequence of the mechanisms of adaptive selection under specific environmental conditions (climate, soil, etc) or that these differences genetically causes. The difference on genetic level usually refers to a possible loss of allele during the postglacial migration, etc. Thus, the estimate of genetic differentiation can be done by calculation of the entire genetic variability (*Hr*) which exists in the group of individuals in all studied populations. *Hr* can be estimated as mean value heterozygosis, which is calculated from the mean allele frequency among studied populations ($Hr = 1 - (p^2 + q^2)$ in case of only one locus with only two allele). The total variability then gets divided to its components within separate populations (*Hs* is equal to the mean value of all heterozygoses which have been calculated for each individual population) and between different populations (*Dsr* is obtained based on differentiation between *Hs* and *Hr*). There is also the level of genetic differentiation (*Gsr*) which represents the percentage of the total variability due to existence of differences between the populations. The *Gsr* assumes the value up to 0, 20 - 0, 25, while already above 0, 05 there can be registered a more significant genetic variability. Based on the represented parameters and their basic information it is possible to calculate the genetic distance between the populations and thus define genetically homogenous areas within which the populations show significant genetic differences (N e i, 1978). Such areas can represent the grounds for an appropriate planning of seeds and planting productivity. Thus within these areas we can freely have at our dispose the seed and planting material, and avoid the risk of genetic pollution of the local populations. In this case special care should be turned to usage of reproduction material which comes from other areas.

MATERIALS AND METHODS

We studies 10 natural populations (figure 1, table 1), which represented diverse climatic areas in which common ash grows in Bosnia and Herzegovina. All selected populations that have been used in this study are natural, and distances between the trees that were selected for the study was minimally 50 m, in order to decrease the possibility of inbreeding between separate selected individuals. At the beginning of February 2004, we collected dormant buds with branches from 24 randomly chosen but equally distributed trees in the population.

Table 1. Locations of common ash populations considered in the study.

Population	Location	Mark	Latitude	Longitude	Altitude(m)
Posušje	Rakitno	A	43°32' 37''	17°23' 16''	940-950
Bosansko Grahovo	Crni lug	B	44°03' 37''	16°35' 22''	740
Bugojno	Zlavast	C	43°53' 35''	17°37' 15''	700-800
Bosanska Krupa	Jasenica	D	44°48' 20''	15°15' 02''	400-450
Sarajevo	Mrkovići	G	43°53' 01''	18°25' 20''	900
Bosanska Gradiška	Podgradci	H	45°03' 21''	17°03' 36''	200-400
Lopare-Brčko	Brezik	E	44°48' 56''	18°51' 55''	130-150
Srebrenica	Srebrenica	I	44°03' 39''	19°17' 07''	600-800
Rudo	Sutjeska	J	43°34' 15''	19°19' 10''	550-700
Kladanj	Stupari	F	44°18' 31''	18°42' 21''	700-800

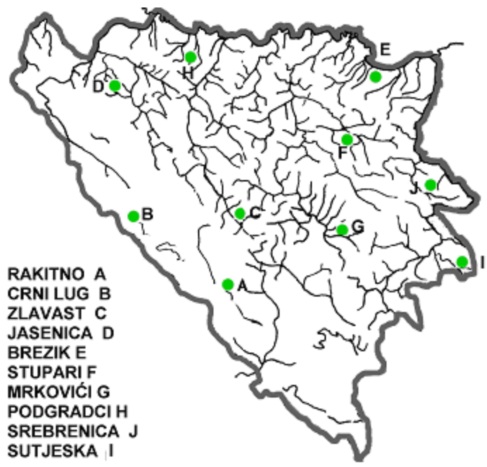

Figure 1. Geographic distribution of the studied populations of common ash

The DNK extraction was performed with usage of the SIGMA ''GenElute™ Plant Genomic DNA Miniprep Kit''.

Table 2. Used six pairs of microsatellite

SSR	Primers	Repeated sequences
FEMSATL 04	TTCATGCTTCTCCGTGTCTC GCTGTTTCAGGCGTAATGTG	(CA)n(AG)m
FEMSATL 10	TTGAGCAACATGTAATTATG AAATATCCGGTGCTTGTGTA	(CT)n
FEMSATL 11	GATAGCACTATGAACACAGC TTCTTGAAGTAGTAGAACTA	(GA)n(TA)m
FENSALT 12	TTTTTGGAACCCTTGATTT ATTAAGAATGCCCGTCCATC	(GA)nCA(GA)m
FEMSATL 16	TTTAACAGTTAACTCCCTTC CAACATACAGCTACTAATCA	(CA)nCG(CA)m
FEMSATL 19	CTGTTCAATCAAAGATCTCA TGCTCGCATATGTGCAGATA	(CA)nCGGC(CA)m

We used six pairs of microsatellite initials (Table 2) fro identification and determination of genetic differentiation. The initials were marked with fluorescent color (IRD 700 and IRD 800) at end 5'. Electrophoresis of the PCR products, amplifications, were performed at polyacridimide gel with usage of DNK sequencer.

For the processing of the levels of the within-population variability and genetic differentiation we used the following software: Genepop 3.4 (Raymond & Rousset 1995), Fstat 2.9.3. (Goudet 1995), Gendist 3.6 (Felsenstein 1986) and Neighbor 3.6 (Felsenstein 2004

Table 3. Elemental climate data of the areas where grow the populations analysed in the study.

Population	Location	Weather station and altitude (m)	Average year temperature °C	Temperature during vegetation period V-IX in °C	Number of days with temperature > 10°C	Year precipitation in mm	Precipitation during vegetation period V-IX in mm
Posušje	Rakitno	Rakitno 915	9,4	14,8	173	1983	624
Bosansko Grahovo	Crni lug	Bosansko Grahovo 861	8,2	13,9	166	1356	558
Bugojno	Zlavast	Bugojno 562	8,8	14,8	174	826	383
Bosanska Krupa	Jasenica	Bosanska Krupa 176	10,3	16,2	198	1304	666
Lopare-Brčko	Brezik	Brčko 96	11,2	17,9	211	781	429
Kladanj	Stupari	Kladanj 560	8,9	15,1	183	1028	602
Sarajevo	Mrkovići	Sarajevo 630	9,7	15,5	186	946	450
Bosanska Gradiška	Podgradci	Bosanska Gradiška 95	10,6	17,2	185	831	457
Srebrenica	Srebrenica	Srebrenica 400	9,5	15,9	192	1027	579
Rudo	Sutjeska	Čajniče 816	8,1	14,6	166	1147	579

RESULTS

Study conducted over within-population genetic structure of common ash in Bosnia and Herzegovina using six micro satellite loci, suggested to the presence of high polymorphism in all studied populations, and the presence of bigger number of alleles in each studied loci. (Table 4).

Table 4: Number of registered alleles

Locus	Number of alleles
FEMSATL 04	20
FEMSATL 10	55
FEMSATL 11	24
FENSALT 12	28
FEMSATL 16	12
FEMSATL 19	26

Confirmation of high polymorphism in populations is also an average number of alleles per loci, which is 10,2, and which are defined according to Lefort et. al. (1999). The average number of alleles in populations ranged from 8,3 what was registered in Srebrenica population to 12,2 in Sutjeska population (Ballian et. al. *in press*). Thus, ten studied populations gave results of quite low heterozygosity ranging from 0,433 in Podgradci population to 0,639 in Mrkovići population (Ballian et. al. *in press*), and the average was 0,542, while quite higher values occurred in cases of expected heterozygosity.

Total genetic differentiation for each genetic loci within 10 studied populations in Bosnia and Herzegovina is quite high, but differentiation between populations is rather low, with the value 0,023 or 2,3 % (Table 5). In the case of genetic diversity as well as in the inbreeding, we could refer to deficiency of species, low gene flow between populations, proneness to inbreeding of individuals that form populations, as well as possible anthropogenic influence.

Table 5. F_{IS} – differentiation within populations; F_{ST} - differentiation between populations

Locus	F_{IS}	F_{ST}
FEMSATL 04	0,393	0,013
FEMSATL 10	0,371	0,047
FEMSATL 11	0,328	0,010
FEMSATL 12	0,426	0,025
FEMSATL 16	0,156	0,033
FEMSATL 19	0,167	0,011
Multi-loci estimation	0,320	0,023

In co-relation with obtained micro satellite sizes and basic ecological parameters, climate, common ash grouped (differentiated) into two groups which means that a part of the populations did not achieved that. Co-relation with the type of soil did not provide any result. First group is a Pannonian with two populations which by their position already belong to a Pannonian basin (Podgradci and Brezik), and Messian one with three populations from eastern Bosnia (Sutjeska, Srebrenica and Stupari), based on climatic factors showed in the Table 3. This shows that between those grouped (differentiated) populations we have possible active movement of genetic material in all directions. Other five studied populations could not be placed into any of these two groups nor in a separate one, so they represent autonomous genetic units.

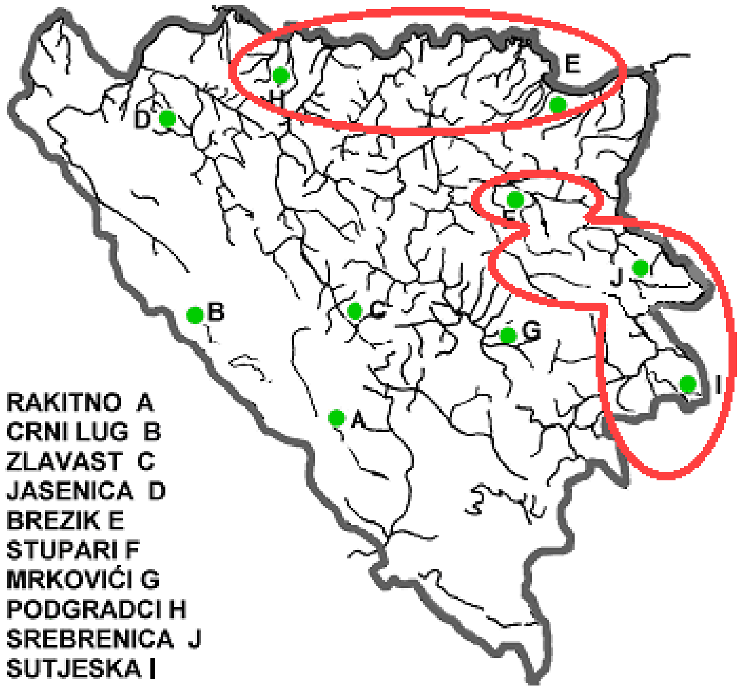

Figure 2. Differentiation of Common Ash in two groups Pannonian and Messian

Thus, we can use seed and seedling material more freely and avoid risk of genetic pollution of the local populations within the area that belongs to the Pannonian (sample of two studied populations), and Messian basin (sample of three populations). In that case, special attention should be given to the usage of reproductive material which can originate from other areas, and have a negative effect to the genetic structure of the local populations. Usage of seed from local populations only is recommended for other populations that did not grouped.

DISCUSSION AND RECOMMENDATIONS

The area of the Dinaric Alps is very specific in the terms of environmental conditions, because there is great variety of climatic, edaphic, orographic, and other factors in the very small area that have a direct influence to differentiation of various ecotypes. Thus, experts believe that types of forest trees from the area of Dinarides demonstrate large variability in contrast to same species from the north. Consequently, results of this study confirmed theory on high polymorphism, which was also obtained in the research with common ash from the area of Bulgaria conducted by Heuertz et. al. (2001).

Taking into consideration that populations of common ash from Bosnia and Herzegovina belong to the Balkan area, it is interesting to compare them with those populations from Bulgaria that were investigated by Heuertz et. al. (2001). Significant fact for the Balkan area is that its south represented one of the glacial refugia during the glacial period (Huntley & Birks, 1983), which implies to possibility of large genetic variability in the western parts of the Balkan. Inbreeding of a variety of genetic structures originating from various glacial refugia is present in the area of Bosnia and Herzegovina, what was determined during studies conducted on common fir (Konnert & Bergmann 1995; Liepelt et. al. 2002; Gömöry et. al. 2004), oaks (Petit et. al. 2002; Slade 2003), and ash (Heuertz et. al. 2004a,b).

Heuertz et. al. (2004b) have found two different haplotypes for common ash in the area of Bosnia and Herzegovina, out of which one is unique for central Europe and other for Balkan, which implies that this is a marginal area with characteristics of two haplotype gen pools. This could be one of the reasons which is directly connected with occurrence of high polymorphism in studied populations.

Analyses of common ash population from the area of Bosnia and Herzegovina demonstrated genetic differences between them, and each of them represents specific population. Based on results, differentiations can be grouped into only two groups with a total of five populations (Figure 2), while other five populations remained ungrouped. However, in this study we could not confirm the results by Heuertz et. al. (2001), which imply to high genetic variability of common ash. Also, individuals that form populations give one specific presentation of studied populations possibly due to constant anthropogenic influence in populations. That is represented by the quite illustrated example by Ducci (1991) in Italy, where anthropomorphic changes in the last 2000 years have had an influence on common fir.

In this study we obtained a very low value of differentiation between the populations, with 0,023 value of the entire genetic diversity, what represents significantly lesser value from the one that was obtained in the study conducted by Heuertz et. al. (2001) in Bulgaria (F_{ST} = 0,087). For that reason, obtained values in our study suggest that this species has weak barriers concerning the gene flow, according to Austerlitz et. al. (2000). That had direct influence to different process of postglacial migration of common ash, which was not the case with other types of trees. In her study, Heuertz et. al. (2001) presented data obtained during several researches conducted on the species *Juglans regia*, *Castanea sativa*, *Ulmus minor*, which are also rare. She suggested that research on pollination of these noble broad-leaved species, as well as of common ash should be conduced, because that is a significant factor that influence on weaker or stronger population differentiation.

Differences between populations or within them are usually visible and obvious using demonstrated genetic parameters. Possible causes of those differences, beside natural selection and anthropogenic activities, are developmental factors or specific processes of adjustment to certain ecological conditions.

When methodical effects (e.g. a number of units in a sample) as well as developmental and anthropogenic factors cannot be excluded, obtained results between populations suggest that adaptation processes in each studied population can have a significant role. Therefore, based on obtained results, following points of view can be presented:

- Obtained differentiation between populations is not so characteristic for the West and South-East Europe, but it is more typical for the western part of the Balkan, if we have in mind proportional size of the territory. For that reason, common ash seed should not been imported from the countries of West and South-East Europe in order not to jeopardise autochthon genetic structure in natural populations.
- Results showed groupings of populations from east and north parts of Bosnia with two climatic different areas, Pannonian and Messian, when the climatic factors were included in the calculations, including only five populations while other five was indifferent, which gave special significance and value to this research. In that way, management of seed and seedling material from seed basis, which are within the area with grouped populations, can be done more freely without the great risk of genetic pollution of the local populations.

Based on the above-mentioned, conclusion can be that there are differences among common ash populations on which the research was conducted. Genetic differentiation was caused by different process of postglacial migration or maybe by adjustment of certain gene-types to some habitats with specific selection processes, and probably by anthropogenic influence in the period of the past 2000 years.

Conclusion from the discussion is that only local genetic resources or better to say local reproductive material should be used in the processes of artificial rehabilitation in the entire area of Bosnia and Herzegovina. Material from the wider area of differentiation can be used more freely in the areas that demonstrated groupings in the process of differentiation.

ACKNOWLEDGMENTS

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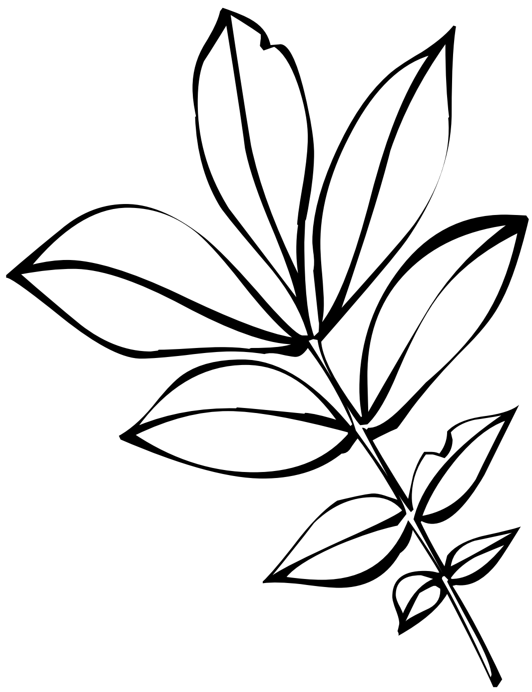

FOREST REGENERATION MATERIAL: STATE OF THE ART AND A NEW EUROPEAN APPROACH FOR PRE-CULTIVATED PLANTING STOCK PRODUCTION

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Abstract: *The last decades, expansion of plantation forestry into a much broader range of species is being observed. Public and private owned nurseries are challenged with the need of producing high quality planting stock of various species in high numbers. Aim of this paper is to describe the current situation in nursery production, as well as the types of planting stock used. New trends in producing high quality forest regeneration materials are also introduced, such as the innovative technology created in the frame of PRE-FOREST project, funded by the European Commission under the CRAFT 6th Framework Research Programme. PRE-FOREST is an innovative research project in the field of forest planting stock material. It is a co-operative project between research foundations and small and medium enterprises (SMEs). Among the project's objectives were the introduction of a new technology based on pre-cultivation (in mini-plugs) of forest regeneration material in a cost efficient and environmental friendly production unit, not affected by outdoor climate; the development of a new, adapted to mini-plugs, grading technology and re-plug robot; the integration of these technologies into a functional prototype unit capable of large scale forest regeneration material production and the introduction of this production system at strategic locations in Europe. The system is developed for year-around production and function in the same way independently of climate variations in different parts of Europe. To obtain our objectives we focused on the interaction among production technology, mini-plug container design and rooting media, in order to develop cultivation protocols for forest reproductive materials of special economic and ecological importance for each participating country. One of the main benefits of the new technology is the environmental friendly production of a large number of seedlings per square meter, leading to a cost efficient result. The produced technology, combined with the scientific support provided by the research foundations participating in the project, will be commercially exploited and in this way, nurseries could be more competitive both at an economic and quality level.*
Keywords: *containerized planting stock, nursery robot unit, mini-plugs, nursery production*

INTRODUCTION

Natural regeneration has traditionally been widely used in the countries of Southern Europe, especially where conditions were favorable. For species with low natural regeneration capacity the production of planting stock from nurseries is needed. This stock is produced mainly for reforestation, restoration, recreational and ornamental uses. The last decades, the need for plantation forestry to expand into a much broader range of species e.g. broadleaved and wildlife-crop species, diverse species and local provenances is increasing in the countries of South Europe (Weber, 2005). In addition, fast growing species (poplars, eucalypts, black locust etc.) are being cultivated as industrial plantations, agroforestry systems, windbreaks etc, meeting a significant portion of the local population needs, and also the national requirements in terms of timber supply and energy demand.

The failure of many reforestation projects in Southern Europe is primarily due to planting of poor quality seedling stock and poor environmental and soil conditions of the land areas where the seedlings are planted. Environments provide a continuous threat to initial plant establishment, especially in some areas of Southeastern Europe, due to the prevalence of high soil and air temperatures, low relative humidity and low rainfall. In such situations, careful selection and planting of high quality stock is crucial to ensure high survival rate in the field (Radoglou, 2001). Planting stock of high quality results in successful seedling establishment (high survival and growth) even when it is transplanted in unfavorable environmental conditions. On the other hand, if planting stock of low quality is used, seedling establishment will be poor even if the environmental conditions are optimal (Table 1).

Table 1. Schematic presentation of the expected seedling establishment in relation to planting stock quality and environmental conditions of the reforestation areas.

Planting stock quality	Environmental conditions	Seedling Establishment	
		Survival	Growth
High	Optimal	High	High
High	Unfavorable	Very good	Very good
Low	Optimal	Very good	Poor
Low	Unfavorable	Poor	Poor

Even though important advances have been done in certain European nurseries, the standard of production technology regarding all types of forest regeneration materials has to be elevated in order to improve biological, physiological and genetic quality, as well as cost efficiency. Lack of capital investments for technical development has led to low cost efficiency in the production of forest regeneration materials, which is sometimes burdened with environmental problems. The forthcoming years nurseries will have to face the challenge of producing high quality and quantity regeneration materials of various species in a cost efficient and environmental friendly way.

In this frame, PRE-FOREST project (Full title: A new European technology for cost efficient and environmental friendly production of pre-cultivated forest regeneration materials) was developed. PRE-FOREST (2006-2008) is an innovative research project in the field of forest planting stock material and was funded by the European Commission under the CRAFT FP6. It is a co-operative project between research foundations and SMEs (small and medium enterprises) and involves six partners, three research foundations: University of Tuscia (Italy); Dalarna University (Sweden); National Agricultural Research Foundation (NAGREF) (Greece); and three SMEs: Vivai Torsalorenzo (Italy); QS Odlingssystem AB (Sweden) and Ditikomakedonika Fytoria (Greece)*. Among the project's objectives was the introduction of a new technology based on pre-cultivation (in mini-plugs) of forest regeneration material in a cost efficient and environmental friendly production unit under control conditions, in other words not affected by outdoor climate; the development of a new, adapted to mini-plugs, grading technology and re-plug robot; the integration of these technologies into a functional prototype unit capable of large-scale forest regeneration material production and the introduction of this production system at strategic locations in Europe.

Aim of this paper is to describe the current situation in nursery production, as well as the types of planting stock used. New trends in producing high quality forest regeneration materials are also introduced, such as the innovative technology created in the frame of PRE-FOREST project.

NURSERY PRACTICES – PLANTING STOCK TYPES

The majority of forest tree seedling production in Greece for reforestation purposes comes from the state nurseries of the Forest Service. The last decades, the number of small or medium sized private nurseries has also increased; they provide planting stock material mainly for restoration, recreational works and for ornamental uses.

Before 1970's, nursery forest production in Greece was mostly concentrated in barerooted seedlings. This material is generally considered of good quality and usually results in successful establishment in the reforestation areas, depending on transportation conditions, the degree of root injury, seedling exposure to wind and careful planting. Barerooted planting stock of conifers and broadleaved species are usually two years old, even though in some cases older seedlings are also used (e.g. for recreational use). These seedlings, produced mainly by private nurseries, need to remain for longer periods in the nursery. Their bigger size makes their planting also more demanding, while they need special treatment after establishment. This planting stock should be generally avoided because of high risk of failure in unfavourable environments.

Containerized seedling production in Greece boomed after the introduction of paperpots in the 70's. This system has been extensively used for producing conifer species for reforestation. Although this system had some advantages compared to barerooted seedling production (e.g. limiting the negative effect of external factors in field establishment) it proved to cause disturbances in the root development due to slow decomposition of the paperpot. The paperpots were soon replaced by rigid plastic containers. The associated problems of root spiralling due to the initial design of these containers led to the introduction of vertical ribs at the container walls in order to guide the roots to the new bottom design (Mattsson, 2005). This second generation of plastic container systems, extensively used in nurseries nowadays (Fig. 1), prevented root spiralling and allowed more intense root egress during field establishment. The third generation of container system, using different concepts of side slits, has been successfully used in other countries (Mattsson, 2005) and also in Greece.

An advantage of planting containerized stock for reforestation purposes is the protection afforded to the root system by the plug during storage, transportation and transplanting of seedlings, which favors survival and early growth at plantation establishment (Barnett & McGilvray, 1993; Mohammed *et al.* 2001; Idris *et al.,* 2004). An additional benefit is that the intact plug around roots may provide an initial source of moisture and nutrients for seedling development soon after field planting (Arnott & Burdett, 1988). Plastic containers can nowadays be found in the market in a huge variety of shapes, volumes and densities.

Figure 1. Common containerised seedling production in forest nurseries. Seedlings are grown in containers of 240/m^2 density (volume 330 cm^3).

Figure 2. Production of black locust seedlings in mini-plugs of 3500/m^2 density (volume 3 cm^3).

Another type of planting stock common both in state owned and private nurseries is the two year old seedlings produced in polyethylene bags. These are used both for conifers and broadleaf species (especially eucalypt, Arizona cypress and some species of cedar). Although this material is considered nowadays as unsuitable for reforestation purposes mainly because it causes root spiralling (Radoglou, 1992) it continues to be produced maily due to its low cost.

Containerized forest nursery stock is produced in nurseries with the use of irrigation systems, peat, and other artificial components. Natural soil is even now used in mixture with peat and perlite in tree forest containerized production. Automated filling and sowing lines do not exist in the majority of forest nurseries. Another important aspect for the production of high quality regeneration material is the use of certified seeds.
During germination and early growth and according to the location of the nursery, seedlings are usually kept in plastic greenhouses. No additional light supply is used. When the climatic conditions allow it then the seedlings are transferred to outdoor areas. Often shading nets are used during the summer period.

NEW TRENDS IN REGENENERATION PRACTICES

Containerized transplants grown in trays with small cells (mini-plugs) (Fig. 2) is a new establishment system with potential for reducing post-planting stress to grow. Main reason for the interest in mini-plugs is the possibility to reduce regeneration costs in nursery production, storage, distribution and planting (Mattsson, 2005). In addition, short growth in the nursery could reduce risks for root deformation and instability of the tree when outplanted (Rune, 2003; Lindström *et al.*, 2005). In the ornamental and vegetable industry, plants have been grown in small plug containers for many years; however, this practice is relatively new for forest trees and other native plants. The published literature is also rather sparse; only a few articles have been published about mini-plugs in forest and conservation nurseries (Riley & Steinfield, 2005). Small cell size offers the nursery the advantage of more plants in the greenhouse space and, consequently, lower cost compared to larger cell volumes (Vavrina, 1995). Mini-plugs are very small container plants grown in predominately stabilized medium in containers less than 33 cm^3 in volume, produced in a variety of shapes, volumes and densities. In contrast to standard container seedling culture, mini-plug seedlings are grown with the objective of producing a fully extractable seedling with a dimensionally stable root plug that will tolerate transplanting within a relatively short growing period (3 to 4 months) (Landis, 1990). Mini-plugs are used in two distinct stocktypes: container-to-bareroot transplants and container-to-container transplants (Riley & Steinfield, 2005). The advantages of mini-plugs are the almost 100% yield, resulting in few culls, the highest plant density per production area, the maximum use efficiency of seeds or cuttings, the shorter crop rotation and the improved stock quality, as plants with large stem diameter and fibrous root systems are produced (Landis, 2007).

One of the main objectives of PRE-FOREST project was to introduce the idea of precultivation in mini-plugs to an innovative, fully automated cultivation system. In this system the seedlings are pre-cultivated in a mobile multiple floor production facility, saving space at the nursery. The dimensions of the prototype unit are 5.1 m x 2.3 m x 2.5 m (length x width x height) and the total space needed for its function at the nursery is 7.0 m x 4.5 m x 3.0 m. The whole system is developed in such a way that is not affected by outdoor climate. The cultivation period, under optimal germination and growth environments extends over a period of 2 to 5 weeks at a density of 1000 to 4000 seedlings per m^2 depending on species. The multiple floor consists of 16 rows; in each row maximum 8 trays of mini-plugs can be placed in such a way that root air pruning is being promoted. The total number of seedlings produced in one cultivation period can vary between 20,000 and 75,000 seedlings depending on the seedling density used (Fig. 3).

Figure 3. The multiple floor facility of the prototype unit developed in the PRE-FOREST project.

Figure 4. The grading and re-plug robot designed in the PRE-FOREST project.

The system provides the possibility of complete control of the growing conditions through a computerised system, connected to an air condition unit. More specifically, the user can select the photoperiod, the stop duration of the rotation of the multiple floor facility, the desired temperature and relative humidity, the irrigation frequency and the fertilization quantity and frequency on a touch screen. No pesticides or insecticides are used and the water and fertiliser are recycled. The system can also utilise 90% of the waste heat from the production. The prototype unit is being manufactured by QS Odlingssystem AB, Njurunda, Sweden and is already established in nurseries in Sweden, Italy and Greece.

The prototype unit is manufactured for year round function. Produced mini-plug seedlings that are destined for spring outplanting can be placed in carbon boxes and stored, either in cooler storage for a short period (0-2°C for 1-2 months) or freezer storage for longer periods (-2 to -4°C for 2-6 months) (Fig. 5-6). At a convenient time, decided by the customer, the pre-cultivated seedlings can be delivered to the nursery. There they can be quality graded and transplanted to bigger trays using a grading and re-plug robot (Fig. 4). This system has a capacity of transplanting 25,000 seedlings per hour and the computer system allows the transplanting of pre-cultivated seedlings to be done using any optional container system. By adjusting the time of transplanting to the local climate at the specific nursery the continuous cultivation of the transplanted seedlings can be done on the nursery outdoor area without the need of greenhouses.

Figure 5. Seedlings of spruce placed in carbon boxes for cold or freezer storage.

Figure 6. Seedlings of black locust placed in cold storage under controlled conditions.

In this new innovative approach the role of research foundations is also very important. In order to obtain the objectives set by the PRE-FOREST project, research was focused on the interaction among production technology, mini-plug container design and rooting media. More specifically, the mini-plug trays tested were of similar size (310 x 530 mm) but varying in density (975 mini-plugs/m^2 – volume 18 cc, 1460/m^2 – 13 cc, 1820/m^2 – 9 cc and 3500/m^2 – 3 cc) (Fig. 7). Two rooting media were studied, peat and a stabilised medium. Our aim was to develop cultivation protocols for forest reproductive materials of special economic and ecological importance for each participating country. In the Forest Research Institute (NAGREF) in Greece basic technological, economical and biological studies were conducted initially, concerning some forest species of special interest for the country (*Robinia pseudoacacia, Cupressus sempervirens, Pinus brutia, Pinus nigra, Picea abies, Abies borisii regis*) (Bellarosa *et al.,* 2007; Dini-Papanastasi *et al.,* 2007; Kostopoulou *et al.*, 2008a; 2008b, see also Kostopoulou *et al.* 2008 and Spyroglou *et al.* 2008 of this conference). Then the produced protocols were applied for the production of forest planting stock material in the prototype unit that was established in the nursery facilities of the second Greek partner, Ditikomakedonika Fytoria. One of the main benefits of the new technology is the environmental friendly production of a large number of seedlings per square meter, leading to a cost efficient result. The produced technology, combined with the scientific support provided by the Forest Research Institute, will be commercially exploited and, in this way, nurseries could be more competitive both at an economic and quality level. It should be noted that apart from forest regeneration material, other plant species (e.g. for ornamental use) could also be produced in the prototype unit, provided that their cultivation protocols have been developed.

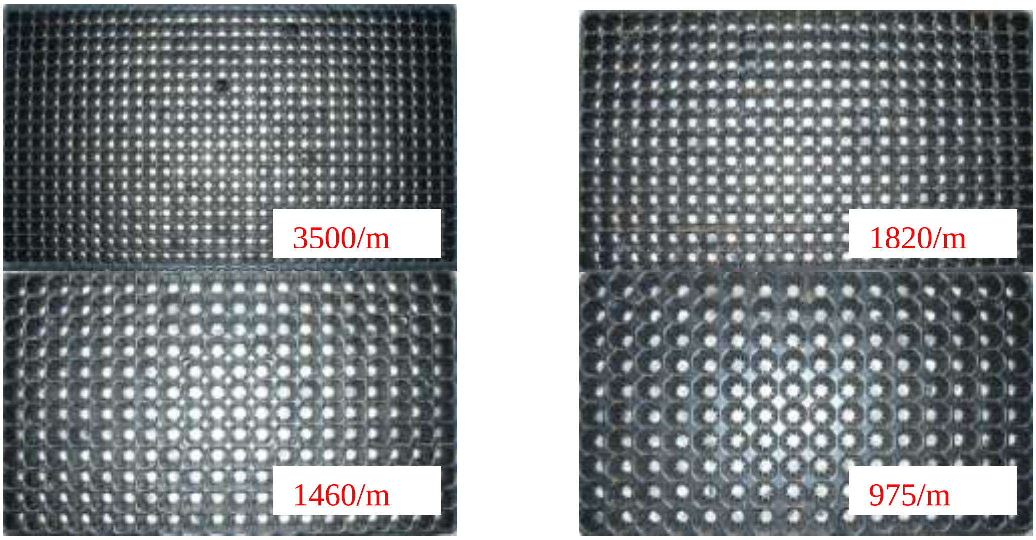

Figure 7. Mini-plug container densities used in PRE-FOREST project for precultivation of forest regeneration materials.

CONCLUSIONS AND FUTURE IMPLEMENTATION

The idea of PRE-FOREST project can contribute to the improvement of dialogue and exchange of experience in Southern Europe in the field of forest regeneration material. It can also enhance the capacity in adopting correct plant domestic strategies and nursery programs and use of suitable propagation and regeneration techniques.

Major advantages of the technology introduced by PRE-FOREST project are:

- Cost efficient production of high quality seedlings, grown from the seed of the customer's choice, with no losses due to containers without seedlings or containers with poor quality seedlings.
- No need for complementary or new capital investments in conventional greenhouse technology with high running costs.
- Nurseries that already have invested in a containerized system can use this system and belonging equipment.
- Environmental stress will be reduced due to less need for greenhouse cultivation.

As a result of the short cultivation period the need of fertilizers and pesticides in the nursery will be reduced which makes the system environmentally friendly. The nurseries will also be able to deliver seedlings continuously for planting and fulfill orders at short notice. The need for greenhouse and storage space will be reduced, decreasing production cocts. The need of overwintering seedlings will be also reduced, which considerably lowers the risk for seedling losses. Finally, the small size of the produced seedlings enables transportation, handling and planting to become more efficient (Lindström *et al.,* 2005), lowering the cost of seedling establishment.

The small dimension of existing nurseries in Southern Europe, some of which are state owned, creates barriers for implementation for technical development. The PRE-FOREST concept leads the way for active cooperation among these small enterprises since nurseries can either buy the prototype unit and expand their business or cooperate with nurseries that already have invested in this automation and order the pre-cultivation of the desired material.

Another important aspect is that this innovative technology can also enhance active cooperation between nurseries and research foundations. The latter will be responsible for developing cultivation protocols for the chosen species through research in seed and vegetative propagation based on technological, economical and biological studies conducted in their facilities.

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APPLICATION OF GENETIC MARKERS IN DETERMINATION OF PROVENANCE REGIONS OF FOREST TREES IN SERBIA

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Abstract: *Separation of seed zones in Serbia based on definition of regions of black pine (Pinus nigra Arnold), spruce (Picea abies Karst), fir (Abies alba Mill.), and beech (Fagus moesiaca /Domin./Czezccott) provenances was accomplished by studying genetic potential and characteristics of different habitats of seed stands of these species. In conveyed studies the testing of structure of the genome of their population was done by protein markers application as the most often used polimorphic markers at the level of gene products and molecular DNA markers based on PCR phenomenon.Results of analyses revealed that interpopulation diversity within these species was greater than that registered among their seed stands. Genetic distances between analyzed seed stands were obvious, so the future trade of seed material should be realized in accordance with these results.On the basis of obtained results the provenances regions were separated, and seeds zones of balck pine, spruce, fir and beech in Serbia were defined.*
Kye words: *black pine, spruce, fir, beech, provenance regions, Serbia*

INTRODUCTION

Study of potential and spontaneous trait variability, which influences the generative multiplication and yield of tree species in a lesser or a greater extent, is necessary for organization of production and seed material trade control. Orientation toward desirable seed and planting material traits intended for forestation programs demands desertion of seed and planting material production technology at the species level, because it is difficult to guarantee the steady quality at this level, Isajev et al. 1999, Isajev et al. 2002. Production, trade and usage of seed of tree species should exclusively be organized at the level of seed stands, seed trees i.e. provenances. Revision of existing, and nomination and separation of new seed stands of economically important species such as black pine (*Pinus nigra* Arnold), spruce (*Picea abies* Karst), fir (*Abies alba* Mill.), and beech (*Fagus moesiaca* /Domin./Czezccott) are permanent activities, and they are of a primary significance for forestry in Serbia. These activities should be synchronized with activities of lumber camps and scientific institutions because some stands are aged or destroyed by climatic factors or insects, diseases and anthropogenic factors, and they should be replaced with new and young stands satisfying prescribed criteria, which should be registered as seed objects, Ćorović et al. 2003.

Success of these activities is associated with separation and limitation of areas where appropriate new selections or provenances can be used. Aimed at running the above mentioned activities, the investigations encompassed the following:

- determination of the precise area of these species in Serbia – geographic distribution, geographical latitude and longitude, scope of heights above sea level, area, location;
- definition of ecological characteristics of their populations – climatic, pedological, phytocenological etc.
- separation of provenance regions, and
- creation of maps of provenance regions in GIS

Spatial distribution of tree seed stands (map 1) encompassed the greatest part of cenological, ecological and population diversity in Serbia. Improvement of forest seed science, application of contemporary methods of forest genetics and forest typology led to better understanding of the type of adaptability, productivity and quality of individual subpopulations, which provided production of quality seed of economically important tree species. Separation of provenance regions was initiated by ecological-geographical studies, and analyses of genetic distance between seed stands run by application of genetic markers made it precise.

WORKING METHODS

Establishment of field experiments network enabled testing of seed stand populations of spruce, black pine, fir and beech, i.e. provenances of these species in Serbia. Multi-annual studies included ecological characteristics and stand condition of seed objects and productivity and yield quality of individual subpopulations, which is the basis for location of seed objects, i.e. separation of provenance regions. Studies at population level – in seed stands and in laboratories (Faculty of forestry, Belgrade, Institute of forestry, Belgrade, and in the Laboratory of biotechnology, Institute of maize "Zemun Polje", Zemun) have been carried out since 2003.

Fieldwork was carried out in seed objects such as:

- 6 black pine objects in the area of L.C. „Forestry" Raška (2), L.C. "Prijepolje" Crijepolje, L.C. "Užice" Užice, L.C. "Stolovi" Kraljevo, TB „Goč" Faculty of forestry, Beograd;

- 11 spruce object in the area of LC Prijepolje, L.C. Užice, L.C. Kraljevo, L.C. Raška, National park Kopaonik, L.C. Kruševac, L.C. Pirot and in national park;

- 7 fir objects in the area of L.C. «Golija-Ivanjica, L.C. «Rasina»-Kruševac, L.C. «Užice» Užice, L.C. «Forestry-Raška, L.C. «Prijepolje» Prijepolje, P.E. «National park Kopaonik», PE »National park Tara»;

- 22 beech objects in the area of L.C. Kruševac, L.C. Ivanjica, L.C. Kragujevac, TB „Debeli Lug" in TB „Goč" Faculty of forestry, Belgrade, L.C. Zaječar, L.C. Užice, L.C. Beograd, N.P. Fruška Gora, N.P. Đerdap, N.P. Kopaonik.

The following activities were completed in seed stands:

- test trees were separated by applied method of individual selections (candidating, evaluation and marking);

Ten test trees were selected as working sample in each seed stand - total of 460 mother trees were separated for analyses.

- plant material for laboratory analyses was collected.
- cones were selected and seeds were extracted for determination of genetic similarity i.e. genetic diversity of different populations of black pine;
- for determination of genetic similarity i.e. genetic diversity of different populations of spruce, fir and beech under laboratory condition one year old assimilation organs were used;
- collected plant material was properly packed and frozen until usage.

In the laboratories of Faculty of forestry and Institute of forestry in Belgrade samples were prepared for biochemical and genetic analyses using krypto technique and mechanical processing, aimed at determining the degree of similarity between provenances within the same and different regions.

Determination of genetic similarity i.e. diversity of different populations of the above mentioned species was done in the Laboratory of biotechnology of the Institute of maize "Zemun Polje" Zemun, using standard methods for protein isolation (according to Wang C.,et al.1994) from seeds and DNA marker technique based on PCR method (according to Jones C.J. et al.1998, and Lučić et al. 2008).

RESULTS AND DISCUSSION

Investigation of genetic structure of black pine, spruce, fir and beech seed stands, acquiring information regarding group and individual variability by applied genetic markers were the tools for bringing nature a bit closer to us:

1. degree of variability of seed quality of studied black pine populations;
2. genetic similarity i.e. diversity of studied black pine populations using analysis of seed proteins and molecular DNA markers based on PCR method;
3. degree of variability of certain morpho metric characteristics of spruce, fir and beech assimilation organs, and existance of interdependence of studied characteristics;
4. genetic similarity, i.e. diversity of populations studied by analysis of protein complexes from assimilation organs;
5. interrelation of ecological traits characteristic for habitats from which samples originated, and results obtained by laboratory analyses.

Analyses results obtained by applied genetic markers revealed that interpopulation diversity was higher than it was registered within studied black pine seed stands. On the basis of obtained results black pine provenance regions in Serbia were defined in accordance with the Law on reproductive material of forest trees (Articles 6 and 7), map 2.

Since Genetic distance was determined between analyzed seed stands intended for nursery production, the trade of black pine seed material should be realized in accordance with defined provenance regions.

Results of investigation revealed the existence of inter population variability of physiological and morphological cone, and black pine seed traits, which confirmed the expressed genetic diversity, which is the basis for differentiation of ecotypes of this kind in the area of Serbia's territory.

From these reasons and with the aim of preserving genetic specificity of natural populations of black pine it is necessary to take care of two aspects when rejonizacije/locating seed objects: genetic differentiation, and preservation of gene fund of this species (*in-situ* and *ex-situ*).

By spatial distribution of existing seed objects of black pine in Serbia, its central part, as well as the eastern and southeastern regions are not supplied with natural seed sources, although they ecologically present entireties of special significance for forestry in Serbia. This state is the consequence of the features of black pine natural populations in these parts of the Republic.

Areas and continuation of black pine forests are such that it is practically impossible to separate greater complexes of seed objects, so the selection must be directed toward separation of group of trees or individual trees, Mataruga et al. 1998, 2005. According to the Spatial plan of the Republic of Serbia and Strategy of forest development, by which the huge work relating to forestation was predicted, it is necessary to separate seed objects in these areas as the carriers of seed production, and as the representatives of the provenance regions.

Analyses of protein content in assimilation organs of 400 test trees were used to test genetic structure of seed stands and some information on population and individual variability of spruce, fir and beech was obtained. Interaction between ecology traits characteristic of habitat population from which assimilation organs originated and the results obtained by analysis of protein complexes was determined.

Analyses results revealed that assimilation organs of different seed stands had different protein patterns. Differences in number, distribution and intensity of protein fractions were determined. On the basis of obtained electroforegrams the protein profile of assimilation organs for each tested seed objects was determined, formula (combination of numbers and letters) given, and coefficient of genetic similarity between tested seed stands was calculated. Genetic similarity within each seed object was calculated on the basis of analysis of needle-associated protein complex taken from mother trees.

Cluster analysis of tested populations revealed expressed genetic diversity of studied populations illustrated by a dendogram. Comparative analysis of constructed dendograms clearly indicated that genetic distances among populations in L.C. Pirot, N.P. Tara, L.C. Užice, and L.C. Raška were estimated to be the smallest. An interesting fact for both dendograms is the connection between L.C. Pirot population and other populations. Spruce in seed objects of L.C. Pirot was connected to other populations, but that connection was extremely loose, which was probably the result of great genetic distance in relation to other population.

Obtained results are the basis for future work on development of model of crossing of test trees of different seed stands, and it is supposed that the obtained hybrid seed will show heterosis. On the basis of ecological characteristics and seed objects stand condition, productivity, and yield quality of individual subpopulation obtained by biochemical analyses, application of math-statistical analyses and determined genetic distances among black pine, spruce, fir, and beech seed stands, the maps of provenance regions for these species in Serbia were created (Maps 2,3,4 and 5).

These maps make the basis for more reliable trade of seed material within Serbia, and they are also needed for issuing documentation for the purpose of exportation.

CONCLUSIONS

Application of genetic markers to study populations of black pine, spruce, fir, and beech seed stands in Serbia, as well as the structure and the function of their genomes enabled more reliable identification of molecular, biochemical and ecophysiological basis of genetic divergence between the populations, and their transfer into the seed traits.

By studying individual or group variability and the genome structure of these populations using protein markers as the most often used polymorphic markers at the level of gene products, and molecular DNA markers based on PCR phenomenon we should get better acquainted with the nature:

- Mutual relationships between ecological traits, which characterize habitats of black pine, spruce, fir, and beech stands, from which analyzed seed and assimilation organs originate, and results obtained by laboratory analyses;
- degree of variability of certain morphometric characteristics of seed, and assimilation organs, and existance of mutual dependence between studied characteristics;
- genetic similarity, i.e. diversity among studied populations;
- separation of black pine (*Pinus nigra Arnold*), spruce (Picea abies Karst), fir (Abies alba Mill.), and beech (*Fagus moesiaca* /Domin./ Czezccott) provenance regions in Serbia.
- separated provenance regions are presented on maps: four for black pine, spruce and fir, and five for beech.

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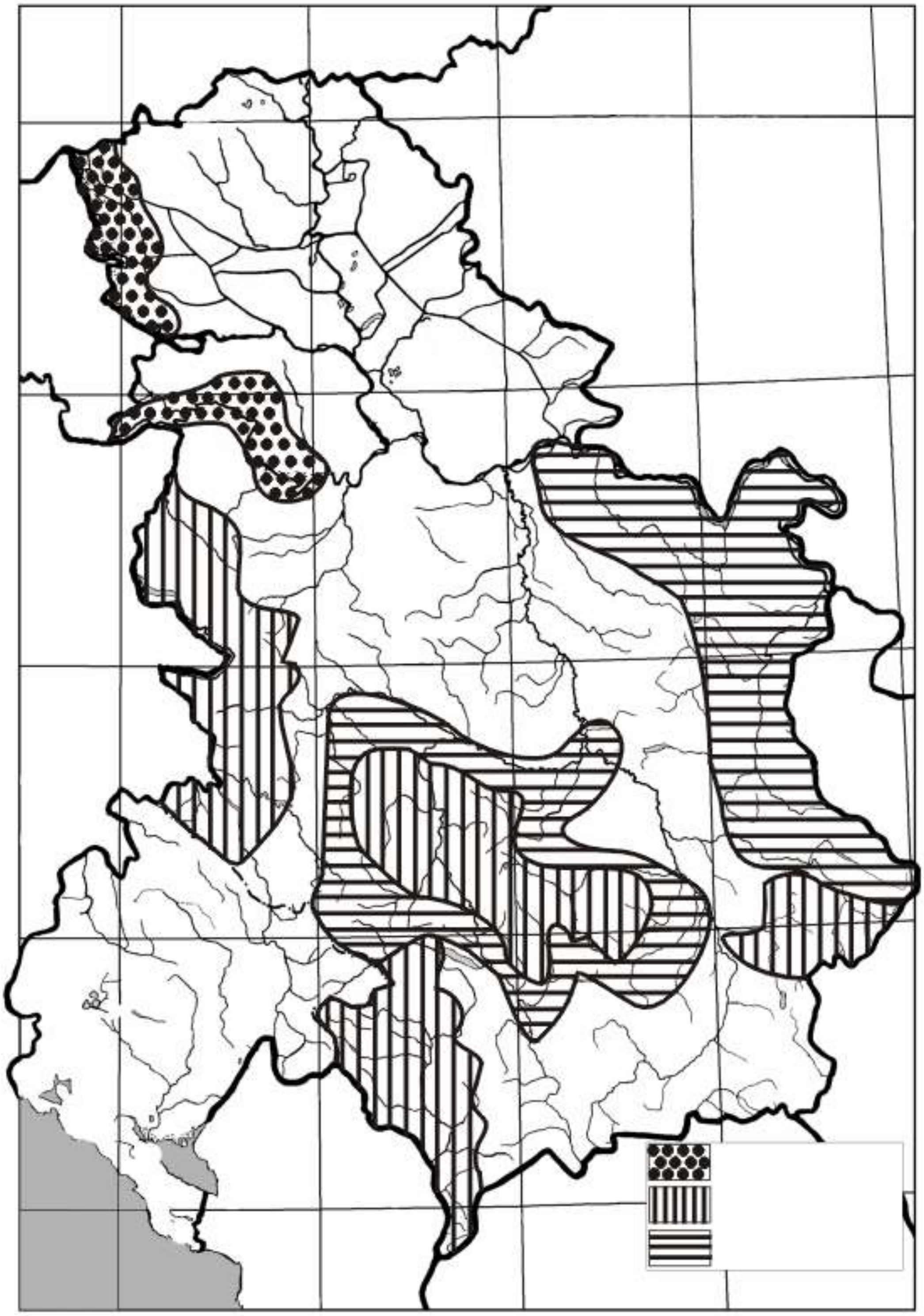
Map 1 - Spatial Distribution of Tree Seed Stands in Serbia

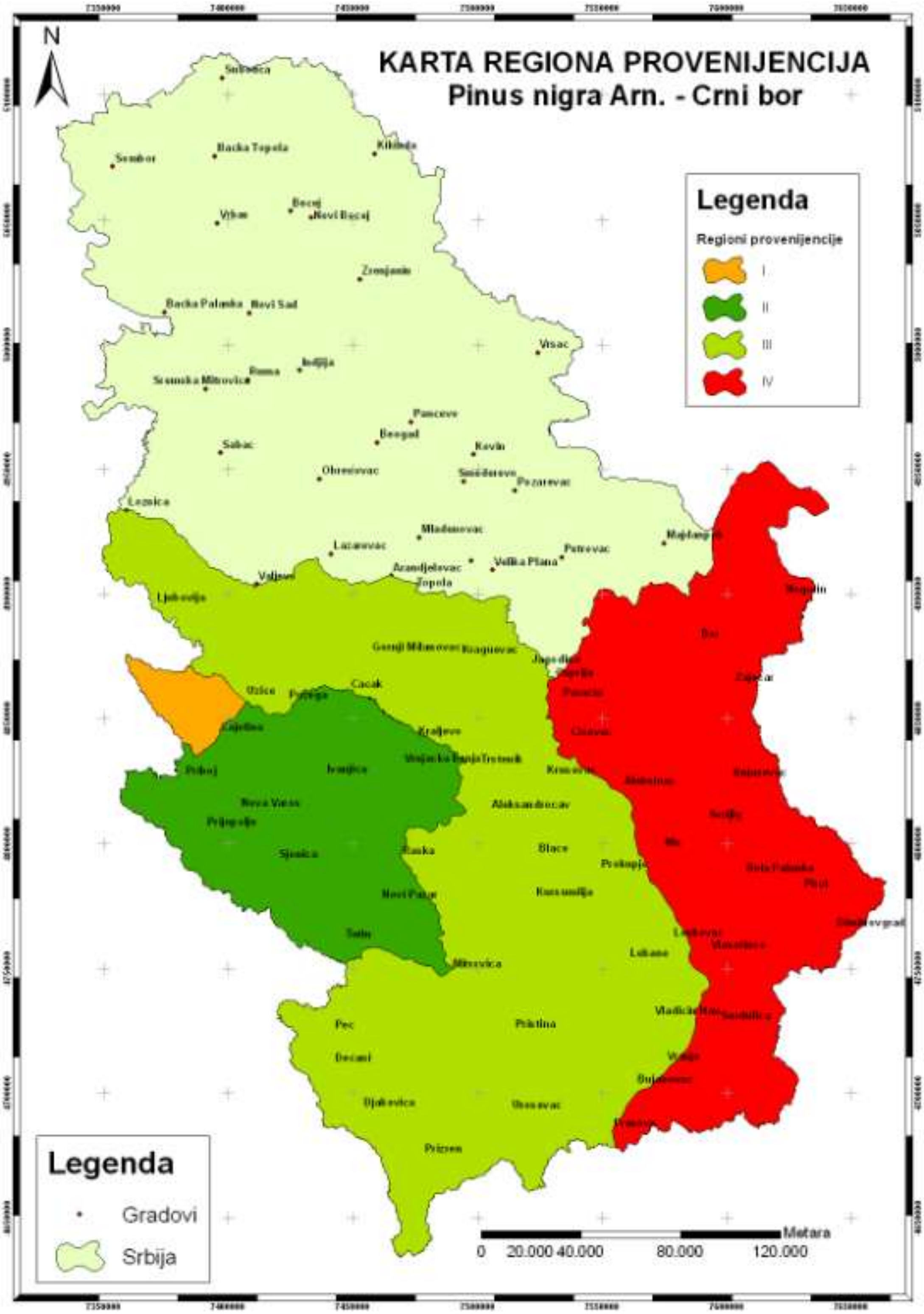


COMMON OAK SEED STANDS

CONIFER SEED STANDS

BEECH AND SESSILE OAK SEED STANDS

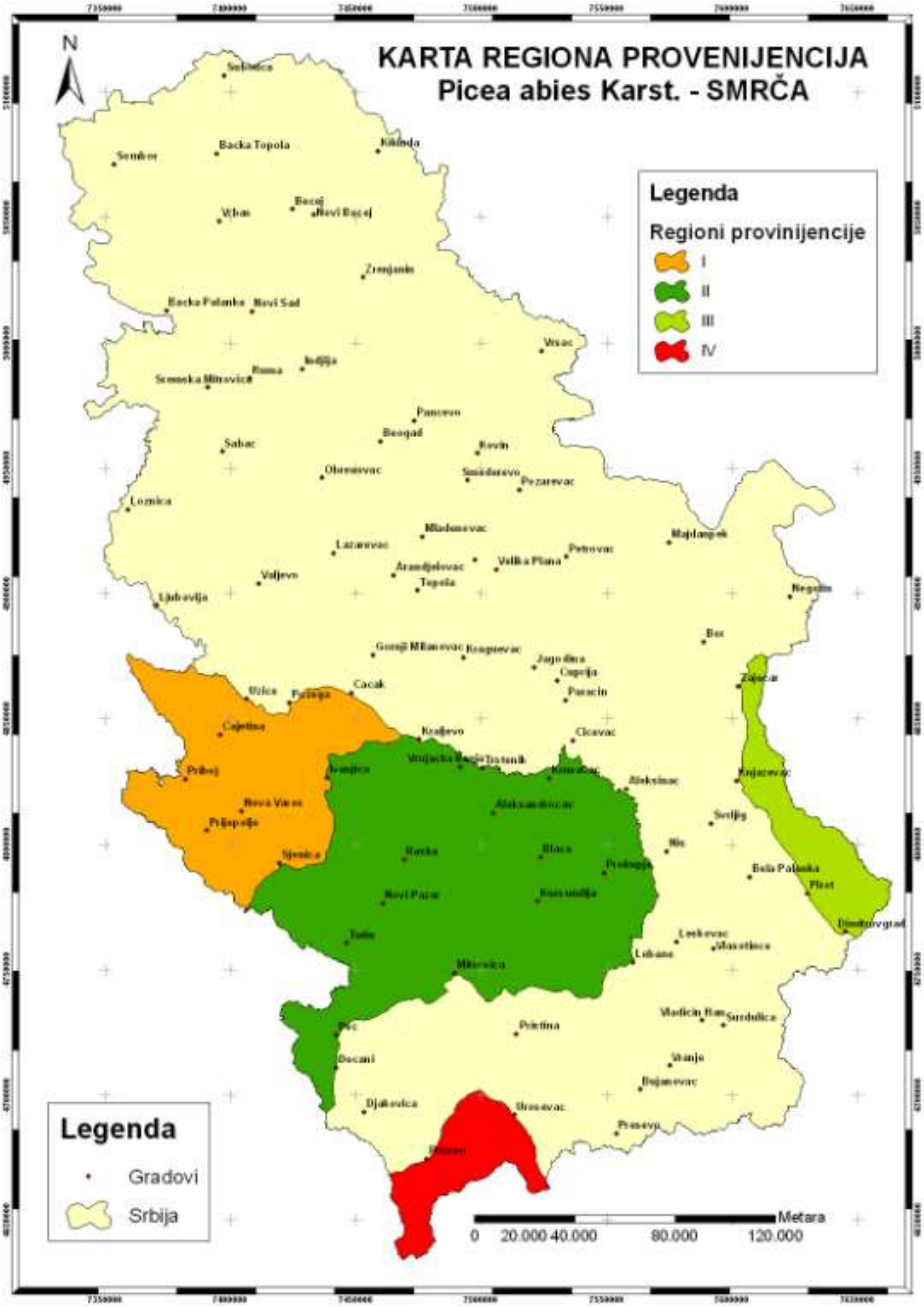
Map 2 - Black pine provenance regions in Serbia



Maps and details of the region of provenance
Legend

- cities
  Serbia

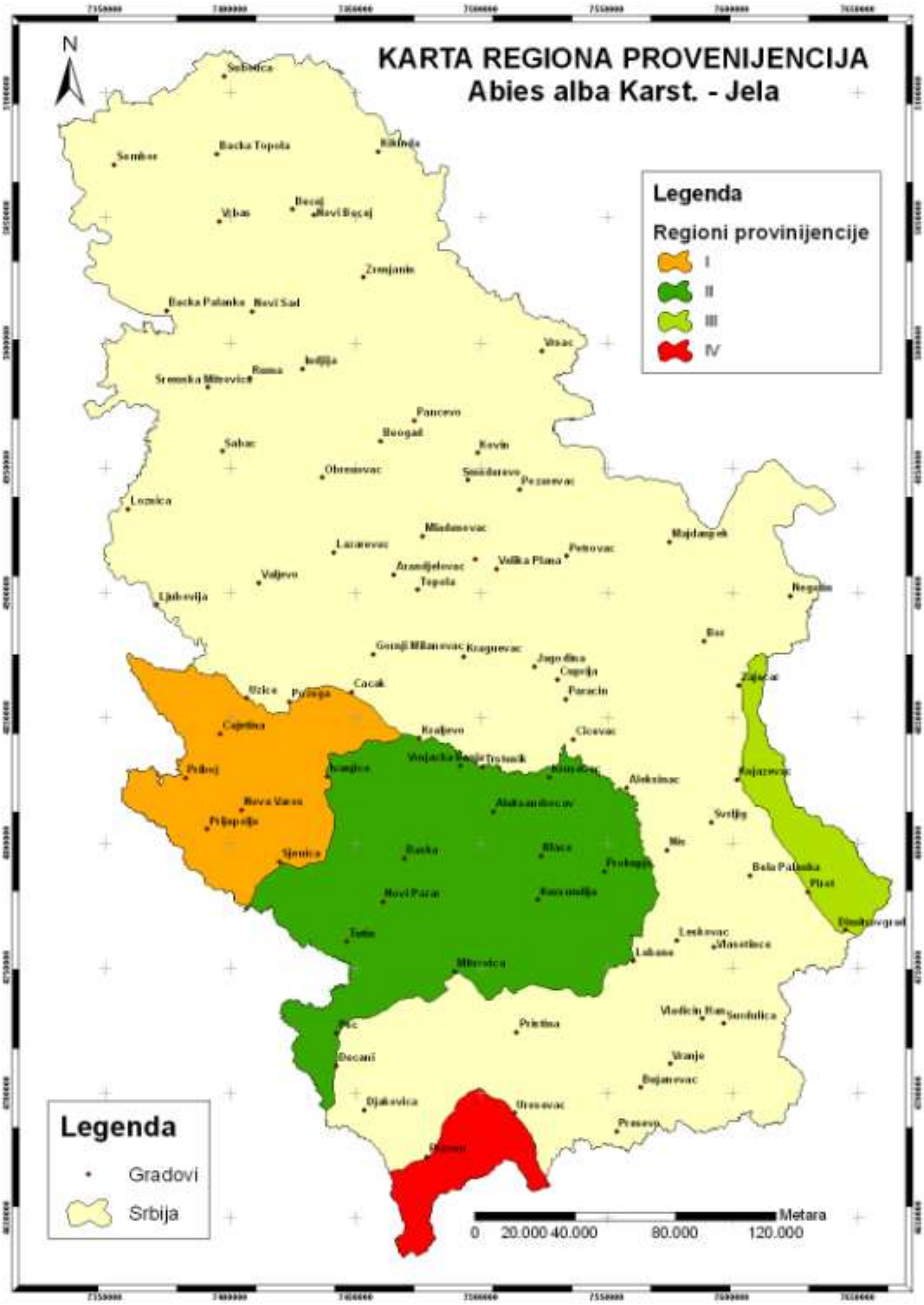
Map 3 – Spruce provenance regions in Serbia



Maps and details of the region of provenance
Legend

- cities
  Serbia

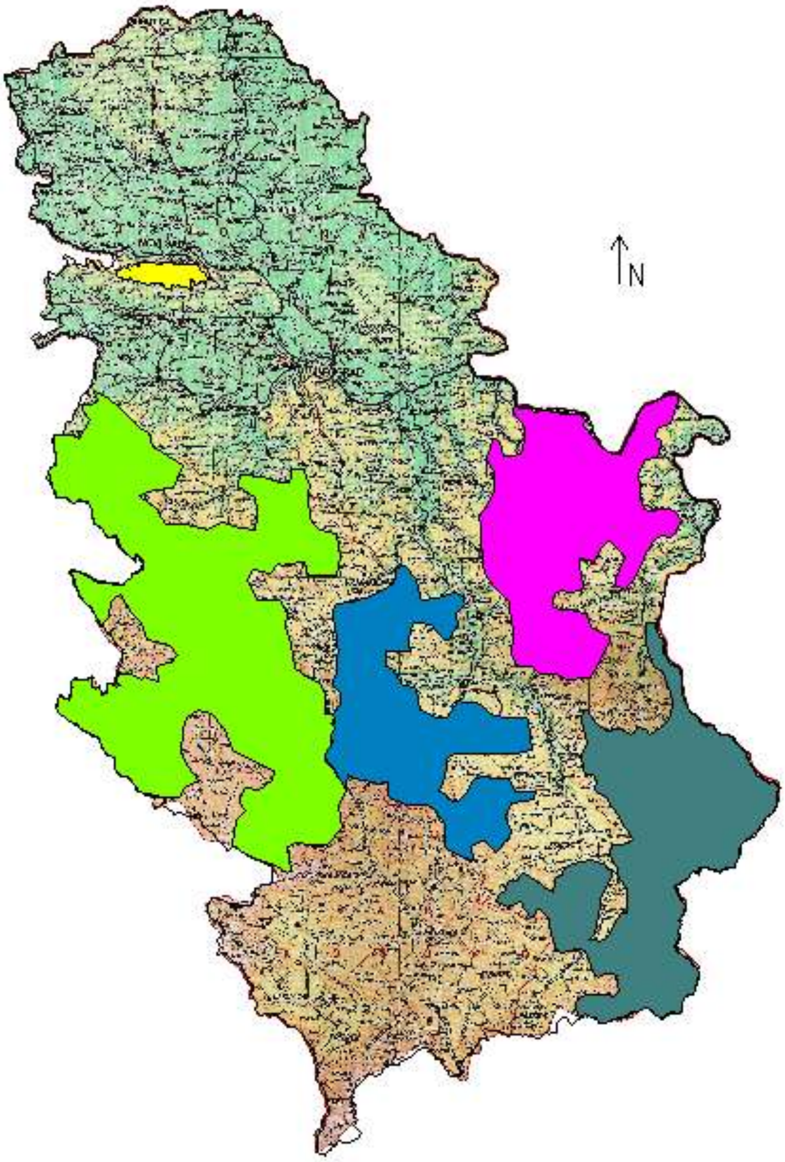
Map 4 - Fir provenance regions in Serbia



Maps and details of the region of provenance
Legend

- cities
  Serbia

MAP 5 – BEECH PROVENANCE REGIONS IN SERBIA



IMPROVEMENT OF BLACK LOCUST *(Robinia pseudoacacia)* STANDS

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Abstract: *Black locust (Robinia pseudoacacia L.) was the first forest tree species introduced and acclimated from North America to Europe at the beginning of the 17th century. It is a fast growing, nitrogen fixing, site tolerant, excellent coppicing species with frequent and abundant seed production and relatively high yielding potential. It has a durable and high quality wood, which is used for many purposes. Although native of North America, black locust is now naturalized and widely planted throughout the world from temperate to subtropical areas. In Hungary, this species has played a role of great importance in the forest management, covering approximately 23 % of the forested area and providing about 19 % of the annual timber output of the country. Due to the increasing interest in black locust growing in many countries, like in Serbia this study has been compiled with the aim of giving a summary on the basis of research and improvement connected with the species over the past decades.*
Key words: *black locust (Robinia pseudoacaia L.), clone selection, silviculture, yield, energy plantations*

INTRODUCTION

Black locust was introduced in Hungary between 1710 and 1720. The first large black locust forests were established at the beginning of the 19th century on the Great Hungarian Plain stabilizing the wind-blown sandy soil. In the country, black locust occupied 37.000 ha in 1885, 109.000 ha in 1911, 186.000 ha in 1938 and 4.000.000 ha in 2005. At present, it is the most widely planted species in Hungary, covering 23% of the country's total forest area. One-third of these stands are high forests and two-third of them are of coppice origin. In the 1960s, Hungary had more black locust forests than the rest of European countries together.

Black locust forests in Hungary have been established on good as well as on medium and poor quality sites. Establishment of black locust stands producing timber of good quality is possible only on sites with adequate moisture and well-aerated and preferably light soils, rich in nutrients and humus. Black locust forests on medium and poor quality sites are utilized for the production of fuel wood, fodder, poles and props, as well as for honey production, soil protection and environmental improvement.

The most important black locust growing regions in Hungary are located in the south and south-west Transdanubia (hill-ridges of Vas-Zala county, hill-ridges Somogy county), the plain between the rivers Danube and Tisza (Central Hungary) and north-east Hungary (Nyírség region).

IMPROVEMENT OF BLACK LOCUST STANDS IN HUNGARY

Clone and cultivar selection

In Hungary, the main goals of the first black locust breeding programme (in the 1960's) were to select new clones and cultivars providing good quality and volume of industrial wood. Superior tree groups have been identified in some seed grown stands. Graft material was taken from the plus trees and planted in test plots at Gödöllő (experimental station of FRI). *Mono- and multiclonal cultivars* were developed and a seed orchard was established from the selections. The Hungarian Forest Research Institute coordinated this research programme. With respect to the volume expected at felling age, the *'Jászkiséri', 'Kiscsalai', 'Nyírségi', 'Üllői'* and *'Szajki'* cultivars proved to be the best (Keresztesi 1988)

In Hungary, the range of sites optimal for black locust growing is rather limited. Therefore, black locust growing is often exercised on sub-optimal sites. Possibilities for black locust growing are highly influenced by climatic conditions and extremes (temperature and precipitation, water supply and unfavourable soil conditions). In the lowlands, which are the most suitable regions for black locust growing, the annual precipitation is not more than 500–550 mm, most of which is outside the growing season. Thus drought is a frequent phenomenon in the summer period coupled with very high atmospheric temperatures (30–35 ^{o}C). Relative air humidity in July is usually between 20–50 %. Due to the filling up of basin-like lowlands in Hungary, site conditions show a mosaic pattern, which changes even over small distances causing widely differentiated growth potential for black locust plantations. For this reason, there are no large, contiguous lands of homogenous site quality for black locust, and their growth and productivity may be very different across a large field. Therefore, the main aim of our *new selection work* is to find and improve black locust clones and cultivars, which perform good shape, provide good-quality wood material for industrial purposes, and which are able to tolerate the changing ecological conditions as well. As a result of our new selection programme 12 black locust clones (*'KH 56A 2/5', 'KH 56A 2/6', 'MB 12D', 'MB 17D 4/1', 'CST 61A 3/1', 'MB 15A 2/3', 'MB 17D 3/10', 'PV 201E 2/1','PV 201E 2/3', 'PV 201E 2/4', 'PV 35 B/2',and 'PV 233 A/2'*) have been recommended (Rédei-Osváth-Bujtás-Balla, 2002, Rédei 2003).

Propagation

In Hungary, black locust plants are commonly multiplied by two methods: by seed and by root cuttings. Growing trees from *seed* is a relatively simple method for reliably producing seedlings on a large scale under a variety of circumstances. There are two state approved seed production stand-regions meeting the requirements for black locust seeds (one in the plain between the rivers Danube and Tisza and the other in the Nyírség region). Seeds are collected by sieving the top 20 cm of soil beneath the selected seed-producing stands. As the seeds of black locust used to remain dormant in the soil for several years, the age of seeds within the lots collected in this way is very variable. This is the reason of viability and germination are so variable. Therefore, before sowing, an accurate seed test is necessary. Seed production for sowing and scarification is carried out by the agency responsible for collecting the seed. It is advisable to treat the seeds against fungi, and this is done in a small concrete mixer. 200–250 thousand seedlings of 40–90 cm high and 5–12 mm in base diameter are raised on one hectare. Mechanization of the method is easy and the production costs are relatively low.

Propagation from *root cuttings* is suitable for reproduction of superior individuals or varieties (cultivars). By applying this method, superior traits of the selected trees can be preserved in the clones. Production of plants in this way demands more care than raising seedlings from seeds. For this propagation method, root pieces cut into 8–10 cm or chopped to 3–5 cm in length are used. Plant spacing in the rows should be 5–8 cm.

Almost 25 new cultivars or selected clones were micro-propagated during the last few years in the Micro-propagation Laboratory of Research Institute for Fruit growing and Ornamentals, Érd in collaboration with the Hungarian Forest Research Institute. *Plant tissue culture methods* provide us with new means to speed up vegetative propagation of recently selected clones and give us the opportunity to establish new clone trials and a seed orchard with them.

Stand establishment, forest tending and yield

Climate, hydrology and genetic soil types are the factors that determine the site type, and this in turn determines the choice of tree species. The water regime of the soil is also highly influenced by the texture of the soil, whether it is humus, coarse sand, loam or clay. Black locust – because of its high requirement for both water and aeration in the soil –

cannot be grown even on any soil composed of humus, coarse sand or clay if the rooting depth is very shallow.

Black locust requires well-drained soils with adequate moisture until the associated nitrogen-fixing *Rhizobium* bacteria are able to thrive. That is why soil preparation (total or partial) to improve aeration and the water regime of the soil and tilling of the inter-row space may become necessary.

Black locust afforestation and artificial regeneration may utilise seedlings. The most popular spacing for black locust in Hungary is 2.4 m by 0.7 to 1.0 m, requiring at least 4000 seedlings/ha. Black locust stands are often regenerated by coppice (from root suckers) as well. In young stands of coppice origin, a cleaning operation should be carried out to adjust spacing when the stands are 3–6 years old and should reduce stocking to less than 5000 stems/ha.

The black locust is a fast-growing tree species, which, up to the age of 10–15 years, is able to close canopy openings caused by tending operations quickly, but the closure is much slower in later years. Height growth peaks within the first five years, while diameter growth culminates in the first decade. The peak of current annual increment is at about the age of 20, whereas that of the mean annual increment is at about the age of 35–40 years.

To find the right cleaning and thinning intensity, the so-called growing space index is a good method. This index expresses the mean distance between trees (in a triangular pattern) as a percentage of mean height after cleaning and thinning. The mean value of the index for black locust stands should be 23–24 %. Pruning of crop trees should also be carried out. After finishing selective thinning, stems must be free of branches up to a height of 4–6 m.

The objective of tending is to produce a high proportion of good quality saw-logs from stands of yield class I and II; some saw-logs and a high proportion of poles and props from stands of yield class III and IV; and poles, props and other small-dimension industrial wood from other yield stands.

According to our yield table (Rédei 1984) the volume of main crop varies between 80 and 280 m^3/ha in function of yield classes at the age of 30 years, which is the average rotation age for black locust stands in Hungary. The black locust stands of Yield Class I–II have a rotation of 35–40 years and an annual increment of total volume of 12–14 m^3/ha/yr. The stands of Yield Class III–IV have a rotation of 30 years and an annual increment of 8–9 m^3/ha/yr. Finally, the poorest stands (Yield Class V–VI) have a rotation of 20–25 years and an annual increment of 4–6 m^3/ha/yr. In first generation coppice stands, growing stock, increment and health are similar to those in high forests.

Black locust energy plantations

More and more agricultural land is being taken out of use for food crops, some of which can be used for wood energy production plantations. Black locust is the very best tree species for this purpose, since it has excellent energy production properties, such as:

- vigorous growing potential in juvenile phase,
- excellent coppicing ability,
- high density of the wood,
- high dry matter production,
- favourable combustibility of the wood,
- relatively fast drying,
- easy harvesting and wood processing.

In the last decade several energy producing plantations have been established in Hungary. In these experiments, several spacing treatments were tested and the common black locust as well as its cultivars were compared.

In Helvécia (central Hungary, sand-soil region) an energy plantation was established using common black locust and its cultivars. The various spacings of the common black locust were: 1.5x0.3 m, 1.5x0.5 m and 1.5x1.0 m. At the age of 5 years the closest spacing (1.5x0.3 m) produced the greatest annual increment in oven-dry mass (6.5 t/ha/yr). This exceeded the increments of the two wider spacings by 33 % and 51 %, respectively. According to the results of the yield trial with black locust cultivars planted at 1.5x1.0 m spacing, at 5 years the highest yield was produced by the cultivar *'Üllői'* (8.0 t/ha/yr), followed by *'Jászkiséri'* (7.3 t/ha/yr) and the common black locust (6.7 t/ha/yr).

Black locust energy forests can also be established by coppicing. Advantages of energy forests of coppice origin are that the cost of establishment is low compared to that of soil preparation, plantation and cultivation. From the developed root system of the previous stand, a large dendromass can be produced within a short time period. Disadvantages of these forests are that the area distribution of trees in coppice stands is not as uniform as in plantations optimized for energy production. In coppice stands the quantity of the produced dendromass is lower and the length of growing time is highly influenced by the uneven distribution of stems.

The first peak of the annual increment in volume of black locust energy forests established from sprouts falls between the age of 3 and 5 years. Then, the annual increment declines and a new peak occurs between age of 9 and 12 years. A further maximum is expected later on, at about 15 years because of an even higher degree of mortality. Approximately one-third of the stems are lost at age 7 and 8. By the 12–13 years, the stem numbers decreased to less than 50 %.

The experiences from both the planted and the coppiced energy plantations and other stands indicate that it is not reasonable to harvest in the first three years, as the yield in oven-dry weight in the fifth year is 2–3 times higher than it is in the fourth year. Harvesting too early may also increase the population of biotic pests.

CONCLUSION

Black locust was the first forest tree species introduced from North America to Europe. Hungary has got much experience in black locust growing, as it has been grown for more than 250 years in the country. Being aware of the importance of black locust, forest research in Hungary has been engaged in resolving various problems of black locust management for a long time, and a lot of research results have already been implemented in the practice.

In the future there are two bigger regions, where the fast spread of black locust can be expected. In Europe the Mediterranean countries (Italy, Greece, Spain and Turkey), while in Asia China and Korea may become the most prominent black locust growers.

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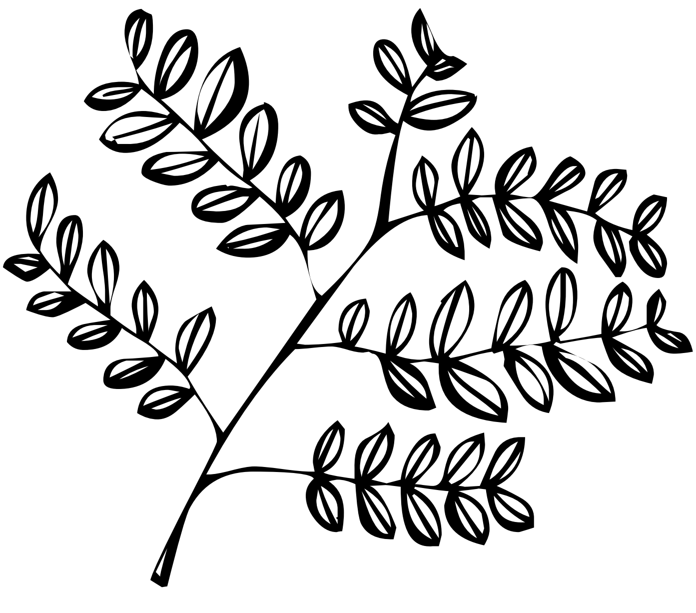

FOREST ECOSYSTEM DEVELOPMENT UNDER A CHANGING ENVIRONMENT & CONCLUSIONS FOR FOREST MANAGEMENT

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Summary: *Lowland forest ecosystems in Saxony and Saxony-Anhalt were impacted for more than a century by heavy industrial pollution. Especially the so called "Dübener Heide", the largest forest area at the border between Saxony and Saxony-Anhalt was affected by depositions from the most important industrial triangle of the former GDR Leipzig-Halle-Bitterfeld. At the same time, management of Dübener Heide underwent high pressure for ensuring the timber supply and the recreation of citizens in this congested urban and industrial region.*

Regional silvicultural management decisions, which were confronted to both, increasing industrial deposition and increasing demand in biomass production, led to large-scale establishment of pure Scots pine stands. These were confronted to extremely high amounts of alkaline deposition (18 Mio. t fly ash) and acidic depositions (12 Mio. t. SO_2*) from regional power plants and chemical industry. This disastrous combination led finally to the break-down of large parts of the regional forest ecosystems and entailed high efforts for conversion and restoration of the forests. Nowadays, a recovery of the forest ecosystems can be observed, which is indicated by large scale re-establishment of original site properties and the original ground vegetation types and which is accompanied by tree growth, which exceeds by far the original regional level. However, climate change poses new challenges to these forest ecosystems. A reduction of 20 % of the actual precipitation and an increase of the mean annual temperature of 3.5 °C are expected for the next 100 years. Beyond this background, the stability and resilience of the actual forest ecosystems must be questioned critically. First studies however show, that the capability of the actual forest ecosystems in buffering such changes allows concluding that changing climate conditions can be responded by respective silvicultural management measures.*

The article introduces (a) the reactions of the exemplary forest ecosystem Dübener Heide on changing environmental frame conditions, where a complex study was conducted in the frame of the research project ENFORCHANGE (www.enforchange.de), supported by the German Federal Ministry of Education and Research. (b)Conclusions on possible management consequences and future challenges are drawn.

Keywords: *Forest ecosystem development, industrial deposition, ecosystem processes, process-oriented forest management.*

INTRODUCTION

Forest ecosystems all around Europe were faced since the very first settlements to an intensive impact of human activities, which led early to changes in the forested area itself and influenced among others the tree species distribution by selective use, furthering or fighting of tree species. Furthermore, human activities influenced the environmental frame conditions under which forests grow. Most actual examples are large-scale deposition of industrial exhalations, especially SO_2, which led to severe forest decline effects up to the 1990ies, ongoing N deposition from agriculture and traffic, which provoke nutrient imbalances and last but not least climate change as the main driver for the actual development of European forest ecosystems. Lowland forest ecosystems are particularly sensible to such impact, as they grow mainly under dry conditions and as their growing area is mostly restricted to sites with unfavourable conditions for other land-use forms. Furthermore, they are often - as a result of favourable topographic conditions - situated near to densely settled and used areas and thus are confronted today to widely spread demands from regional society. Fig. 1 records as an example 200 years history of human impact on the so called Dübener Heide, a well investigated Lowland forest ecosystem in North-Eastern Germany.

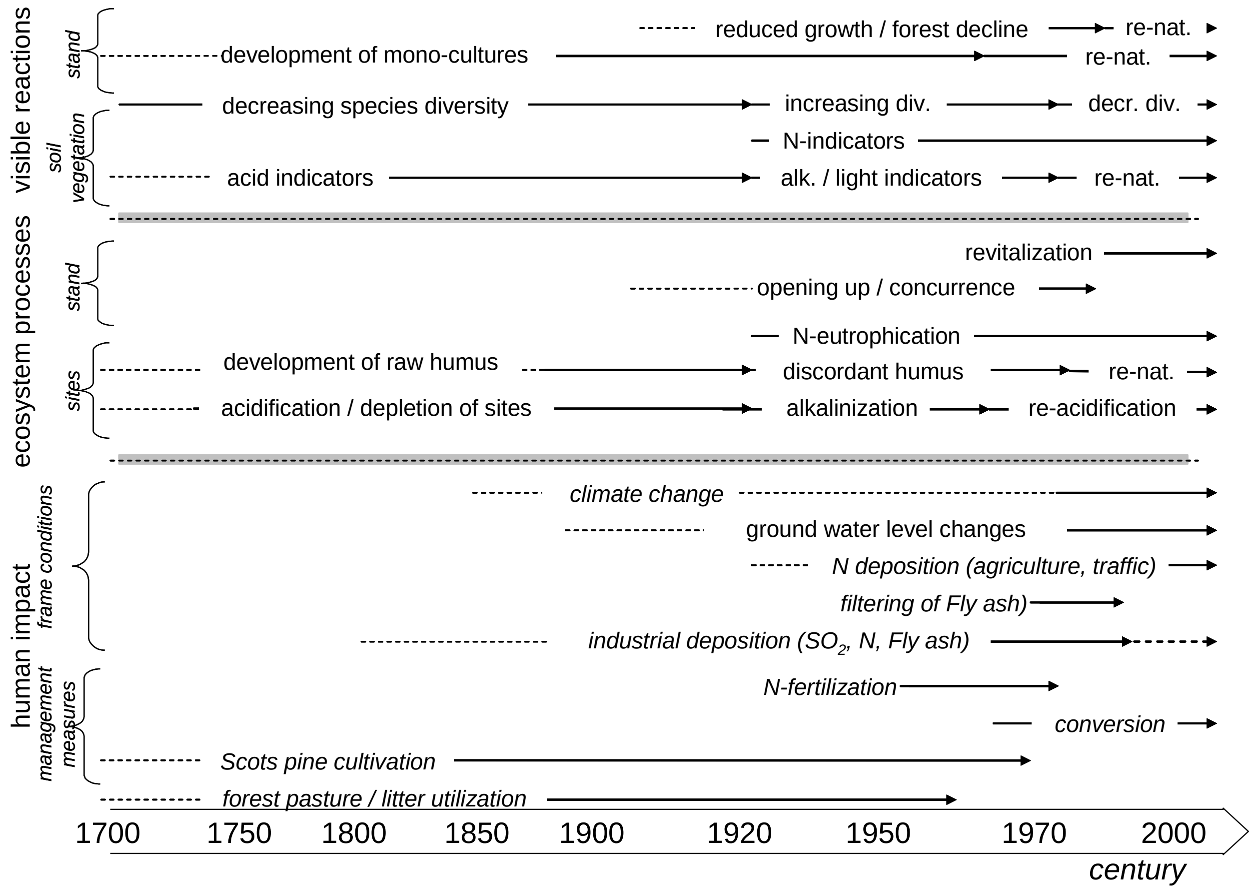

Figure 1. History of human impact on the Lowland forest ecosystem Dübener Heide (acc. to Fürst et al., 2007). Main processes and visible reactions are resumed. Arrows show the duration of the impact factors, processes and ecosystem reactions. Dashed lines are used when the exact start of an impact, an ecosystem process or a visible reaction is not known. The impact factors shown in Fig. 1 are separated into impact by management measures and impact by change of the frame conditions for the ecosystem development. Ecosystem processes are separated into site processes and processes on stand level. Visible reactions are shown for the soil vegetation and the stand level. (alk. = alkaline, div. = diversity re-nat. = re-naturalization).

In Dübener Heide, systematic cultivation of Scots pine (Pinus sylvestris, L.) started from the middle of the 18th century on. This led in combination with local forest pasture and litter utilization to poor Scots pine forests, which were characterized by acidophilic soil vegetation groups (dwarf shrubs, mosses, lichens) and raw humus forms (Bendix, 2001).

Due to the regional lignite occurrence, industrialization started early in the immediate vicinity of Dübener Heide, but was limited at the beginning to small power plants with local deposition effects. From the 1920s on, an intensification of industrial energy production led finally to incredibly high deposition loads, which amounted from 1910 – 2000 to 18 Mio. t fly ash and 12 Mio t SO_2. N deposition from industry and agriculture amounted up to 300 kg / ha * a, and was even surpassed in the 1960ies by local N fertilization of up to 990 kg / ha. Fly ash was deposited along a characteristic distance and wind direction in dependent gradient, whereas SO_2 and N deposition were more evenly distributed (Lux, 1965; Klose and Makeschin, 2004).

Deposition and fertilization dependent vegetation types appeared in the Dübener Heide from the 1920/1950ies on. Basophilic and light preferring species started to settle in the immediate vicinity of the former power plants. They indicated alkaline dust deposition and the hereby provoked opening-up and die back of the Scots pine stands. Raising regional vegetation diversity and high species diversity near to the power plants went along with a decreasing vitality of the Scots pine stands. This process was accompanied by artificially elevated pH (KCl) values (up to 7) and base saturation (up to 100 %) in the humus layer and upper mineral horizons. Based on a visual assessment of forest decline, up to four deposition zones were distinguished, which formed the basis for a spatially differentiated ecosystem management intensity. Efforts to convert the Scots pine stands started from the 1970ies on in the zones with heavy forest decline phenomena and they were widened to the total area from the 1980ies until now (Lux, 1964 a, b; Kopp, 2003).

From the 1980s on, fly-ash filters were introduced and the still increasing acidic deposition components NO_x, SO_2 / SO_x were no longer buffered by alkaline dusts. Herpel et al. (1995) documents for some sample plots in Dübener Heide a decrease of pH(KCl) of 0.4 units and base saturation decrease of 17 % until 1988 compared to the situation in the 1970s. From 1988 to 2000, a further reduction of 0.7 pH-units was reported by Kurbel (2002).After 1989, SO_2 and fly ash deposition were more or less stopped, whereas N deposition in a magnitude of 28 – 45 kg/ha*a from animal husbandry and traffic amplifies the long-term effects of the former N-deposition and N-fertilization in terms of dense grass layers and nitrophile soil vegetation. However, N-eutrophication was balanced to a certain degree by ample ground vegetation development, improved tree growth, and vital natural regeneration of mainly noble hardwoods (Lux, 1964 b). Kopp (2003) expects also a re-development of humus forms, which represent the original site potential.

Nowadays, re-immigration of acid indicators and disappearance of the dense grass layers are observed due to vanished base deposition and ongoing acidic deposition (Augustin et al., 2005). The health of regional forests has improved as a result of lower industrial deposition and conversion, but they are still threatened by increasing N deposition (Materna & Fiedler, 1994).

One of the major challenges for future management of the forests in Dübener Heide is the change of the regional climate. Here, considerable decrease of precipitation and increase of temperature are expected. This raises the question, if the actual tree species composition and noble hardwood regeneration are well adapted for the future.

MATERIALS AND METHODS - FOREST ECOSYSTEM STUDY ENFORCHANGE

"ENFORCHANGE" (Environment and Forests under Changing Conditions, www.enforchange.de) is a research project supported by the Federal Ministry of Education and Research (BMBF, Germany).Within the project, long-term forest ecosystem reactions and processes as a result of changing frame conditions and the consequences for a sustainable forest ecosystem management are studied.

ENFORCHANGE tested the hypothesis that forest sites in Dübener Heide are still impacted considering potentials such as nutrient availability and water balance as well as specific risks such as heavy metal release. Furthermore, it was assumed that forest growth is still superposed by the spatially differentiated effects of former depositions along a historically documented regional deposition gradient. To support forest ecosystem management, ongoing ecosystem processes under special consideration of climate change effects are modelled and finally up-scaled from a number of sample plots by statistic regionalization approaches. Figure 2 resumes the monitoring - modelling - management information and support chain, which is realized in the project.

A combination of available GIS-data, data from climate and environmental monitoring, field trials and own measurements is used to feed the process model BALANCE, which is coupled with the stand climate model HIRVAC and the tree growth model SILVA. The model output delivers information on the effects of changing environmental frame conditions and different management alternatives on ecological and economic parameters, which are given as feed-back to the user. Two reference regions serve for the validation of the modelling results and the development of process-regionalization approaches.

12 project plots were installed in Dübener Heide along the regional deposition gradient. These plots represent major (terrestrial) soil type and stand type combinations in the region and are chosen from a pool of 150 study plots, which was established in the 1960ies. At the key plots, chemical and physical site properties including magnetic susceptibility are measured depth level-wise with focus on the humus layers and the upper mineral horizons, and forest growth and yield characteristics are assessed. The project plots deliver the data base for the multiple-regression based regionalization of environmental data. The plot-wise data set is complemented by grid-based screening of spatial differentiation in magnetic susceptibility with two different grid densities (1*1 km^2 and 4*4 km^2). Magnetic susceptibility describes the amount of magnetizable iron-oxides, a component of fly ash from lignite combustion. By correlation with heavy metal or base

cation content, this indicator supports the spatial transfer of respective plot-wise measurements in regions with high deposition amounts. In the project context, magnetic susceptibility is used, to identify spatial strata, which are differentiated (a) by the intensity of former deposition load and (b) by the speed of ongoing processes, such as re-acidification and base cation leaching. These strata are proposed to be used for differentiated handling in regional forest management.

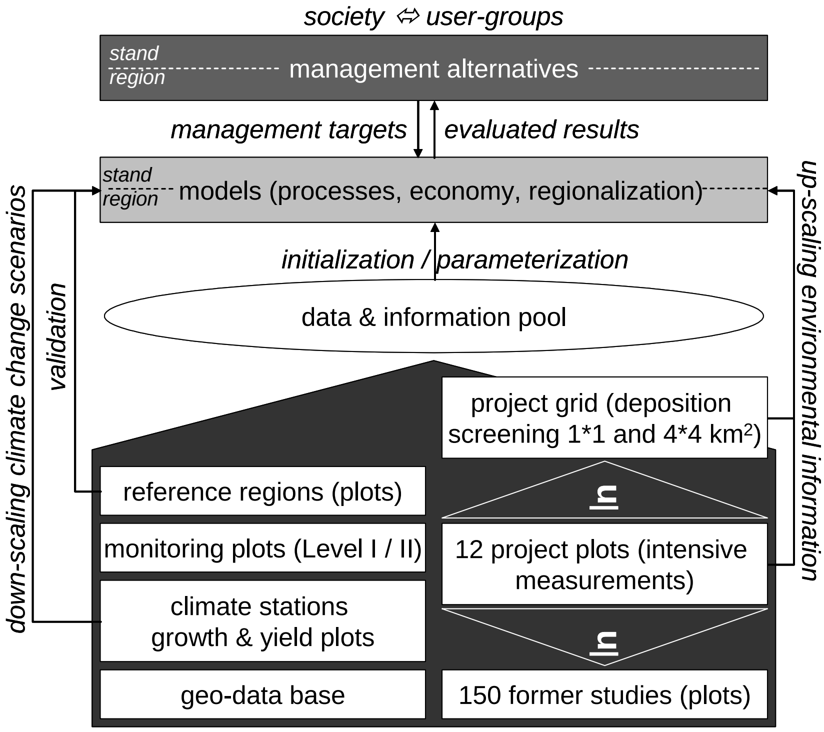

Figure 2. Monitoring - modelling - management information and support chain in the project ENFORCHANGE.

RESULTS

Deposition impact on the site potential

The assessment of chemical and physical parameters at the project plots and the screening of magnetic susceptibility revealed that the spatial differentiation along the historically documented deposition gradient still exists. This gradient is mainly driven by former fly ash deposition. SO_2 deposition impact could not be detected anymore and N deposition affects all parts of Dübener Heide. Differences in the nutrient equipment and the pH values as well as differences in physical humus properties, such as content of mineral matter in the humus layer (Klose et al., 2002; Koch et al. 2002) and last but not least different levels of magnetic susceptibility support a stratification of maximally three spatially distinct areas instead of four as proposed by Lux (965): in up to 8 km distance to the former emitters, pH (KCl) values and base saturation are clearly elevated far beyond the original potential of the sites. Here, fly ash impacts also the physical properties of the regionally dominating raw humus forms with their high hydrophobicity. Hartmann et al. (2007) revealed that fly ash reduces the water repellency and hydrophobicity of the humus layers and increases the water conductivity. At the same time, the available water for plant growth, expressed by the field moisture capacity becomes smaller due to the fly ash caused decrease of the fine pore volume. Additionally, a tendencially decreased depth of the root zone due to high nutrient availability at the humus layer and upper mineral horizon at fly ash influenced sites might amplify the future risk of drought stress (Koch et al., 2002; Klose et al., 2002). In a zone up to 15 km, only pH-values are elevated and in a distance of more than 15 km, no measurable effects could be found.

Based on first tentative extrapolations, pH values tend to approximate the original regional values in a time period of around 30 - 50 ys, where the re-acidification rate in the zone up to 8 km distance is 5 - 10 times higher compared to the zone in more than 15 km distance. In the zone up to 8 km distance, the stock of extractable Ca in the humus layer and upper mineral soil until a depth of 30 cm reaches up to 4,000 kg / ha (Fritz & Makeschin 2007). This is 10 to 20 times higher compared to the plots in a distance of 30 km, which are farthest to the former emitters and whose chemical properties represent more or less the original regional potential. Until now, it is not foreseeable, until when base cation leaching scales down the extremely high base cation potential and especially the Calcium stock in the zone up to 8 km distance to the regionally characteristic level.

While the plot-wise measurements were arranged along the formerly documented deposition gradient, which assumed a strong influence of the power plant "Zschornewitz" in the immediate vicinity of Dübener Heide, the magnetic susceptibility screening was based on regular grid measurements. These measurements revealed (1) that the spatial effect of the deposition went especially at the humus layers much farther than proved by the assessment of chemical and physical properties at the project plots (Fig. 3 a). Furthermore, the measurements highlightened that (2) the impact of the industry site Bitterfeld, which is situated farther from Dübener Heide was clearly stronger than originally believed (Fig. 3 b).

Deposition impact on forest growth

Forest health and growth were extremely affected by the former depositions. Table 1 shows exemplarily the volume increment ($m^3/ha*a$) and volume increment reduction (% of reference value in zone IV) along the regional deposition gradient (Lux 1964 b modified, 1965).

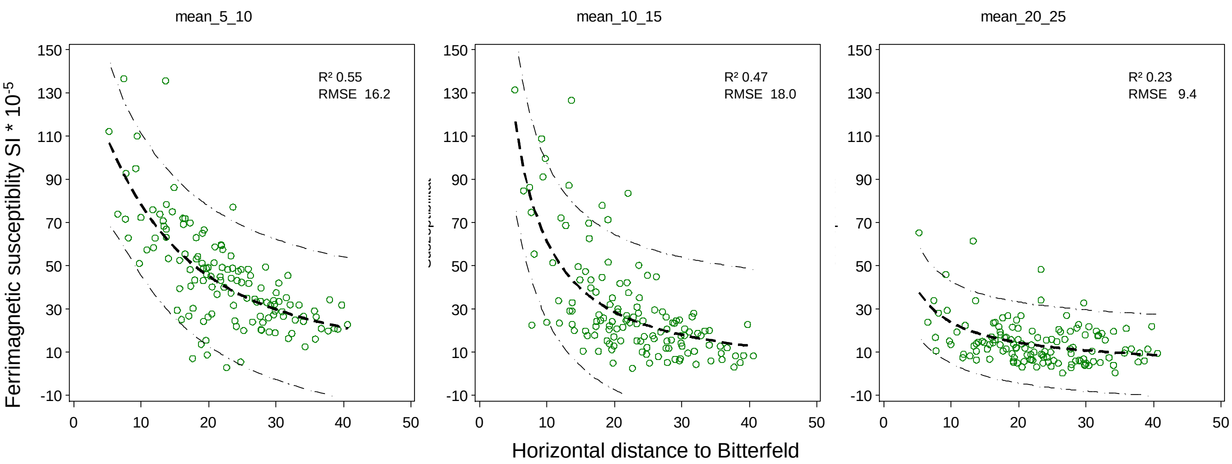

Figure 3a. Spatial range of detectable fly-ash deposition in the depth levels 5 - 10 cm, 10 - 15 cm and 20 - 25 cm, mean values. The correlation between distance and magnetism decreases from 5 - 10 cm to 20 - 25 cm. Fly ash deposition affected mostly the upper 0 - 15 cm.

Comparing the late 1960ies until 1980 with the 1980ies, deposition showed only at the first time period the expected spatially differentiated negative impact on tree growth, with decreasing intensity with increasing distance to the power plants. In the period 1982 - 1988, the former spatial differentiation disappeared. This period was characterized by beginning fly ash filtering, where at the same time, SO_2 deposition even increased. Comparing tree growth parameters between 1940 - 1991 and 1975 - 1991 nearest and farthest to the power plants underpins that the influence of the deposition does not show the extreme spatial differentiation, which was assumed in the 1960ies, the time, were the deposition zones were defined. Of course, forests in the immediate vicinity of the former emitters reacted first on the emissions and thus supported the stratification into four deposition zones at least at the beginning of heavy deposition (Lux, 1966). Later on, the extremely high and spatially widely distributed SO_2 deposition might have been the driving factor for the observed large scale impact on tree growth (Hüttl & Bellmann, 1999) From the 1990ies on, the last power plants were closed or were equipped with modern fly ash and SO_2 filtering techniques. Investigations from intensive forest growth monitoring plots have proved that height growth of Scots pine recovered after the 1990ies. Ongoing measurements at the ENFORCHANGE key plots show that nowadays, height and diameter growth of forests in Dübener Heide follow the general trend to be superior to the benchmark data in the regionally valid growth and yield tables. This applies to all relevant stand types and especially for the regionally dominating Scots pine stands.

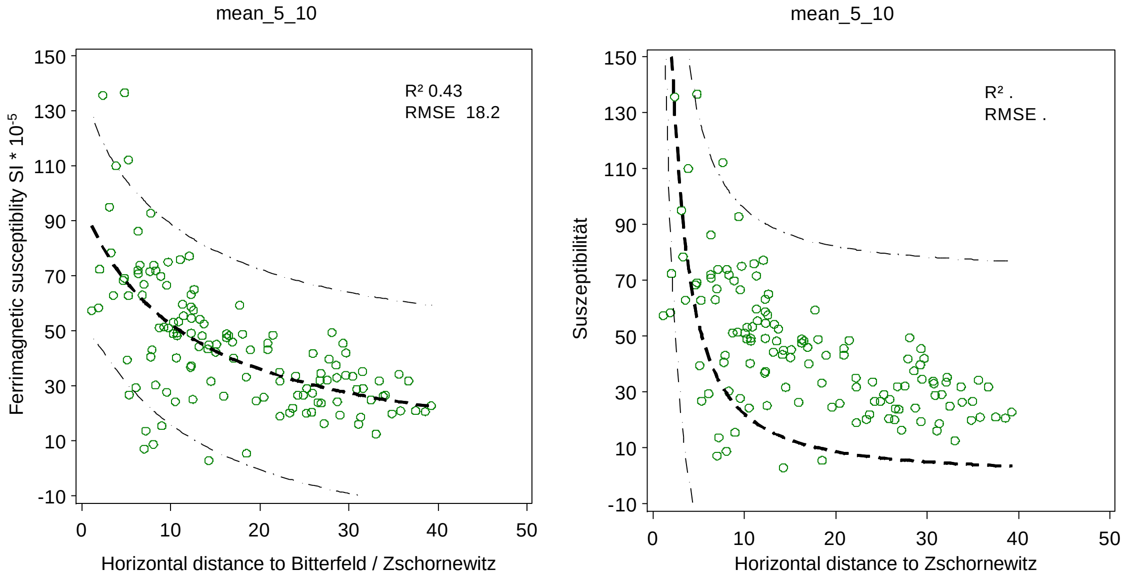

Figure 3 b. Spatial influence of fly ash at the depth level 5 - 10 cm in dependence from the distance to Bitterfeld and Zschornewitz. The spatial correlation between magnetic susceptibility values and horizontal distance is lower for Zschornewitz and the spatial range of detectable fly ash deposition is smaller compared to the impact of Bitterfeld.

Table 1. Volume increment (m^3/ha*a) and volume increment reduction (% of reference value in zone IV) in the deposition zones. DI highest, DII high, DIII medium, DIV low deposition.

Deposition zone		**DI**	**DII**	**DIII**	**D IV - regional reference value**
growth parameters	height (m/a)	0,09	0,15	0,16	*0,23*
	basal area (m^2/ha*a)	0,38	0,53	0,53	*0,64*
	volume (m^3/ha*a)	4,6	5,9	6,2	*8*
growth reduction (referred to zone D0)	height growth reduction (%)	-61	-35	-30	*0*
	basal area increment (%)	-41	-17	-17	*0*
	volume increment (%)	-43	-26	-23	*0*

At a glance, fly ash deposition effects on Dübener Heide can not only be considered as damaging factor, but resulted in double-edged effects on regional forests: positive aspects are a higher nutrient availability (base saturation) and cation exchange capacity and an improvement of physical humus properties like texture and sorption capacity. On the other hand, a disturbance of ground vegetation composition and organic matter decomposition can be observed (Kopp, 2003). A visible consequence of fly ash deposition is the ample noble hardwood and European beech regeneration, which can be observed in the zone of 8 - maximally 15 km distance to the former emitters. Its potential to be integrated into silvicultural concepts must be discussed critically beyond the background of ongoing re-acidification and worsening of climate conditions. Especially re-acidification has a negative impact on the stability and development of the fly ash-adapted stand types and the quality of by-products like water, biodiversity and socio-economic functions.

Climate change impact and forest growth

The down-scaling of global climate change scenarios for Dübener Heide proved that a reduction of the mean annual precipitation of up to 100 mm and an increase of the mean annual temperature of up to 3.5 °C can be expected. Even worse, the water deficiency during the summer period is estimated to become aggravated, which affects especially the regionally dominating poor sandy soils (Goldberg et al., 2007; Franke & Köstner, 2006). The temperature increase and precipitation decrease will not follow a regular trend. Until 2010 an increase of precipitation and small temperature decrease is expected, while from 2010 on a clear worsening of climate frame conditions for forest ecosystem development is predicted.

Modelling of the impact of climate change impact on growth parameters revealed that growth under the recent regional climate conditions is highest. Taking the IPCC scenario A2 and European beech stands as an example, height growth reduction can reach up to 4 m during the stand life, which means a decrease of one yield class in the regional yield tables with respective consequences for future timber production. This applies especially for the young stands, while the consequences in elder stands are considerably lower.

On the other hand, forest ecosystems are able to buffer the predicted decrease of precipitation and increase of temperature almost completely considering inner stand climate. This depends from the stand type: Scots pine stands aggravate the predicted development by their high evapotranspiration rate and low Albedo. Mixed stands or pure broadleaved stands reduce the admission of solar radiation to the soil surface and thus are characterized by clearly lower evapotranspiration rates and temperature compared to open land climate. The ability to regulate the stand climate of mixed and well structured stands is the highest and countervails against the predicted shortening of precipitation and temperature increase (Fischer et al., 2008).

DISCUSSION - CONSEQUENCES FOR MANAGEMENT

Environmental frame condition changes as described for Dübener Heide trigger complex ecosystem processes. Forest management planning should adapt to these ongoing processes instead of basing decisions to descriptions of the actual status, which are delivered by forest inventory and site classification (Schoenholtz et al., 2000; de Vries et al., 2003). Process-oriented forest management planning respects natural dynamics in (forest) ecosystem management on landscape level. In Dübener Heide e.g., fly-ash deposition provoked a homogenisation of site quality differences, and a differentiation of formerly comparable sites and vegetation types along the regional deposition gradient. These modifications are superposed by N deposition and climate change.

The realization of process-oriented forest management planning demands to identify main forest ecosystem processes (see Fig. 1). Development targets and management measures should be based on area-related process-indicators (vegetation types, change ratios in chemical and physical parameters) as discussed by Scheuner and Makeschin (2005), Kopp (2003), and Schoenholtz et al. (2000). This allows for a better appraisal and consideration of future on- and off-site potentials and risks in strategic development targets and short-term management measures.

In a second step, a regionalization of process-information (speed / intensity, direction) based on the process-indicators and results from process modelling should be realized. Respective regionalization techniques for up-scaling of processes from monitoring and inventory plots are still under development. The regionalization of process parameters is a major topic in most landscape related sciences (Diekrüger et al., 1999; Volk & Steinhardt, 1999). Some promising approaches were yet presented by Zirlewagen & v. Wilpert (2004) and Saborowski & Jansen (2002).

Finally, process-homogeneous planning units must be identified, which allow for drawing process-sensitive and spatially differentiated decisions on type and intensity of management measures. Although the delineation of process units is a general aim in landscape ecology (Haber, 2005), no generalizeable approach has been developed so far. The dynamics within a planning unit and the spatial relation between different planning units under regionally changing management concepts or local variations of environmental changes are of special interest. They should be pursued and tested considering their practical suitability for spacious application. This would support a statistically valid process-oriented forest management, which could easily be integrated into ecological landscape management approaches on different scale levels (Volk & Steinhard 1999).

Integrating processes into management concepts could help to avoid unreasonable investments, e.g. furthering of tree species and stand structures, where the future development provokes ecological risks or financial losses. In Dübener Heide, spatial differentiation of re-acidification rate and base cation leaching demands for respective silvicultural responses. In the nearest distance to the former emitters, natural (noble) hardwood regeneration can be used as cost-efficient countermeasure, as the elevated base cation stock is expected to be available for more than one forest generation. In medium distance, it is expected that the actually still improved site potential will soon be lost. At the same time, natural hardwood regeneration occurs less frequently in this zone, while Scots pine regeneration struggles still with grass dominated soil vegetation and thick moder - raw humus layers. Conversion efforts could help to slow down the re-acidification, but are faced to the problem of future water scarcity, which in this case cannot be compensated by the positive effects of fly ash on hydrological humus properties and high nutrient availability. Fly ash deposition brought also a higher heavy metal input into the sites, where the mobility is expected to increase with ongoing re-acidification. Consequently conversion with tree species, which do not support re-acidification is recommended, where magnetic susceptibility values let expect a locally higher fly ash input. In the far distance zone, fly ash deposition and re-acidification ratio are low. With regard to climate change, the actual Scots pine dominated stands can be considered as well adapted and no additional efforts are demanded.

Silvicultural management in both parts of Dübener Heide, in Saxony and Saxony-Anhalt starts to consider the idea of a process-oriented forest management by leaving the stand type as decision basis and replacing stand type by "development type" with s close reference to indicators for actual ecosystem and site development trends.

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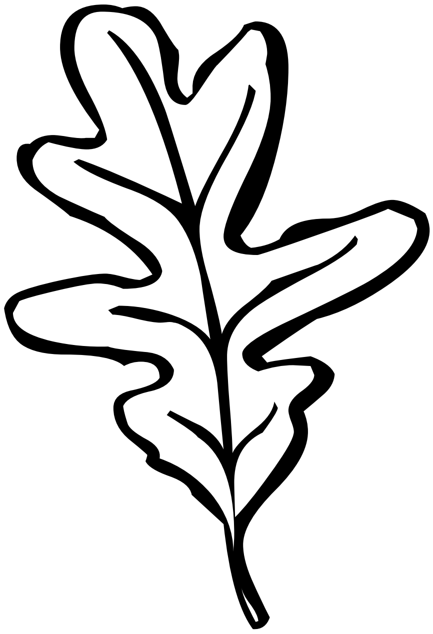

SEED QUALITY ASSESSMENT OF FOREST SPECIES USED IN NURSERIES

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Abstract: *The production of high quality planting stock material should be based on high quality seed source. The application of any new technology of large-scale seedling production demands the use of certified seeds of high quality and quantity. Our objective was to point out the need for developing standard protocols of seed quality assessment of forest species. This paper describes the experience gained from developing such protocols in the frame of the European project PRE-FOREST (CRAFT FP6). Seed quality assessments for Robinia pseudoacacia L., Pinus nigra Arn., Pinus brutia Ten., Abies borisii regis Mattf., and Cupressus sempervirens L. seed lots of different provenances were tested according to the International Seed Technology Association standards. The assessments comprised purity analysis, seed weight, moisture content and germination capacity tests. From the seed lots examined, R. pseudoacacia exhibited external dormancy because of the impermeable seed coat. This dormancy was overcome by mechanical scarification and immersion in hot water. P. nigra and P. brutia seeds germinated easily without any pre-treatment and at high percentages. Limiting factor on germination capacity of C. sempervirens and A. borisii regis seeds was the existence of empty seeds. This problem can be solved if special care is taken during cone collection; only mature cones should be collected. Good storage conditions and seed moisture content below 12% are also important factors that can affect seed germination from the first place.*
Key words: *Seed quality, germination capacity, 1,000 seeds weight, seed purity analysis, seed moisture content, seed dormancy*

INTRODUCTION

Plant production comprises a series of procedures like seed collection, its conservation, pre-treatment before sowing, sowing and finally maintenance of seedlings up to planting time. Mass production of stocking material often becomes problematic because seeds of many species exhibit dormancy and their germination is difficult. The use of high quality seeds is the key to successful crop production, but both biological and environmental factors can reduce seed quality.

Seeds are a living biological end-product of genetic and environmental interaction and their behaviour cannot be predicted with certainty. Seed condition explains the large variability that has been observed between species and seed lots, and even within seed lots.
Seeds of many plant species germinate easily when exposed in favourable environmental conditions. Seeds of many other plant species, though, do not germinate even if there are optimum environmental conditions because they exhibit some kind of dormancy. Up to date at least six types of dormancy have been defined: 1. Embryo dormancy, 2. Physiological dormancy, 3. Secondary dormancy, 4. Hardseedness (external dormancy), 5. Immature embryo and 6. Double dormancy, which is a combination of two or more of the above mentioned types of dormancy (Turnbul, 1975).

Seed dormancy and germination are complex adaptive traits of higher plants that are influenced by a large number of genes and environmental factors. Studies of genetics and physiology have shown the important roles of the plant hormones abscisic acid and gibberellins in the regulation of dormancy and germination (Kornneef *et al.,* 2002).
Plant growers expect genetically pure seed of high quality. As a result, seed companies maintain quality control programmes that monitor seed from harvest to purchase. An array of 'traditional' seed quality tests, including mechanical and tests of genetic purity, seed germination and vigour, and seed health, are used, and seed quality assessment techniques continue to evolve (McDonald, 1998).

In Europe, nursery plant production has improved significantly the last years. The research project PRE-FOREST is based on pre-cultivated forest planting material in mini-plugs, on a prototype production unit that functions under controlled conditions. Major advantage of this technology is the potential to produce high quantity of planting material per m^2 independently of environmental conditions (Radoglou *et al.*, 2009).

The objective of this study was: 1) to assess seed quality (germination capacity, moisture content and 1,000 seeds weight) of the species: Black locust (*Robinia pseudoacacia* L.), brutia pine (*Pinus brutia* Ten.), black pine (*Pinus nigra* Arn.), Italian cypress (*Cupressus sempervirens* L.) and fir (*Abies borisii regis* Mattf.) of different provenances used for seedling production in mini-plug containers in order to determine the right number of seeds needed to fully cover the mini-plug containers with seedlings, 2) to conclude in a threshold of seed quality needed to be used in seedling line production under the new technology developed by the PRE-FORET project and 3) to propose a protocol.

MATERIALS AND METHODS

Seed material

The origin of seed lots that have been used in tests was as follows: One seed lot of fir of Kalambaka provenance, collection year 2006. Four seed lots of Italian cypress, two of them were from the same provenance of Cyprus collected in 2005 and 2006, and the other two of Lagadas and Drama provenances, central Macedonia, Greece, collection year 2004. Two seed lots of brutia pine of Thasos and Soufli provenance, collection years 2005 and 2006 respectively. One of black pine of Grevena provenance, collection year 2006. Finally, two seed lots of black locust; one from Serres, East Macedonia, Greece, collection year 2004 and the second of Hungarian origin, which came from a small seed orchard established near Thessaloniki (40° 46΄ 09΄΄ N, 22° 21΄ 03΄΄ E, altitude 10 m a.s.l.) with seedlings raised by seeds from three different seed crop stands of Hungary (the original seeds were provided by the Forest Research Institute of Budapest), collection year 2006. Seed lots were provided by the Section of Forest Nurseries & Seed Production, Athens. The Italian cypress seeds were provided by the Forest Service of Cyprus and by the Reforestation Directorate of Thessaloniki.

Seed germination

Four replicates of 100 seeds per provenance per species were placed in plastic Petri dishes on the top of filter paper moistened with de-ionized water. The germination protocol was chosen according to the International Rules for Seed Testing (Table 1) (ISTA, 2008). The first measurement was carried out on the 7th and the last on the 14th or 28th day, depending on species.

After the end of each germination test, a biochemical test for determination of seed viability was conducted using the topographical tetrazolium test (TTZ) procedure (ISTA, 1993). The hard, fresh and generally the un-germinated seeds were pre-moistened by soaking in water for 24 hours at room temperature. Then the seed coats were opened and removed. A solution of 2,3,5-triphenyl tetrazolium chloride 0.5% was prepared and the seeds were immersed in it and left in dark for a period of 24 h at 30°C. After the above-mentioned period the seeds were prepared for the final evaluation of viable and non-viable seeds.

Table 1. Duration and chamber conditions for germination tests according to ISTA (2008) rules

Species	duration	conditions
black locust	14 days	Light 8h at 30°C, Dark 16 h at 20°C
Italian cypress	28 days	Light 8h at 20°C, Dark 16 h at 20°C
brutia pine	28 days	Light 8h at 20°C, Dark 16 h at 20°C
black pine	28 days	Light 8h at 30°C, Dark 16 h at 20°C
fir	28 days	Light 8h at 30°C, Dark 16 h at 20°C

Seed moisture content

Approximately 5 g of seeds per species and provenance were randomly drawn from the working sample of the seed lot. The seeds were weighed and placed in glass Petri dishes in the oven at 117°C for 17 h. After cooling at ambient temperature, the seeds were weighed for second time and the moisture content was calculated:

$$MC\% = (M_2 - M_3)x\frac{100}{(M_2 - M_1)}$$

where:
MC%: is the seeds moisture content (%)

M1: is the weight (g) of the container
M2: is the weight (g) of the container and its contents before drying
M3: is the weight (g) of the container and its contents after drying

Seeds weight

The weight of 1,000 seeds and the number of seeds per kg of each seed lot were determined. On eight samples of 100 seeds the weight was recorded. If the variation coefficient of the mean was ≤ 4 for non chaffy seeds, then the 1,000 seeds weight was calculated as the arithmetic mean of those eight samples.

RESULTS AND DISCUSSION

Germination capacity of black locust seeds

This germination test was carried out on un-treated seeds in order to evaluate the degree of their dormancy and on pre-treated seeds by mechanical scarification for 75 minutes. Un-treated seed lots, from both provenances exhibited low germination percentage due to the impermeability of the seed coat (Hartmann & Kester, 1983; De Gomez & Wagner, 2001; Correal *et al.*, 2008; ISTA, 2008). However, the Greek provenance showed better germination capacity (64.3%) than the Hungarian one (27.3%) (Figure 1d). The Greek provenance achieved 50% of germination capacity in 8 days, indicating medium germination energy while the Hungarian provenance did not reach 50% germination capacity. At the end of the germination test, 56.8% of the Hungarian provenance comprised of hard seeds while that of the Greek one 20.5%. The test of tetrazolium chloride (ISTA, 1993) showed that 43.8% and 13.8% from those hard seeds were viable respectively. Both seed lots exhibited high external dormancy. Scarified seeds exhibited a considerably higher germination capacity (85.6%) and germination energy; 67.8% of germination capacity was achieved earlier than the first counting day (seventh), indicating very fast and uniform germination. When scarified seed is soaked, water enters exclusively through the strophiole in a controlled manner. This regulated entry of water to the embryo is associated with even pressure on underlying seed tissues (Khadduri *et al.,* 2002). For nursery practice, production of maximum number of quality seedlings with minimum cost, time and labour seed pre-treatments are required (Mossain *et al.,* 2005). Because black locust seed coat is hard and impermeable, it takes more time to germinate in nursery and with lower germination capacity. Scarification of seeds has been proven to give good potential for seedlings production.

Germination capacity of Italian cypress seeds

Italian cypress seeds are normally sown without pre-treatment. Low germination capacity usually reported is due to high percentage of empty seeds contained in seed lots. High percentage of empty seeds may be a result of non careful selection of mature cones. Schrönike *et al.* (1975) found that most of Arizona cypress grown in South Carolina came from commercial seed dealers with poor or nonexisting records of seed origin and, thus, results from year to year were so erratic. Recommendations should be made to growers to obtain seeds from known certified origins.

The germination test of Italian cypress seeds of two Greek provenances (Drama and Lagadas) was carried out on un-treated seeds and showed very low germination capacity. Drama provenance showed very low germination capacity (2.8%) (Table 2, Figure 1c). The empty seeds in Drama provenance were 82%, indicating that cone collection was not based on some quality cone collection criteria, like avoiding picking up second year immature cones and extremely old, grey cones. These types of cones yield seed with significantly lower germination values than seed from mature cones of an intermediate age (Goggans *et al.,* 1974). Cone handling during and following collection can dramatically influence final seed quality, as well as, the incidence of seed-borne disease (Kolotelo *et al.,* 2001). 15.3% of the seeds were "fresh" (imbibed, apparently viable seeds but without any sign of germination). TTZ test for viability determination revealed that 12.5% of fresh seeds were dead and only 2.8% viable. Lagadas' provenance germination capacity was higher than that of Drama's (35%). The number of empty seeds was high (54%) but less than Drama's provenance. Fresh seeds represented 6.8% of the total, and from these, TTZ test showed 5.5% dead and 1.2% viable seeds.

The practical meaning for such low seed quality for nurseries is that in each mini-plug cell three to five seeds must be placed in order to ensure more or less one seedling per cell. This costs in time and money and it is not profitable for the new sophisticated automated prototype for plant line production suggested by the PRE-FOREST research project. The new technology requires clean, filled, sized, germinable seeds to make the investment in equipment worthwhile (Bonner, 1996).

The germination test on un-treated seed lots of Cyprian provenance showed medium to good germination capacity. Fasouri 06 seed lot showed germination capacity (39.3%) (Figure 1c). The percentage of empty seeds was 51.3%, 4.8% of which were fresh. After the end of the germination test, TTZ test for viability revealed that 89.5% of the un-germinated seeds were dead and only 10.5% viable.

Germination capacity for Fasouri 05 seed lot was much higher (58.9%) than that of Fasouri 06 (39.3%) with 50% of the germination capacity achieved in 12 days. The percentage of empty seeds was again high but less than the first seed lot (38.5%); fresh seeds represented 0.8% of the total and TTZ test showed that all were dead. 50% of the germination capacity was achieved in 12 days.

Germination capacity of brutia and black pine seeds

Pine seeds normally do not need any pre-treatment before germination. Low germination percentages that are usually reported are due to high percentage of empty seeds contained in seed lots. High percentage of empty seeds is a matter of careful selection of mature cones, and there is close relation between seed size and germination capacity in pine seeds (Catalán Bachiller, 1991).

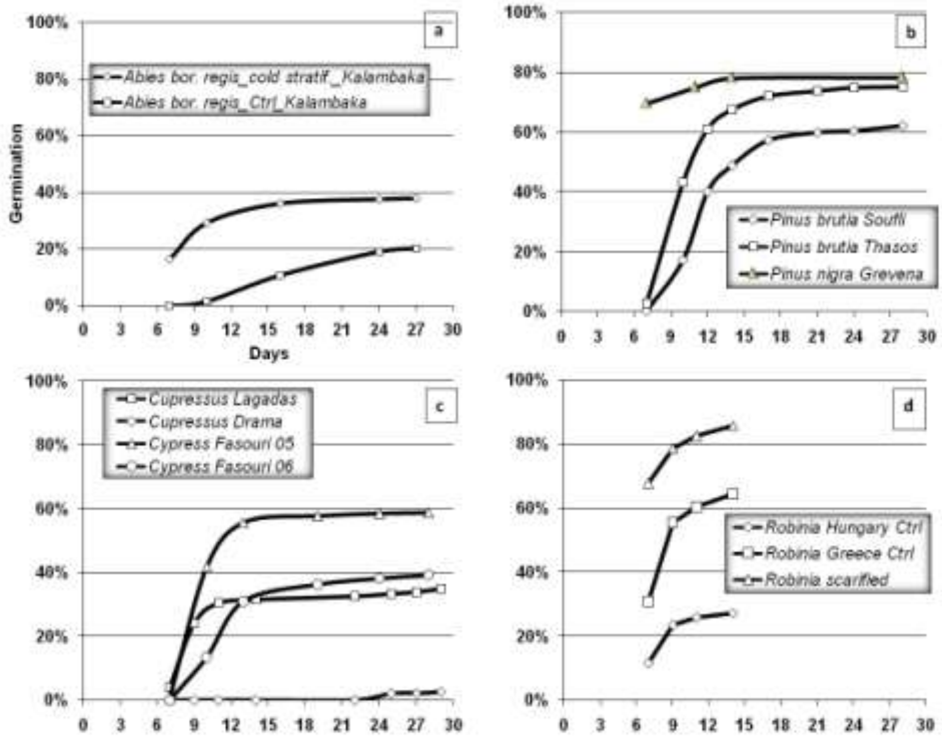

Figure 1. Germination capacity of fir (a), brutia and black pine (b), Italian cypress (c) and black locust (d)

Germination test on brutia pine seeds of two Greek provenances (Thasos and Soufli) was carried out on un-treated seeds and showed medium to high germination capacity. In Soufli provenance the germination capacity was lower (62%) than in the Thasos one (75%) (Figure 1b). The percentage of empty seeds was 6.2%, which is considered a common value because the seeds are usually collected from natural populations and not from genetically improved seed orchards. 15.3% of the seeds were fresh. TTZ test showed that 7% of these seeds were viable and 8.2% dead. Dead seeds represented 10.6% of the total seeds tested. If the 8.2% of dead seeds determined by TTZ test is added to the percentage of dead seeds then the total is around 19%, which is high but if we consider that the seeds were collected in 2004 we could attribute it to not ideal storage conditions.

Thasos provenance showed better results in terms of germination percentage (75%). Empty seeds were 9% and fresh 9%. Fresh seeds were further classified by TTZ test as 3.7% dead and 3.5% viable. Totally, the percentage of dead seeds was 9.2%, which is much less than that of Soufli provenance. In addition, Thasos provenance reached 50% germination capacity in 12 days while the other in 14 days, indicating that the former seed lot was of better quality.

Black pine seeds have been tested without any pre-treatment (un-treated) and after being pre-treated for 21 days in cold stratification (prechilling) according to the double test procedure determined by the rules of the International Seed Testing Association (ISTA, 2008).

Germination capacity was 78.3% in un-treated and 77.8% in pre-treated seeds. Therefore, it can be inferred that there was no internal dormancy within this seed lot. The percentage of empty seeds was 7.2% and 8% accordingly and, finally, the percentage of dead seeds was practically the same for control (14.5%) and for cold stratified seed (14.2%). It must be mentioned here that both tests of this seed lot reached 50% of germination capacity within 5 days exhibiting high germination energy.

No pre-treatment is required for germination of black pine, as well as, for brutia pine seeds. These results agree with those of Calamassi *et al.* (1984), who found out that cold stratification of nine provenances of Aleppo pine (*Pinus halepensis* L.) seeds did not improve their germination capacity but only their germination energy. It must be noticed that the un-treated black pine seeds germinated at 69% and the pre-treated at 75% at the 7th day, indicating that prechilling did not promote germination capacity but enhanced the speed and the uniformity of the germination, contrary to the provenances of brutia pine.

Romanas (1977), in his study on black pine plants reported that this species presented a wide adaptation in different climatic conditions, geological formations and many site types. So it does not seem strange that the seeds of certain provenances responded positively in moist cold stratification while others did not. When seeds are collected in the appropriate season and are stored properly, the germination capacity is usually high enough (70- 90%) so that no further seed treatment is needed for its improvement.

Table 2. Germination capacity, seed weight, number of seeds per kg and seed moisture content of various species and provenances

Species	Germination capacity (%)	Weight of 1,000 seeds (g)	Number of seeds per kg	Seed moisture content (%)
fir (Thasos)	20.3/37.8*	52.81	18.935	10.7
Italian cypress (Drama)	2.8	5.02	199.203	11.4
Italian cypress (Lagadas)	35.0	5.40	185.185	11.8
brutia pine (Soufli)	62.0	44.57	22.436	10.1
brutia pine (Thasos)	75.0	46.15	21.668	10.1
black locust (Greece)	64.3/85.8*	21.03	47.551	7.6
back locust (Hungary)	27.3/85.8*	19.30	51.813	7.6
Italian cypress (Fasouri06)	39.3	7.62	131.234	9.4
Italian cypress (Fasouri05)	58.9	7.57	132.100	8.9
black pine (Grevena, Greece)	78.3/77.8*	21.54	46.425	7.3

* Germination capacity of: un-treated/pre-treated seeds.

Germination capacity of fir seeds

The fir seeds presented great response to prechilling. The fir seeds usually are of lower quality in terms of germination capacity. The seeds of this species usually are of lower quality in non mast years while seed quantity contained in each cone is independent of mast years. Care must be taken when fir cones are collected (Catalán Bachiller, 1991). Another crucial parameter for fir seed viability is storage conditions; if seeds are stored in room temperature and their moisture content is above 10% then the seed lot loses quickly its viability. Fir seeds sometimes exhibit internal dormancy so the recommended test by ISTA (2008) is a double test: prechilling for 21 days at 2-4°C and no pre-treatment.

Figure 1a shows the results of the double test carried out in growth chamber. The overall germination capacity was low; 20.3% for un-treated seeds and 37.8% for pre-treated seeds. This seed lot exhibited internal dormancy and prechilling increased germination capacity by 17.5%. Empty seeds' percentage was high for un-treated (42.3%) and for pre-treated seeds (43.7%), indicating that seed lot collection was made either on a non-mast year or the cones, or from natural stands of low quality. Fresh seeds represented 9% in no pre-treated and 1% in pre-treated seeds. Finally, the percentage of dead seeds was 27.2% for no pre-treated and 17.3% for pre-treated ones, indicating that the storage conditions were probably not the best for fir seeds.

Positive effect of pre-treatment on the energy and germination capacity of *Abies fraseri* Pursh. seeds was reported by Adkins *et al.* (1984). The seed germination percentage increased significantly in 90 days pre-treatment and test temperatures 20/10°C (day/night). In temperatures 30/20°C, the best pre-treatment duration was that of 60 days. They also observed a strong relationship between pre-treatment duration, germination temperatures and light.

Seed weight

Determination of thousand-seeds weight is an essential part for the estimation of the number of seeds contained in one kg. This parameter helps the determination of the appropriate weight of seeds needed for the production of a certain number of plants and can reduce the cost of the seed purchase. In table 2, the thousand-seed weight, as well as, the number of seeds per kg of the examined seed lots are presented. Seed weight is a function of seed size, moisture content, and proportion of full seed in a given lot, and therefore it also gives an indication of seed quality. The thousand-seed weight is required for calculating sowing rates in bare root nurseries but is less important in container facilities because sowing is done on a numerical basis (one or more seeds per container) (Landis, 1998). The thousand-seed weight can easily be converted to seeds per kg (or seeds per pound), which traditionally is used to describe seed lots. Comparison with the respective variable in Catalán Bachillers' (1991) book on seeds of forest trees and shrubs in Spain and in Piotto and Di Noi (2001) showed that these numbers are within the range of the observed number of seeds per kg in Spain and Italy.

Moisture content determination

Moisture content determination is an index of storage conditions. In order to determine the moisture content, the oven method has been followed. Moisture content for black locust, cypress of Cyprus provenance and black pine seeds was below 10%, which is appropriate for storage. For fir, Greek provenances of cypress and brutia pine seeds moisture content was above 10% and this interferes with the seed viability in the long term. The seeds of the present study belong to the orthodox seeds category (Roberts, 1973) because they can be desiccated to a low level of moisture content (3–5%) and be stored at low temperature without damage.

CONCLUSIONS

The germination test has immediate implications for seed quality and for estimation of the number of seedlings that can be obtained from a quantity of seeds.
Only pre-treated seeds of black locust should be used in mini-plug containers because they germinate uniformly and rapidly at a percentage more than 80% giving high quality seedlings.

Pine seeds performed generally good exhibiting germination capacity above 75%.

The limiting factor on germination capacity of Italian cypress and fir seeds was the existence of many empty seeds. Careful seed collection and storage conditions (moisture content less than 10%) are important factors that affect seed germination from the fist place. Cone handling during and following collection can dramatically influence final seed quality.

Improved seed quality is an essential factor of seedling production in mini-plug containers under the new PRE-FOREST technology. Thus, a threshold of 80% germination capacity for seeds collected from natural populations is prerequisite for optimal seedling production.

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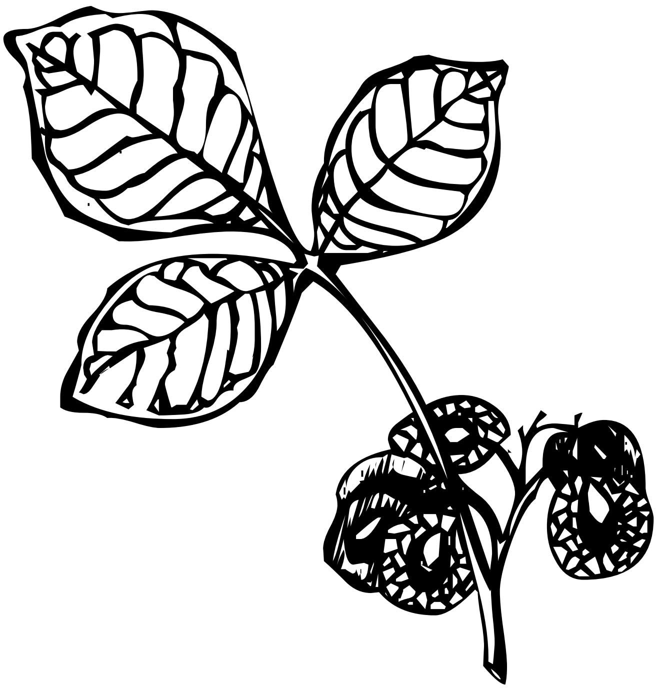

WILDLIFE CONSERVATION GENETICS: EFFECTIVE TOOL FOR CONSERVATION AND MANAGEMENT

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Summary: *Conservation genetics became in the last two decades a useful tool for all decisions concerning nature conservation. Information on population size, gene diversity, differentiation of populations, and population structures is an essential prerequisite for qualified wildlife conservation and management.*
The Carpathians and mainly the Balkan Peninsula are, for many species, considered to be refugia of their further migration during the postglacial period. This applies especially to Rupicapra rupicapra and Sus scrofa which are the species with three different subspecies remaining within this region, and Ursus arctos and Cervus elaphus with two different genetic lineages. Similar pattern can be expected also in other large mammals and/or animal species (fish, birds, insect). Comparative studies of genetic diversity and differentiation of protected and managed wildlife species within the larger areas of Carpathians provide guidelines for decisions in their management and conservation practices. Such comparison for brown bear and chamois populations in Slovakia and Romania would be greatly beneficial as populations of these species greatly differ in population sizes and densities.
Key words: *conservation genetics, Ursus arctos, Cervus elaphus, Sus scrofa*

INTRODUCTION

The last two decades are in many respects considered as the decades of biodiversity and wide application of molecular methods not only in genetics itself, but also in diverse fields of science *e.g.* population biology, ecology, nature conservation etc. There appeared new field of science *e.g.* molecular ecology, ecological genetics, conservation biology and conservation genetics which use molecular methods and serve to wide range of scientific fields as ecology, nature conservation and wildlife management.

Under the term conservation genetics we understand the application of genetic methods in the conservation of animal or plant species and *in sensu lato* it is a part of the molecular ecology. It works on various levels of the DNA variation but most commonly on the population level.

Correct estimates of population size, gene diversity and differentiation of populations, and population structures are necessary for all decisions concerning species conservation and/or their management. The best tool for investigation of the population structures, genetic diversity and differentiation as well as of the processes running on the population level (*e.g.* mating system and gene flow) are the molecular methods which enable the use of non-invasive methods without catching or disturbing the animals.

The early years of the application of the population genetics for wild animal species have been hampered by the lack of suitable gene markers which would enable to collect larger number of samples as isozyme analyses require the use of fresh material. The discovery of the polymerase chain reaction (PCR) by K. Mullis in 1984 enabled the use of small amount of biological material and thus allowed to avoid destructive sampling or killing the animals just for sampling purposes.

The aims of the conservation genetics could be classified as follows:

- Population genetics – effective population size, gene flow, „bottleneck“, paternity, etc.
- Phylogeografy – historic origin of populations and their phylogenetic relations evolutionary significant units (ESU), mostly neutral variation.
- Special topics – relation of the gentic diversity and population viability, experimental „conservation genetics“, selection traits (adaptive and detrimental).

The population genetic applications deal with the study of populations structures (mostly using microsatellites), estimation of genetic diversity (observed and expected heterozygosities), effective population size *Ne*, gene flow, recent „bottleneck“, mating systems, hybridization, and/or with the identification of subpopulations or management units, etc.

The study of phylogeography means the application of the phylogenetic methods on the populations level, the study of population history and evolutionary age, detection of the ESU („evolutionary significant units“) and local adaptations significant for reintroductions.

The special topics are aimed at the study on the detrimental variation (detection of inbred depression), identification of adaptive variation (local adaptations) and experimental consevration genetics (mainly the relations of host – parasite, prey). Under this category we may also include the loss of genetic diversity in small populations, selection power and genetic drift in small populations and the significance of environmental stress on the expression of functional genes.

MATERIAL AND METHODS

Samples

Tissue and hair samples were collected from 260 legally hunted and accidentally killed brown bears in Slovakia and Romania. Tissue samples of red deer were collected from 480 individuals culled within the regular hunting in Slovakia, Hungary, Poland, Czech Republic and Bulgaria. Additional 340 samples were collected by drilling the antlers from private and/or museum trophy collections in Slovakia, Romania and Hungary. Tissues, blood and bone samples were collected from 301 hunted or trapped wild boar individuals originating from 10 countries (Slovakia, Bosna and Hercegovina, Serbia, Germany, Morocco, Romania, Slovenia, Hungary). Tissue samples prior DNA isolation were stored in 96 % ethanol; the blood samples were sampled and stored in tubes containing EDTA as anticoagulant.

Genetic analyses

Genomic DNA was extracted using various methods. The DNA from bones was extracted using DNeasy tissue kit (Qiagen). DNA isolation from whole blood was done using *prep*GEM™ (ZYGEM). Soft tissues were isolated using modified method Doyle & Doyle (1987) which was furthermore modified for using for DNA extraction from animal tissues by Oliveira *et al.* (2007) or by Chelex (Walsh *et al.* 1991).

For analyses of brown bear populations a panel of 13 polymorphic microsatellite markers (Mu10, Mu23, Mu50, Mu51, Mu59, G10L, G10B, G10C, G10D, G10J, G10M, G10P, G10X) used previously by several authors (*e.g.* Bellemain 2004, Petkau *et al.* 1998) have been used.

A panel of 16 microsatellite markers (BM1818, Cer14, CSPS115, CSRM60, CSSM14, CSSM16, CSSM19, CSSM22, ETH3, ETH225, Haut14, Haut24, ILSTS006, IOBT965, IOBT918, MM12, Kuehn 2004) was used to analyze the red deer populations.

A panel of 14 polymorphic microsatellite markers previously used by VERNESI *et al.* (2003) and FERREIRA *et al.* (2006) was used for analysis of wild boar samples. DNA microsatellite markers SW461, SW841, SW1465, SW1492, SW1514, SW2021, SW2496, SW2532, S008, SW1701, SW828, SW986, SW1129, SW1517 were combined in multiplex-polymerase chain reactions using fluorescently labeled primers.

Amplification reactions were performed with the Qiagen Multiplex PCR Kit® with variable concentrations of primers adapted according to the signal. Q-Solution® was also added to each PCR reaction.

Microsatellite alleles were sized on automatic capillary sequencer ABI PRISM 3130. The GeneMapper software (Applied Biosystems) was used to analyse electrophoretic data.

Data analysis

In an attempt to assess the possible level of genetic structuring in the wild boar population, allele frequencies, expected and observed heterozygosities were estimated for all markers, based on the overall set of samples, using POPGENE version 1.31.

In order to evaluate levels of genetic heterogeneity among sampling areas, Weir and Cockerham’s estimator of F_{ST} (Weir & Cockerham 1984) was computed using the program GENETIX (Belkhir *et al.* 1996. Significant deviations from zero were tested over 1,000 permutations.

Pairwise genetic distances between sampling areas were calculated by the program Phylip (Felsenstein 2003). The UPGMA algorithm for clustering the populations, based on the matrix of D_A distances (Nei *et al.* 1983) was used to visualize the relationships among the groups. A consensus tree was obtained by bootstrapping (1,000 cycles) distance values over all loci.

A Bayesian cluster analysis was carried out using the method implemented in the STRUCTURE version 3.1 (Pritchard *et al.* 2000). The number of clusters (*K*) was determined by the methods proposed by Evanno *et al.* (2005). Once defined the most reliable value of *K*, the genetic contribution of each inferred cluster to the predefined populations as well as to each individual was investigated.

RESULTS

Brown bear

The main aim of this case study was the comparison of two brown bear populations along the Carpathians. The Slovak population originated from about 20–30 individuals which remained in the Western Carpathians in the between-war period, while the Romanian populations have been developed from the population of about 1,000 individuals living in the Eastern and the Southern Carpathians prior the World War II.

Brown bear was almost extirpated from the Western Carpathians due to strong hunting pressure, consequence of which was also splitting the western and the eastern populations. After the strict protection in 1932 a steady population growth started in Slovakia, with the consequence of the enlarging the natural range and forming several metapopulations. The present population size is estimated on about 700 individuals and annually about 10 % of the population are legally allowed to culling with limitations to the brown bear size (age).

The population size of the brown bear population in Romania increased from about 2,000 individuals in 1958 to 8,000 individuals in 1990 and the present estimates are roughly 6,000 individuals.

The results of the analyses have shown that most of applied markers were suitable for the population genetic analyses. The number of alleles ranged between 5 (G10B) and 9 (Mu59) in Slovak population, while between 5 (Mu59) and 10 (G10X) in Romania population. The effective number of alleles (n_e) ranged between 1.685 (IOBT918) and ILSTS006). The observed (H_o) and expected heterozygosities (H_e) ranged in Slovak Carpathians between 0.50 (G10L) and 0.79 (Mu23) and 0.55 (G10L) and 0.81 (Mu23), respectively, while in Romanian Carpathians between 0.58 (G10M) and 0.83 (Mu50) and 0.66 (G10M) and 0.88 (Mu59), respectively.

Comparison of the expected heterozygosities among the studied populations in Slovakia and Romania and populations from Scandinavia and North America based on ý common loci have shown that the expected heterozygosity (h_e = 0.77) of the Romanian population was the highest among all observed populations, while the value of expected heterozygosity of the Slovak population was h_e = 0.66 and corresponded to the values of Scandinavian populations.

The clustering of Slovakian and Romanian populations was the closest, in comparison with the clustering of the Bosnian and Slovenian samples from the Balkan lineage. On the other hand network inferred from allele-sharing distances between samples from Romanian and Slovak populations showed very nice pattern of clustering the Slovak and Romanian populations in two different clusters. The same pattern was shown comparing both populations with the Bayesian allocation of individuals into two different clusters, although there occured three individuals which could be easily allocated into the other group.

The bottleneck of the Slovak populations was not proven and the heterozygosities of both populations are comparable.

Red deer

The aim of this investigation was to analyze the genetic structure and differentiation of red deer populations within the Western Carpathians (Slovakia) and adjacent Polish Carpathians and to compare these populations with the adjacent territories *e.g.* southern Hungary, Romania, northern Poland and the Czech Republic. Special attention has been paid to the comparison of two different subspecies of red deer *Cervus elaphus hippelaphus* (Western European red deer) and *Cervus elaphus montanus* (Carpathian red deer).

In the XIXth century the territory of Slovakia was almost without red deer. The present populations are the result of the red deer migration from the Eastern Carpathians

(Carpatho-Ukraine) and/or translocation to the game parks and free wildlife ranges. Due to intensive translocations at the end of the XIXth and beginning of the XXth century and by migration and consequent hybridization with local red deer the present red deer populations could be considered as the mixture of individuals and their progenies originating from different parts of the Eastern European natural range. With regard to this fact the limits of the occurrence of the Carpathian red deer, morphologically also characterized by the longer skulls and larger body size, should be defined. Mainly the limits between the Western European and Carpathian red deer in the contact zone of Western Slovakia are of importance.

The results of the analyses have shown that most of applied markers were suitable for the population genetic analyses. The number of alleles ranged between 6 (IOBT918) and 16 (CSSM19) and the effective number of alleles (n_e) ranged between 1.685 (IOBT918) and ILSTS006). The observed (H_o) and expected heterozygosities (H_e) ranged between 0.147 (CSSM14) and 0.829 (ILSTS006) and 0.164 (CSSM14) and 0.874 (ILSTS006), respectively.

Within the dendrogram two clear clusters of Slovak populations were distinguished: (1) cluster of two Eastern Slovakian populations Medzilaborce and Bardejov, and Poľana, thus the populations which could be attributed to *Cervus elaphus montanus*, and (2) cluster of the three populations with the largest translocations at the end of XIXth century (Rožňava, Revúca and Vysoké Tatry). The remainder of the Slovak populations (Central and Western Slovakia) were clustered to these mixed populations and showed transitional position.

Comparison of the genetic differentiation of the Slovak populations showed a clear genetic similarity among the red deer populations from Slovakia, Southern Poland, Romania and Southern Hungary. The genetic similarity of populations from Southern Hungary (Baja and Zala) with Carpathians ones could have the origin at the translocations of red deer from Maramures to other part of Hungary during the Austro-Hungarian Empire. Populations from Northern Poland, Czech Republic, and Austria show larger genetic dissimilarities to the Carpathian populations.

High degree of genetic diversity of Slovak red deer populations could have two reasons: (1) they represent a contact zone of two different subspecies as it was formulated in the aim of this investigation, and (2) they are a consequence of frequent translocations in the past century.

Wild boar

Wild boar is the most common game species in Europe with a continuous natural range over Europe, except Sweden, Norway, Finland and the British Isles. Within the European range the occurrence of several subspecies were described in the past: *Sus scrofa scrofa* and *S. s. atilla* for the Central and Eastern European part of the natural range and *S.s. meridionalis, S. s. algira* and *S.s. lybicus* for the Mediterranean part of the natural range. This study was aimed to the investigation of the genetic diversity and differentiation of wild boar populations in Central and Eastern Europe and to delimitation of the occurrence of *Sus scrofa scrofa* and *S. s. atilla* for the Central and Eastern Europe.

Observed number of alleles ranged in individual loci between 4 (SW1465) and 19 (SW1517), while the effective number of alleles varied between 2.12 (SW986) and 8.91 (SW 1517). With some exceptions almost all effective numbers of alleles reached higher proportions than 45 % of the total number of alleles.

The values of observed heterozygosities for individual loci ranged between 0.40 (SW986) and 0.82 (SW461) and those of expected heterozygosities ranged between 0.53 (SW986) and 0.89 (SW1517). Only three loci (SW986, SW1465, SW1492) reached the values of observed heterozygosities below 0.60 or expected hetorozygosites below 0.67 a these were also characterized by the lowest number of observed alleles. All remaining loci were characterized by higher heterozygosities with the mean 0.7123±0.1257 for observed and 0.7966±0.1104 for expected heterozygosisites.

Two analyses were applied for characterization of population differentiation. Cluster analyses run on allelic frequencies (UPGMA with bootstrapping) using PHYLIP (Felsenstein 2003) showed no genetic differentiation between two of three Slovak regions (west – east transect), while the eastern Slovakian group was genetically more similar to the Romanian one. The western European group (Germany and Morocco) were the last branches attached to the cluster.

Another analysis (using the software STRUCTURE) structured the entire set of individuals among the predefined number of groups. For the given data set the optimum number of two groups was found – eastern European and western European. The correct classification of individuals for populations belonging to the first group ranged between 70 and 94 %, while the correct classification of individuals belonging to the second group ranged between 78 and 94 %.

DISCUSSION

Basic aim of all three case studies was to investigate the genetic structure, diversity and differentiation of brown bear, red deer and wild boar populations along the Carpathians and to compare them with adjacent population of different subspecies and/or different genetic lineages.

What concerns the fullfilment of the first task all microsatellite markers of nuclear DNA used for our investigation could be successfully used to investigate the genetic diversity and differentiation of populations in studied part of natural range. They are sensitive enough and possess high polymorphism both on the level of observed as well as effective number of alleles. All loci originally developed for *Ursus americanus* by Petkau *et al.* (1998) have been shown as suitable also for *Ursus arctos* from Scandinavia, western Europe and the Carpathians. They have been characterized by high polymorphism (Bellemain 2004, Bellemain & Taberlet 2004).

Similarly, all loci used in this study for wild boar showed rather high number of alleles, although both sets of primers were used earlier in Mediterranean or Atlantic part of Europe – Italy (Vernesi *et al.* 2003) and Portugal (Fereira *et al.* 2006).

The presence of two red deer subspecies (*Cervus elaphus hippelepahus* and *Cervus elaphus montanus*) could not be proven with this data set. The transition towards from the Slovak populations towards the eastern ones (Ukraine and Rumania) seems to be rather continuos. The existence of both subspecies can only be proven based on mitochondrial DNA and/or on historical samples originating from the period when translocation of red deer was rather limited.

The presence of two wild boar subspecies – *Sus scrofa scrofa* and *S. s. atilla* in the territory of Central Europe has not been proven yet. The transition from Western Carpathian populations (Slovakia) towards Romania seems to be continuous. Additional analyses of samples from Ukraine, Moldova and Bulgaria in one direction and the samples from the Czech Republic and Austria in the other direction would be required to enlighten the intraspecific structure of wild boar populations from Central and South-eastern Europe.

Furthermore, the admixture of genes of domestic pigs is expected in populations from Balkan, which may be the consequence of the random mating of wild boars with rural domestic pigs or the consequence of purposeful hybridization in the past.

Lack of differentiation of wild boar and red deer populations from Western Carpathians (Slovakia) and those from Eastern Carpathians (Ukraine and Romania) could be ascribed partly to translocations in red deer at the beginning of the XXth century and partly also to gene flow due to population growth and their movement westwards along the Carpathians. More detailed study in this respect has to be done especially using uniparentally inherited markers (mtDNA) and application of historic samples from museums and private collections.

The Carpathians are stretching from the Danube in Western Slovakia to the Iron Gates on the Danube in Southern Romania. The length of the main ridge is approximately 1,500 km and they cover approximately 190,000 km^2.

The Balkan Peninsula and the Carpathians belong in Europe to one of the centers of genetic diversity both for plant and animal species. The Balkan posses more plant species diversity and in many tree species more gene diversity than the remaining continental part of Europe. The same is known for species diversity in animal species and it is also expected to be the case gene diversity.

The Carpathians on the other hand, are considered as the corridor for species migration after the last glaciation. Many plant and animal species migrated from their Balkan refugia along the Carpathians northwards and further on also westwards, lost part of their genetic diversity during their migration process, but in comparison with the western and northern parts of natural range they still possess a high degree of genetic diversity. At present the Carpathians are considered also to be centers of diversity and harbor rather diversified natural ecosystems along their mountaineous ranges.

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EU SYSTEM FOR FOREST REPRODUCTIVE MATERIAL: EXPERIENCE AND CHALLENGES

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Summary: *The EU system is governed by the Directive 105/1999/EC on the marketing of forest reproductive material. It requires harmonization of national laws and procedures and:*

- *Quality of basic materials (sources) guaranteed by common minimum requirements;Regions of provenance delineated;National registers and national lists of basic materials following common rules;Classification of the forest reproductive material (FRM) into 4 categories;Clear identification of each lot of FRM from collection to delivery to end-users, using unified master certificates and information documents;*
- *National designated authorities and control systems in each member state to guarantee identity (trueness to name) and traceability of FRM;*
- *Designated authorities inspect registered suppliers, and assist each other to ensure proper functioning of the system.*

Most of the EU states participate also in the OECD Forest Seed and Plant Scheme, which is the only international standard for FRM outside the EU.
The EU is a common market for FRM. The knowledge about the effects of long-distance transfer of FRM is not considered sufficient, however, and many member states take pre-cautionary measures favoring local materials. Definition of the EU-wide breeding zones is needed but it would require careful collation of outcomes of field experiments with information about the phylogeny, climate and biogeography. The climate change further challenges the research of adaptability of forest trees.
Two case studies are presented to demonstrate the effects of transfer on the provenances of 2 important forest tree species: Norway spruce and common beech. Their results indicate clearly that not only species- but also area-specific approaches need to be incorporated in guidelines concerning the transfer of forest reproductive materials.
Key words: *forest reproductive material, basic material, Directive 1999/105/EC, common market, provenances, Norway spruce, beech*

INTRODUCTION

Regeneration of forest trees is the prerequisite for maintaining the forested land permanently as forests. Although natural regeneration contributes to conserving the genetic and species diversity, their structure and ecological dynamics, a considerable part of forests in Europe are regenerated artificially. The statistics available in the Reports of the Ministerial Conferences on the Protection of Forests in Europe (MCPFE 2007. p. 49-50) indicate that despite of the trend in favour of natural regeneration and somewhat increasing coppice sprouting, still more 50% of forests in Europe are regenerated artificially. Its proportion is still high not only for economic reasons and traditions of forest management in many countries. It proves to be indispensable also in the patches of forests cleared by the heavy storms in Europe since 1990 and by air pollution. It is also used in forests under conversion to more stable forest types.

The purpose of the system is to 1) guarantee the quality of basic materials of FRM, (2) the trueness to name and (3) traceability of forest reproductive material. Upon prevailing artificial regeneration, the guarantees of the genetic quality and origin of forest reproductive material are key factors predetermining future productivity and stability of forest stands.

Reasonable gains are achievable while the right reproductive material - in regard to the quality of basic material and origin - is used in a right place: it is about 5 % when the seed comes from quality seed stands, 10% for the first generation and 15-30% (Lindgren et al. 2008, Reid 2008, Pöykkö 2008) in advanced generation seed orchards. The improvement includes bigger stem volume, reduced branch thickness and taper, frequently also a better wood quality, together with the same or higher resistance to stress.

Obvious ecological and economic losses due to reduced stability and production of forest stands follow frequently the use of inappropriate reproductive materials in regard to the provenance and basic material. All artificial forest regeneration works as expected, while the quality reproductive material is used on a right place. Potential losses, however, are a multiple of possible gains.Recent situation in forest regeneration turns to be increasingly difficult in relation to the weather extremes, outlining the climate change. While it progresses further and climatic ranges shift rapidly, artificial regeneration will be indispensable as a tool making tree species structure of forests correspondent to climatic conditions. Maintaining ecological and production potential of forests would require a more intense care of basic materials, managements of the climatic factors influencing reproduction processes, and knowledge-based guidelines for the transfer of forest reproductive material. All it requires to carry out carefully planned and coordinated tests across larger areas.

SYSTEM FOR FOREST REPRODUCTIVE MATERIAL IN THE EU

The EU system for Forest Reproductive Material is governed by the Directive 105/1999/EC on the marketing of forest reproductive material. The directive is further transposed into the national law of individual member states.

The first EU directives dealing with forest reproductive material were adopted in the 1960´ies. The Council Directive 66/404/EEC of 14 June 1966 covered the marketing of forest reproductive material while the Council Directive 71/161/EEC of 30 March 1971 dealt with external quality standards for forest reproductive material marketed within the Community. In spite of manifold amendments, principles of the system remain largely the same:

- The quality of basic materials is guaranteed by their selection according to the common minimum requirements
- National registers and national lists of basic materials are according to the common rules.
- Forest reproductive material (FRM) is classified and marketed in one of 4 categories.
- Master certificate for each lot of FRM is issued right after its harvest.
- All suppliers of FRM are registered by the national designate d authorities.
- Control system is established in each country to guarantee the identity and traceability of each lot of FRM in all stages from collection, processing, storing through nursery production to the marketing and delivery to end-users.
- National designated authorities appointed by the government inspect suppliers of FRM. They provide mutual administrative assistance.

Production and marketing of FRM must be consistent regarding the plant health with Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community.

Categories in which the forest reproductive material (FRM) can be marketed:

- Source identified: from a source not selected according to its quality, just its provenance needs to be known. Not allowed or restricted in many member states.
- Selected: originating in seed stands registered on the basis of their phenotypic quality.
- Qualified: its basic material can be parents of families, seed orchards, clones or their mixtures, resulting from individual selection.
- Tested: coming from tested basic materials of all types.

Main implementation tools and infrastructure of the system include:

- Harmonized national laws and procedures.Common EU list of approved basic materials based on harmonized national registers.Common Maps of provenance regions (is expected).Harmonized master certificates and information documents.
- National designated authorities assisting each other to ensure functioning of the system.
- Standing Committee on Seeds and Propagating Material for Agriculture, Horticulture and Forestry, European Commission – Directorate General Health and Consumer Protection.Common approach concerning reproductive material produced in third countries.The Community Plant Variety Office (CPVO) since 1995 (www.cpvo.europa.eu).

Detailed rules for application of the directive are provided by a series of regulations and decisions. Regulations lay down rules regarding (1) the format of the national list of basic material (1597/2002), (2) provision of mutual administrative assistance of official bodies (1598/2002), (3) authorization of member states to prohibit the marketing of specified forest reproductive material to end user (1602/2002), and (4) small quantities of seed

(2301/2002). Further details concerning operation of the system forest reproductive material in the EU are available at:
http://ec.europa.eu/food/plant/propagation/forestry/index_en.htm

OECD FOREST SEED AND PLANT SCHEME

The OECD Scheme for the Control of Forest Reproductive Material Moving in International Trade aims to encourage the production and use of forest reproductive material that have been collected, processed, raised, labeled and distributed in a manner that ensures their trueness to name. Established in 1967 and fully revised in 1974 and 2007, the Scheme reflects the wish for governments to have these materials correctly identified, with a view to minimizing uncertainty in achieving successful afforestation. Detailed technical information about functioning of the scheme (and also its full text) is available at www.oecd.org/agr/forest. Most EU members support and participate in the OECD Scheme because it is the only international standard for FRM outside the EU.

The scheme contains 7 rules, which constitute minimum requirements on: Categories of forest reproductive material, Delineation of regions of provenance, Approval of basic material, registration of basic material, production of reproductive material, Inspection, sealing and labeling of reproductive material and Method of operation of the scheme.

Due to the similarity of the OECD Scheme with the EU system, most non-EU members of the Scheme are taken as equivalent third countries. Following the expression of interest, fulfillment of the legal and technical requirements, and positive evaluation by the verification mission from the OECD Secretariat, Serbia was admitted to the OECD Scheme together with Croatia in autumn 2008.The OECD Scheme covers only 2 categories of forest reproductive material: "Source Identified" and "Selected". Since mid 1990´s, discussion goes on concerning specific procedures for genetically modified (GM) reproductive materials of forest trees under the OECD Scheme. Due to the lack of consensus between the United States and European Union, the Scheme was restricted to only those categories, where genetic modifications are unlikely. It appeared inevitable while a specific treatment of all GMOs is required by the Directive2001/18/EC on the deliberate use of GMOs in the European Union.

FOREST REPRODUCTIVE MATERIAL AND THE COMMON MARKET

In spite of the requirements on free movement of forest reproductive material, the knowledge about the effects of its long-distance transfer is incomplete to sparse for many forest tree species. Mostly in order to avoid eventual undesirable consequences of large-scale, long-distance transfer, most EU members apply pre-cautionary measure favouring local reproductive materials. It is implemented **i**ndirectly: by maintaining subsidiary schemes, national forest certification standards and guidelines. Some states do it directly, maintaining legal rules partly to fully regulating the use of forest reproductive material.Common EU rules towards third countries exist. According to Article 19 of Directive 1999/105/EC, two new decisions replaced the earlier ones by January 1, 2009:

- Council Decision 2008/971/EC of 16 December 2008 on the equivalence of forest reproductive material produced in third countries: It applies to the Source-identified and Selected material from Canada, Croatia, Norway, Serbia, Switzerland, Turkey and the US.
- Commission Decision C(2008)8589 of 23 December 2008 authorising Member States to take decisions on the equivalence of the guarantees afforded by forest reproductive material to be imported from certain third countries. It enables, in a case of need and upon notification, import of Source-Identified material from Belarus, Bosnia and Herzegovina, Macedonia (FYROM) and New Zealand in 2009-14.

EU-wide breeding zones need to be defined for responsible marketing of FRM
It is a complex task, however, requiring systematic cooperation and a careful synthesis of:Information about biogeography, climate and predictions of climate change with.

- Ecogenetic data coming from provenance, progeny and clonal tests and
- Knowledge of the phylogeny, genetic diversity and differentiation of forest tree species.

The eco-genetic information seems to be sufficient for definition of breeding zones of Norway spruce, Scots pine, European larch, beech, many poplar clones and possibly also sessile and pedunculate oak. From among admixed species, thanks to existing provenance experiments and marker-aided studies, reliable conclusions can be made for common ash. It could also be possible to define related rules for Maritime pine, Sitka spruce and Douglas fir, which are subject to intensive long-term testing and breeding. Common EU-wide testing of poplar clones has not taken place and needs to be started, however.

The EU-funded project TREEBREEDEX - A working model network of tree improvement for competitive, multifonctional and sustainable European forestry is expected to provide relevant contributions towards:

- Creation of the EU-wide map of provenance regions,
- Definition breeding zones for most important forest tree species in Europe.The project is a Coordination Action aimed at the establishment of the European forest tree breeding infrastructure network within the 6th Framework Programme, Research Infrastructures Action Integrating activity. It has 28 partners coordinated by the French National Institute of Agricultural Research, France, and will run until 2010. More information is available at http://treebreedex.eu/

Conservation of forest genetic resources is not a part of the EU system for FRM

Related activities are covered by Regulation of the Council 870/2004, establishing a Community programme on the conservation, characterization, collection and utilization of genetic resources in agriculture. The only action dealing with forest genetic resources within the EU Programme RESGEN is the project EUFGIS – Establishment of a European Information System on Forest Genetic Resources. It is coordinated by the Bioversity International, inter-governmental organization coordinating also the EUFORGEN – European Programme for Forest Genetic Resources. Its purpose is to strengthen documentation of forest genetic resources . EUFGIS aims at establishing a Web-based information system to serve as a documentation platform for national FGR inventories and to support practical implementation of gene conservation and sustainable forest management in Europe. The project will create a network of national focal persons in European countries (including the non-EU states of Europe). The project will also provide training on FGR documentation to national focal persons and will run until 30 September 2010.

Gaps and needs

There is an obvious need to define the EU-wide breeding zones and draw recommendations for transfer of FRM. Predicted climate change, however, demands a high priority assigned to the study of the adaptability of forest tree species, especially at their xeric and lower limits (MÁTYÁS 2007). While the climate change progresses as it is expected, climatic ranges of many tree species will be change largely in Central and Southeast Europe. Not only isolated but also currently large forest tree populations may turn endangered. In such circumstances, practical application the available knowledge about adaptability and its genetic background, and the synthesis of ecology and genetics will be of vital importance. It seems, however, that there were only few studies which results would be applicable for the conditions of severe change, where populations are reaching their tolerance limits.

CASE STUDY I: EFFECTS OF THE ALTITUDINAL TRANSFER ON NORWAY SPRUCE

MATERIAL AND METHODS

The long-term effects of the transfer of Norway spruce provenances to new sites were studied on the series of 5 plots, each with the same 49 provenances, located along the altitudinal gradient 480 - 1,310 m in Slovakia. The experiment is a spin-off of the IUFRO Norway spruce inventory experiment 1964/68. Plots were left without silvicultural intervention until the age of 40 years. Responses were tested in 12 autochthonous provenances from Slovakia, where more detailed and reliable data about the climate in the place of origin were available.

<u>Predictor variables</u> were the differences between the place of origin and place of translocation (5 provenance plots) of provenances in regard to the

- Altitude,
- Mean temperature: Annual, April-September, July;
- Mean precipitation: Annual, April-September, July;

<u>Response variables</u> were the survival rate and mean height provenances.

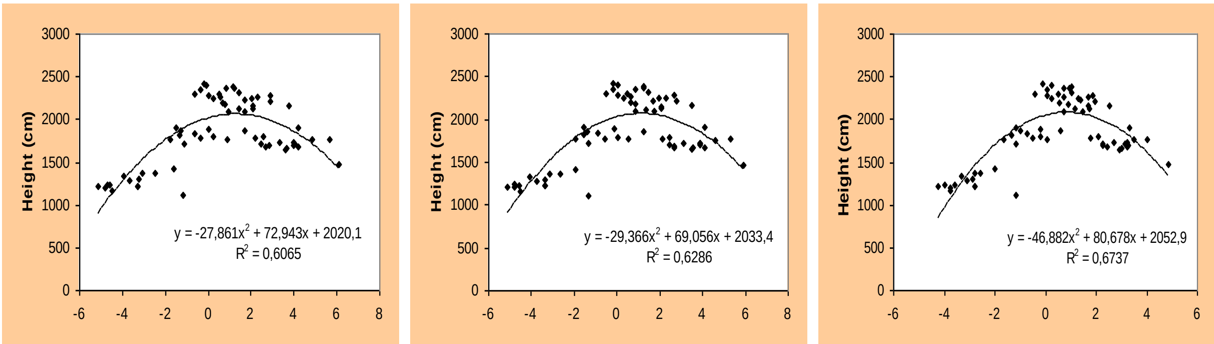
RESULTS

<u>Survival</u>: The survival at the age 40 was higher towards lower elevations, with higher mean temperatures, longer vegetation and smaller (but still sufficient) precipitation. It could be noted that the lowermost plot of the series is at 480 m in forests naturally dominated by oaks.

<u>Growth responses</u>: Regarding the effect of altitudinal transfer, the mean height of provenances reached its maximum when the plantation site was 211 meters lower than the source population. Regarding the effect of temperatures, the maximum mean height was at the mean temperature higher by 1.4 °C in July, 0.9 °C in the period April-September and 1.3 °C over the year. At the same time, precipitation was lower by 24.5 mm in July, 120.5 mm in April-September and 106.3 mm per year.

Figure 1: Regressions between the mean height and the differences in altitude, length of vegetation and mean annual temperature at the place of origin (source population) and translocation for 12 provenances of Norway spruce planted at 5 sites.

Mean July temperature | Mean temperature April-September | Mean annual temperature



Difference between places of origin (source populations) and planting sites plots in °C

CONCLUSIONS

- The international experiments prove that Western-Carpathian provenances of Norway spruce are adaptable, performing well in Northwest Europe and southern Scandinavia.
- Provenances able to benefit of longer vegetation and better soils and tolerate temporary water deficit, come from the lower part of the local natural range of Norway spruce (PACALAJ et al. 2000). Their genetic resource are increasingly threatened by the climate change, however. Due to storms and extreme weather, they need to be backed-up ex situ.
- Norway spruce from higher altitudes is rather a specialist to colder sites with higher precipitation (LONGAUER 2007). It will have still more problems at its present sites while the climate change advances further.
- The present results of provenance experiment indicate that increased average temperature and lower precipitation do not pose a direct threat to Norway spruce. They become limiting, however, in combination with pollutants (NO_x, ozone), pressure of fungal parasites and their change into pathogens and, afterwards, insect pests.

CASE STUDY II: EFFECTS OF THE LONG-DISTANCE TRANSFER ON COMMON BEECH

The range-wide provenance experiment with beech was established in two series 1995 and 1998 under the coordination of the Federal Research Centre for Forestry and Wood Products Hamburg, Germany. The effects of transfer on the growth and phenology of common beech were studied at the Slovak plot of the series 1998 with 32 provenances (Figure 2). Provenances were planted in 3 repeats by 50 individuals. The plot is located in Central Slovakia at the altitude of 600 m.

The following characteristics of provenances were measured and assessed in 2007: (1) Diameter at the height of 20 cm, survival, (2) Spring flushing (7 phenological phases assessed 13 times from April 1 till June 1), (3) Autumn phenology (6 phases assessed 16 times from September 1 till November 1), (4) Length of vegetation (from flushing midstage till autumn discoloration midstage), (5) Late frost damage (5 degrees of intensity, 2 observations).

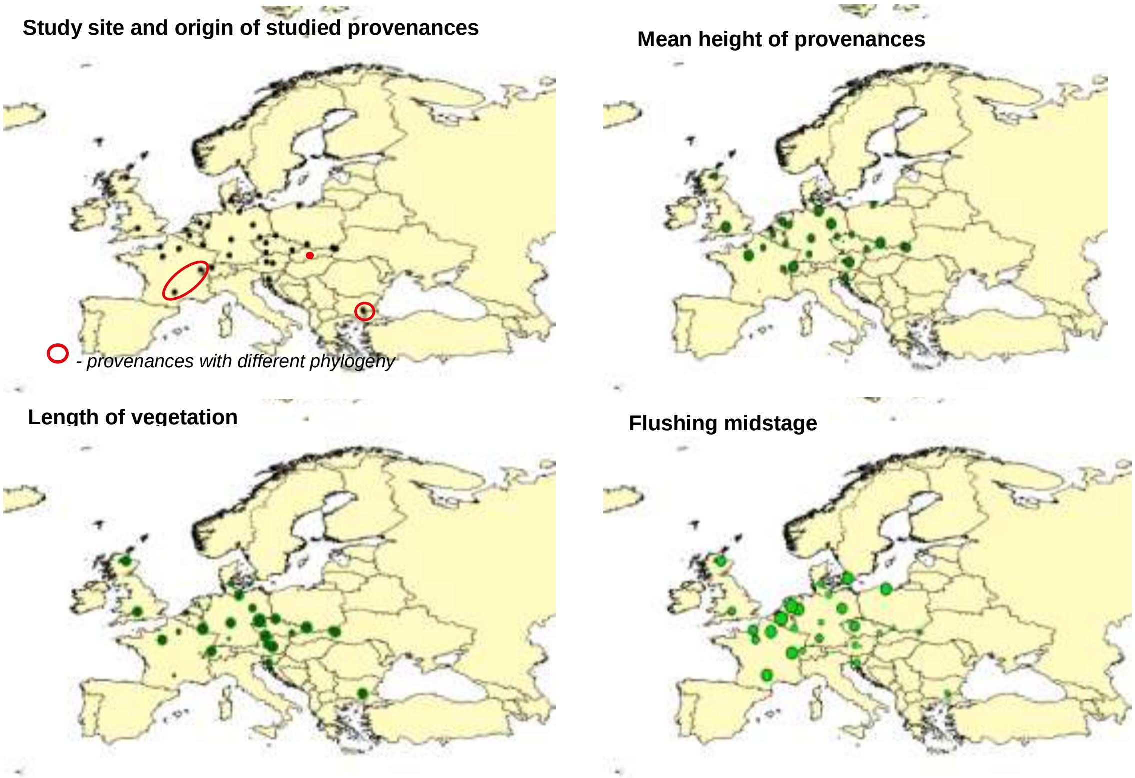


Figure 2. a) Location of the provenance plot and provenances (phylogenetically divergent provenances highlighted), b), height c), length of vegetation and d) flushing of the studied provenances of common beech.

Table 1: Studied parameters and their phenotypic (individual level) / genetic (provenance level) correlations. * P < 0,05; ** P < 0,01; *** P < 0,001; NA – not assessed

	Mean height	Mean diameter	Late frost damage
Flushing midstage	-0.342*** -0.195	-0.236*** -0.152	-0.786*** -0.903***
Length of vegetation	0.462*** 0.504***	0.414*** 0.429**	NA
Late frost damage	0.147*** 0.045	0.045* 0.051	NA

RESULTS AND CONCLUSIONS

Transfer of the forest reproductive material of common beech in Central Europe is possible but with caution for the following reasons:

- Mortality, height growth and length of vegetation of common beech reveal mosaic distribution without clear trends or geographic patterns.
- Phenological traits including spring flushing and autumn colouring show non-random trends, however, with later dates in provenances from oceanic climate and lower altitudes.
- Provenances from more continental areas of Central-Europe as well s those from higher altitudes exhibited earlier spring flushing, which increased (significantly) the risk of a late frost damage. This phenomenon is explainable by their smaller temperature sum requirement for flushing (VON WUEHLISCH et al., 1995).

- There is an imprint of the phylogeny: provenances from Bulgaria and Southern France descending from smaller glacial refugia (other than the main one) showed poorer growth and lower survival.

ACKNOWLEDGEMENT

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LIGHT SHARING AMONG DIFFERENT FOREST STRATA FOR SUSTAINABLE MANAGEMENT OF VEGETATION AND REGENERATION

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Summary: *There is a current trend towards managing forests with multiple objectives, in particular to preserve or increase biodiversity and sustainability. There is renewed interest in understorey vegetation as a way both to increase the number of species and, indirectly, to favour fauna, including game, or improve soil quality. However, this stratum of herbaceous or shrubby species can also compete with young tree seedling and jeopardise tree regeneration. Hence a compromise has to be found among the different management objectives for the forest and in particular the understorey.*
Light is one of the main environmental factors controlling ecological and biological processes in forests. For example, light quantity and light quality control the success of seed germination and the establishment and growth of tree seedlings in the understorey. Light also promotes the development of the floor vegetation, the composition of which varies with site conditions in addition to light. Modifying light availability in the understorey, for example with thinning operations, thus interferes with tree regeneration and flora cover and composition. The understorey plants can intercept a significant fraction of the light and so the resulting amount of light available for tree seedlings varies greatly depending on species and composition of the floor vegetation. Light is successively intercepted and transmitted by the different strata composing the forest stand, i.e. overstorey trees, midstorey trees, shrubs and herbaceous species. The different strata interact, the development of each one being controlled by the others, often through modification of light availability. Foresters have to control this chain of light sharing to steer forest stands towards different objectives. We report results concerning the light sharing chain and discuss their implications for management.

INTRODUCTION

For a long time forests of the temperate zone have been managed solely for the production of wood. In his book on sylviculture, Perrin (1963) noted that "earlier foresters considered herbs as enemies and managed stands for a clean soil", a clean soil meaning without any vegetation, "evidence that the trees were using all the available radiation". The current trend is more towards managing forests with multiple objectives, in particular to preserve or increase biodiversity and sustainability. The understorey vegetation has received renewed attention as a way both to increase the number of species in the forest, and indirectly, to favour fauna, including game, or improve soil quality. However, this stratum of herbaceous or shrubby species can also compete with young tree seedling and so jeopardise tree regeneration. In addition, a dense layer of vegetation, particularly thorny species, is not always appreciated by urban forest walkers. Hence a compromise has to be found among the different managing objectives for the forest and in particular the understorey. Foresters need tools to implement such forest management.

Light is one of the main environmental factors controlling ecological and biological processes in forests. Clearly, factors such as soil water availability, nutrients and temperatures are also fundamental, but these are all linked to some extent to light availability (Barbier et al., 2008). Light and water from rainfall are both related to tree crown density and gap percentage in the canopy, so that an increased light penetration implies an increase in rainfall reaching the forest floor. Less light or water interception by the canopy in turn means a smaller leaf area index (LAI) and so lower tree transpiration (Bréda, 1999).

This condition can improve soil water content, but the effect is offset by the water consumption of the understorey vegetation, which can increase with greater light availability. Light also drives the energy balance of the stand. More light means a significant increase in air and soil temperatures during the day and a small decrease in night temperatures by radiation loss. The net increase in day temperatures and a higher soil water content improve the biological activity of the soil; the humus is therefore of better quality and nutrients are recycled more efficiently. Hence light can be considered as a synthetic indicator of resource availability and microclimate in forests (Barbier et al., 2008).

Light quantity and quality are among the processes that control the success of seed germination and the establishment and growth of tree seedlings in the understorey. The shade-tolerance of young tree species varies according to their successional status, the more shade-tolerant species being potentially better able to regenerate in the dark understorey than the more intolerant ones. Light is consequently a fundamental key to forest regeneration. Light also promotes the development of the ground vegetation, composed of graminoids, forbs, shrubs, and young trees, the composition of which varies with site conditions and overstorey tree species, in addition to light. The understorey vegetation can compete with tree seedlings for resources to varying degrees depending on the species present, and may seriously impede tree regeneration in some cases (Balandier et al., 2006). These understorey plants can intercept a significant fraction of the light in the understorey and so the remaining light available for tree seedlings can vary greatly depending on species and composition of floor vegetation.

The light path into the forest is as follows: the incident light above the canopy is first intercepted by the overstorey trees, and then by the midstorey trees when these are present. The light that reaches the understorey controls the development and composition of the floor vegetation, which in turn intercepts some light. Finally, the tree seedlings use what light remains. The forester has to control this chain of light sharing to direct forest stands towards different objectives. We present here some results on this light sharing chain and their implications for management.

MATERIALS AND METHODS

Light transmitted by the overstorey and understorey vegetation was measured using different methods. Hemispherical photographs were used to indirectly estimate light transmission through the overstorey of adult trees. The equipment used was a camera fitted with a 180° aperture lens with which it was possible to take a photograph of the whole sky hemisphere, with the zenith at the centre of the photograph and the horizon at the edge. The photograph was taken without direct radiation from the sun, i.e. under full cloud cover, or at sunrise or sunset, which considerably restricted the time periods when photographs could be taken. The camera was set perpendicular to the sky zenith about 1.5 m above the ground, if possible above the understorey vegetation. The principle for computation of transmitted light from the photograph was as follows; the colour photograph was converted into a black (vegetation) and white (sky) picture using a local thresholding procedure (Adam et al., 2007). The ratio of white to black pixels (sky to vegetation) gave the gap fraction from which the transmitted light could then be computed (Adam et al., 2008). The transmitted light could be divided into diffuse light from the whole sky vault using the whole photograph, and direct light from the sun using the fraction of the photograph corresponding to the path of the sun, which could be calculated for any day of the year. The method thus enabled us to compute the transmission of light for the whole year, certain months or a particular day. The drawback of the method is the difficulty obtaining a quality photograph with no over-exposure in which all parts of trees are clearly represented. This is possible only if no direct sun is present on the photograph and the sky is perfectly homogeneous, and the colour pixels are correctly classified into sky and vegetation. This last is the most severely limiting part of the method.

Transmitted light can also be measured directly using light sensors. In many experiments we used either linear (length 1 m) or point light sensors in the total solar radiation spectrum (300-3000 nm) or the PAR spectrum (400-700 nm). Sensors were set 1.5 m above the ground and if possible above the understorey vegetation for at least 24 h, to account for the complete sun path over the entire day. In the same 24 h period a sensor of the same type was set nearby in an open place to measure incident radiation. The transmittance of the forest stand was then calculated as the ratio of light in the understorey to incident light during the 24 h period. This process enabled us to compare, at least to some extent, measurements made on different days of the year (i.e., with different sun flux) and with different weather conditions (i.e., cloudy or sunny). The drawback of the method is that a single day of measurement cannot account for light interception by the overstorey during several months or the whole year, particularly if the canopy is very heterogeneous, i.e., with large gaps where direct sun radiation can reach the ground on some days or in some months in the year.

Light transmitted by the understorey vegetation was measured using a linear PAR sensor of length 80 cm and width 1 cm (Accupar, Decagon). Its long-stemmed shape was easy to slide into the vegetation, generally a few cm above the ground, without disturbing the structure of the plant cover. It was composed of 80 photocells to measure local variations in light interception. In the same way as overstorey transmittance, light recorded under the understorey vegetation is related to a measure of incident radiation during the same period. The drawback of the method is the same as for the overstorey, and a hemispherical photograph is not easy to take from within the vegetation.

Light measured either under the overstorey or under the understorey vegetation was related to vegetation characteristics such as tree height, diameter, basal area, crown characteristics, or herbaceous height or cover.

RESULTS

Light interception by the overstorey

In regular, even-aged stands, composed of a single species, light interception by the overstorey is relatively easy to predict. Light measurements and stand characteristics showed that transmittance (T) of the overstorey was related exponentially to stand basal area (G), by a Beer-Lambert law (Balandier et al., 2006; Sonohat et al., 2004), $T = \exp(-kG)$, where k is the extinction coefficient: this is a function of species, stand age, and time lag and intensity of the last thinning (Sonohat et al., 2004). Figure 1 illustrates the effect of species identity; for the same stand basal area and age, light intercepted by different species during the leafy period can vary by a factor of 1 to 4.

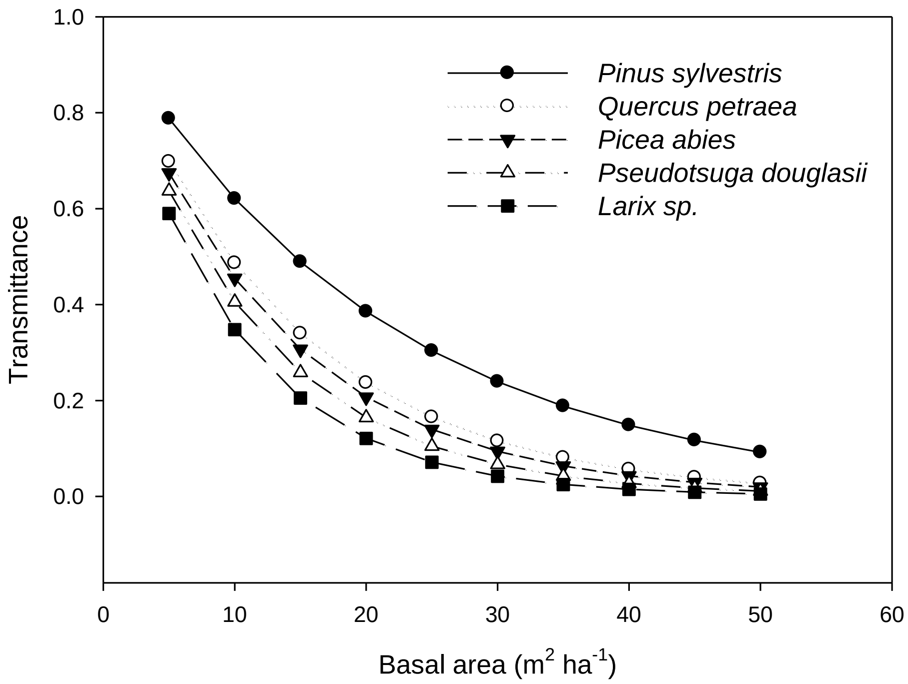

Figure 1. Simulations of light transmitted during the leafy period by different species according to their stand basal area for stands in France and Belgium. Transmittance is expressed as the quantity of light measured in the stand understorey relative to the incident light above the canopy

In these regular, even-aged stands, the quality of light, i.e. its spectral composition, can be deduced from measurements of a considered wavelength band as there are relationships between total solar radiation (300-3000 nm), photosynthetically active radiation (PAR, 400-700 nm) and red (R, 660 nm) or far-red (FR, 730 nm) radiation (Balandier et al., 2006). Of particular interest is the R / FR ratio, which drives many plant morphological processes.

In uneven, irregular stands, composed of one or more species, light measurement, prediction and simulation is not easy because of the large gaps in the overstorey canopy, which leads to a very wide variability of transmitted light, in both quantity and quality. In that case, a single variable at the stand scale, such as basal area, is no longer sufficient, and more local variables must be considered such as individual tree size and position, or at least information on tree spatial distribution (Goreaud et al., 2007). The difficulty of the approach is staying at a level of description that allows forest managers to actually use the model, i.e. it must not be too complex, at least for the information required as input. With this in mind, we built a model with a multiscale representation of the forest (Da Silva et al., 2008), i.e. from the tree branch scale, crown scale, tree scale to stand scale, that can be used to study the worsening of light prediction with the scale of representation (Da Silva et al., 2007). This model is being evaluated.

Development and composition of the understorey vegetation

Light availability in the understorey promotes the development of the floor vegetation. Globally, at the community level, there is a logarithmic increase in the vegetation cover with light availability, from no vegetation for very low levels of transmittance (≅ 0.02) to a maximum for an asymptote of 0.4 of transmittance, which means that increasing light above this value does not correspondingly augment vegetation cover or biomass (Balandier & Pauwels, 2002). At the species level, patterns of plant cover or biomass with light availability are not simple and depend on the light requirements of species. For example, a recent study showed a near-linear increase in *Cytisus scoparius*, a light-requiring species, with light availability, whereas *Rubus idaeus*, a more shade-tolerant species, showed a bell-shaped curve in response to light, with a maximum cover for a transmittance of 0.45 (Gaudio et al., 2008). We are studying the response patterns of other common species that colonise gaps in temperate forests, such as *Rubus fruticosus*, *Molinia caerulea*, *Calluna vulgaris* and *Pteridium aquilinum*.

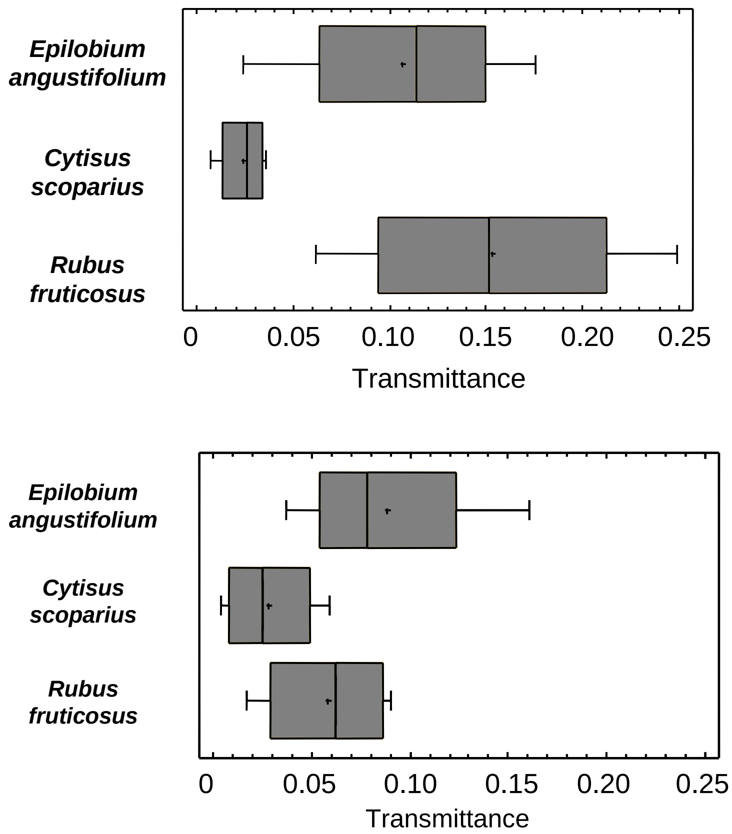

Figure 2. Light (PAR) transmitted to soil level by three common plants colonising gaps in temperate forests in May (top) and July (bottom) 2005 at different sites in uplands of central France (for each species the centre box covers 50% of the data, the vertical line in the box being the median)

Light availability modifies not only the cover of the floor vegetation but also its species composition. However, the response pattern of species composition with respect to light is not simple. Some studies have reported a bell-shaped curve with a maximum number of species for intermediate light levels (e.g., Balandier & Pauwels, 2002), others a continuous increase in species richness with light. The first response is observed more frequently at a local scale, whereas the second is often recorded for larger geographical scales (Rajaniemi, 2003). The bell-shaped curve from shade to full light is explained by the progressive enrichment of a flora made up of shade-tolerant species by more and more light-requiring species as light increases up to a maximum species richness; thereafter one or more monopolistic, strongly light-requiring species colonise the whole space at the expense of all the other species. In terms of tree regeneration management, those monopolistic species (e.g. *Rubus fruticosus*, *Molinia caerulea* and *Pteridium aquilinum*) are highly competitive for resources and can jeopardise tree seedling establishment and growth.

Light interception by the understorey vegetation

Depending on their development, the understorey plants can intercept a non-negligible amount of light (fig. 2). For example, the transmitted light recorded under *Cytisus scoparius*, *Rubus fruticosus*, and *Epilobium angustifolium* can be as low as 0.01, 0.03 and 0.05, respectively (Sivade, 2005). These are very low values, which can seriously compromise tree regeneration when tree seedlings are small and surrounded by such species. Another study on *Rubus fruticosus* for different stands in France and England suggested that transmittance could range from 0.5 to less than 0.02 for plant LAI values ranging from 0.5 to 4, respectively (unpublished data).

DISCUSSION: IMPLICATIONS FOR TREE REGENERATION

Tree seedling establishment and growth are affected to varying degrees by light availability according to species shade-tolerance; for example *Fagus sylvatica*, a late successional shade-tolerant species, is able to survive and grow under very limited amounts of transmitted light, as low as 0.03. It grows more vigorously with increasing light up to a value of about 0.4, beyond which no further improvement in growth is usually seen (e.g. Balandier et al., 2007). By contrast, an early successional species that is not very shade-tolerant, such as *Pinus sylvestris,* needs more light to establish and grows more vigorously with more light linearly up to a transmittance of 1. Hence forest managers have to run the stand differently, especially as regards thinning operations, to regenerate and promote particular species. However, as shown above, depending on the light availability in the understorey, vegetation of varying density can develop and intercept a large amount of the available light (in addition to water and nutrients, Balandier et al., 2006). This understorey vegetation, depending on its density and species composition, can strongly compete with tree seedlings, severely impeding their growth, or even causing their death (Balandier et al., 2008; Coll et al., 2003; Provendier & Balandier, 2008). Therefore the forest manager has to find a compromise between a light level that promotes optimal tree seedling growth, but also generates a dense competing layer of understorey vegetation that can jeopardise tree regeneration, and a very low light level that prevents all understorey plant growth, including tree seedlings. The point of equilibrium that allows significant tree seedling growth while preventing too-dense understorey vegetation is often also the point where species richness is at its maximum; for a natural sustainable management, this point should be sought by forest managers adjusting light sharing among the different strata of the forest.

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SHORT ROTATION COPPICE WITH WILLOW FOR ENERGY AND PHYTOREMEDIATION IN SWEDEN

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Summary: *Short-rotation willow coppice is cultivated in Sweden to produce biomass for energy. The crop is commercially grown on agricultural land and the biomass produced is used in district heating plants for combined heat and power production. In recent years, nutrient-rich waste residues– mainly municipal wastewater, landfill leachate and sewage sludge– have been successfully applied to willow coppice fields to reduce the content of pollutants in waters and soils through plant uptake, and to facilitate enhanced biomass production used for energy purposes. The benefits of such practices are both environmental and economic: this method of treating waste products (which can be considered more as resources than as wastes) is more cost effective than conventional treatments, and the nutrients contained in the waste products serve as low-cost fertilisers to increase biomass production.*
Keywords: *Energy forest, willow, Salix, Sweden, phytoremediation*

SHORT ROTATION COPPICE WITH WILLOW FOR ENERGY IN SWEDEN

Cultivation of short-rotation coppice (SRC) with willow was introduced in Sweden after the oil crises in the 1970s, with the intention of replacing fossil fuels by renewable energy sources. Comprehensive research to identify suitable intensively grown fast-growing species for energy suggested that willows grown in coppice systems (re-growth after harvest) were the most suitable for such purpose (Sirén et *al.*, 1987). During the first decade, the land available for short-rotation willow crops was abandoned farmland and appropriate wetlands. In the middle of 1980s, however, it became obvious that the surplus production of agricultural crops left an opening for use of more productive farmland, and therefore more productive agricultural land was chosen for cultivation of short-rotation willow coppice for energy. Research then had also shown that willow cultivation on wetlands was extremely difficult for several reasons, e.g. low soil pH and spring frosts. In the first half of the 1990s, investigations had shown that willow crops were suitable also for phytoremediation applications (Aronsson & Perttu, 1994), and that a combination with biomass production for energy was a cost-effective method for wastewater treatment (Rosenqvist et al., 1997).

Today, about 15 000 hectares of short-rotation willow coppice crops are grown on Swedish arable land using mainly different clones and hybrids of *Salix viminalis*, *S. dasyclados* and *S. schwerinii*. Willow cultivation is fully mechanised from soil preparation, planting and management to harvesting and combustion. In the initial phase, approximately 12 000 cuttings per hectare are planted in double rows to facilitate easier management (weeding, fertilisation, etc.) and harvesting. Short-rotation willow coppice fields in Sweden are harvested every three to five years, during the non-growing season, preferably when the soil is frozen, using specially designed machines (e.g. converted Claas Jaguar corn harvesters). The above-ground biomass is chipped on-site, then stored or directly transported to and burned in combined heat and power plants. The average production of commercial willow plantations in Sweden nowadays is ca 5-6 tonnes dry matter per hectare and year depending strongly on site conditions, although much higher biomass productions (up to 20 tonnes) from well-managed sites have been reported [Christersson & Sennerby-Forsse, 1994; Bergkvist & Ledin, 1998]. After harvest, the plants coppice vigorously, and replanting is therefore not necessary. The estimated economic lifespan of a short-rotation willow coppice stand is 20 to 25 years.

Despite a considerable fertilisation effect, Swedish commercial willow plantations are rarely fertilised. This is most likely due to the high costs for fertilisers (i.e. around 1 Euro/kg N) and the presently relatively low price for the willow chips in Sweden (i.e. around 33-40 Euro/tonne dry matter).

SHORT ROTATION COPPICE WITH WILLOW FOR PHYTOREMEDIATION IN SWEDEN

For a cost-efficient and sustainable cultivation of short-rotation willow coppice for energy, it is necessary to fertilise, but also to recycle phosphorus and other mineral elements exported from the field at harvest. Therefore, the use of nutrient-rich residues as an alternative, cost-efficient fertilisation method was proposed from the early stages of the development of short-rotation willow coppice, and has been successfully practiced in Sweden (Aronsson & Perttu, 2001; Dimitriou & Aronsson, 2005). The benefits of using short-rotation willow coppice for phytoremediation, i.e. the use of plants for treatment of contaminated air, soil or water (Licht & Isebrands, 2005) are both environmental and economic. The residues, which are considered more as resources than as wastes, are dealt with more cost-efficiently as compared with conventional treatments, and the nutrients contained in the residues are used as fertilizers to increase plant production. The basic idea is to reduce the content of pollutants, nutrients or both in waters and soils by plant uptake and by filtration, and to facilitate microbial transformation. The high evapotranspiration rate of short-rotation willow coppice (Persson & Lindroth, 1994) and the tolerance of their root systems to anaerobic conditions (Jackson & Attwood, 1996), make it possible to apply high irrigation rates. The ability of willows to take up in their shoots substantial amounts of heavy metals (e.g. Cadmium) is also beneficial for reducing the input of heavy metals in the system (Dimitriou, 2005). Municipal wastewater was the first waste product used for irrigation of short-rotation willow coppice since it was considered a balanced fertiliser with regard to willow plant nutrient needs (Perttu & Kowalik, 1997). However, it was soon realised that other residues rich in nutrients could also be successfully recycled to enhance willow biomass production, if properly used. Nowadays, the principal waste residues used in Sweden are municipal wastewater, landfill leachate, various industrial wastewaters (e.g. log-yard runoff) and solid residues as municipal sewage sludge.

Despite the obvious advantages of the system, environmental hazards such as nitrogen and phosphorus leaching, plant toxicity, greenhouse gas emissions (NO_X), accumulation of heavy metals in soils and sanitary aspects could be some of the hinders for a successful performance if the applications are not conducted with caution. Research has been and still is conducted for the evaluation of possible environmental problems as well as for the rationalisation of the waste applications when SRWC is used for phytoremediation purposes. There are currently around 40 such large-scale systems in Sweden treating the different kind of wastewaters mentioned above and more than 10 000 hectares with SRWC fertilised with municipal sludge. In the case of wastewaters, sprinklers or pipes for drip irrigation are installed in the field and the irrigation is conducted during the growing season. Lined ponds are constructed for water storage during the winter period. Sludge in most cases is mixed with wood-fuel ash in order to get a more nutrient balanced fertiliser. A general description of some large-scale phytoremediation systems currently in operation in Sweden and some general research results are presented in the following text. The intention is to give a general overview of how this technology works and briefly describe research conducted to monitor potential environmental hazards and to evaluate their sustainability. Some general conclusions from the research results obtained so far will be also briefly reported and described in the text below.

EXAMPLES OF LARGE-SCALE PHYTOREMEDIATION SYSTEMS IN SWEDEN

Municipal wastewater

Municipal wastewater (wastewater from water flushing toilets) generally contains substantial nutrient amounts of nitrogen and phosphorus and is in most cases a well-balanced nutrient solution that can be used for fertilisation of plants (Aronsson & Perttu, 2001; Angelakis et *al.*, 1999). For sanitary reasons, however, it is preferable to be irrigated on non-food, non-fodder crops such as short-rotation willow coppice. In Sweden, during the 1990s, several large willow plantations equipped with drip or sprinkler irrigation systems were established adjacent to wastewater treatment plants to improve the nitrogen treatment efficiency while producing biomass irrigated with wastewater. The hypothesis was that if biomass production were 10 tonnes of dry matter per hectare and the nitrogen concentration in the willow shoots 0.5 percent (which is approximate the case), then 50 kg of nitrogen per hectare would be removed from the field with harvest each year. However, research has shown that nitrogen retention in short-rotation willow coppice can be more than 200 kg of nitrogen per hectare per year because of denitrification, *i.e.* the microbial transformation of nitrate to nitrogen gas, and long-term binding of nitrogen in the soil, namely the build-up of nitrogen-rich soil organic matter (Aronsson & Perttu, 2001). Therefore, higher amounts of nitrogen can be efficiently treated in a certain area cultivated with willow.

In Enköping, a town of about 20 000 inhabitants in central Sweden, a novel system has been introduced. The nitrogen-rich wastewater from dewatering of sludge, which formerly was treated in the wastewater plant, is now distributed to an adjacent 75-ha willow plantation during the growing season. This water contains approximately 800 mg nitrogen per litre and accounts for about 25 percent of the total nitrogen treated in the wastewater treatment plant. The water is pumped into lined storage ponds during the winter and used for irrigating short-rotation willow coppice during the summer (May to September). The system was designed so that conventionally treated wastewater can be added to promote plant growth. The willows are irrigated for about 120 days annually. The system treats about 11 tonnes of nitrogen and 0.2 tonnes of phosphorus per year in an irrigation volume of 200 000 m^3 of wastewater, of which 20 000 m^3 is water derived from dewatering of sludge after sedimentation and centrifugation. Irrigation ceases automatically on rainy days. Irrigation rates reach a daily mean value of about 2.5 mm during the growing season.

Possible environmental hazards associated with such applications, e.g. nitrogen leaching and nitrous oxide (N_2O) emissions into the atmosphere, are continuously monitored. The results so far indicate minimal environmental risks and increased biomass production in the irrigated parts of the willow field.

Landfill leachate

Landfill leachate (water that has percolated through landfills) is usually treated together with municipal wastewater in wastewater treatment plants. This conventional way of treating landfill leachate is generally costly and involves high energy consumption since the leachate is transferred away from the site for treatment. Therefore, landfill operators are becoming increasingly interested in alternative solutions for on-site treatment of leachate. One method is to aerate leachate to reduce ammonium and organic substances after oxidation and then pump the leachate to short-rotation willow coppice grown either on restored parts of the landfills or on adjacent arable fields. The objective is to sustain plant growth and minimize the potentially negative effects of the usually high ionic strength of landfill leachate with chloride concentrations often in the order of 1000 mg/l. The main advantage of this method is the low establishment costs compared with conventional on-site, engineered systems (Rosenqvist & Ness, 2004). A willow plantation established on a restored cover of the landfill decreases leachate formation by means of high evapotranspiration. A near-zero net discharge of landfill leachate can be achieved by recycling this wastewater into a short-rotation willow coppice plantation, even in the humid climatic conditions of northern Europe. Simultaneously, hazardous compounds in the leachate (e.g. ammonium and a range of persistent and potentially toxic organic substances) are taken up by the willows or retained in the soil-plant system. A high concentration of ammonium ions in water is an environmental hazard, but if it is carefully monitored, ammonium can also be considered a source of nitrogen for the willow plants. Decision on irrigation loads should be based on estimated evapotranspiration rates and nutrient retention as well as an assessment of the potential ecological impact on the surrounding water bodies receiving elements which are not retained by the soil-plant system. Differences in leachate composition from various landfills under different soil and climatic conditions, as well as differences in uptake of chemicals by different willow clonal materials, need to be considered in the design and management of leachate treatment systems involving irrigation of short-rotation willow coppice. Therefore, site-specific factors as the chemical composition of the leachate, the climate, the soil conditions, the irrigation method, the plant material selected, and their interactions, must be taken into account for successful implementation.

There are currently about 30 sites in Sweden where landfill leachate is used to irrigate short-rotation willow coppice. The plants are usually grown for at least one year before any leachate irrigation occurs, to ensure good plant establishment and thereby reduce possible toxic effects on the plants and promote high treatment efficiency. Irrigation is usually conducted with temporary drip irrigation system, and the irrigation pipes are placed in the middle of the double rows planted with willow. In other cases, and when the plantations are already established, irrigation with low-sprinklers can be used. Irrigation is practiced during the vegetation. For example, at Högbytorp, central Sweden, a system operated by the company Ragnsells Avfallsbehandling AB stores and aerates the landfill leachate in ponds. The company is planning a full-scale implementation of on-site leachate treatment (c. 90 000 m^3 per year). Currently, a part of the leachate is pumped into a 5-ha short-rotation willow coppice field which is irrigated daily during the growing season with approximately 2 to 3 mm of wastewater, and in the future more willow plantations will be cultivated to treat larger amounts of the leachate. Laboratoty and field experiments are conducted to identify maximum water loads applied in relation to a series of factors as plant stress due to hazardous compounds (chloride and ammonium), nitrogen leaching to the groundwater and willow growth. Moreover, different willow clones are tested to evaluate their growth performance under leachate irrigation. Furthermore, different diagnostic physiological tools for rapid identification of plant stress are tested. So far, preliminary results have shown that willow growth in the field seems to be undisturbed due to irrigation and nitrogen leaching is relatively low, considering the high amounts applied (unpublished data). Clone differences are evident with regard to stress reaction to leachate irrigation and leaf length seems to be a good rapid indicator of plant stress after irrigation with leachate (Dimitriou et *al.*, 2006).

Sewage sludge and wood ash

About 10 000 ha of short-rotation willow coppice in Sweden have been fertilised with sewage sludge. Sewage sludge contains some nitrogen (mainly organically bound) and high amounts of phosphorus. However, it is not a balanced fertiliser in terms of plant nutrients, since potassium concentrations are very low. Therefore, sewage sludge is mixed with wood-ash before applied to willows when wood-ash is available. This more balanced fertiliser replaces conventional inorganic fertilisation. The idea is that the effects of hazardous heavy metals and phosphorus in sludge-ash mixtures should be minimised by plant uptake and retention in the soil-plant system. At harvest, shoot parts that contain heavy metals are removed from the system and burned, and this material is partly recycled by applying the ash to the willow coppice stands. Only bottom-ash (i.e. ashes left at the bottom of the boiler) is applied, since it has a lower heavy-metal content than the fly-ash which is precipitated in the smoke-gas filters in the chimney.

Table 1. Balance between supply via sludge–ash application and potential output via willow plantation for several sludge and ash treatments, if a potential harvest occurred annually or every three years. The potential output with a stem harvest was calculated for a 30:70 bark:wood ratio for year 1 and a 25:75 bark:wood ratio for year 3. Changes indicate potential changes in the soil pool (In Dimitriou et *al.*, 2006).

		Treatments									
		sl+ash		**(sl+ash)x2**		**sl**		**ash**		**control**	
	Harvest interval (yrs)	**1**	**3**	**1**	**3**	**1**	**3**	**1**	**3**	**1**	**3**
Cd	Supply	*1.2*		*2.5*		*1.2*		*1.3*		*0*	
	Potential output	5.6	5.2	8.9	7	8	5.7	6.9	7	6.7	5.2
	Change	**–4.4**	**–4**	**–6.4**	**–4.5**	**–6.8**	**–4.5**	**–5.6**	**–5.7**	**–6.7**	**–5.2**
Cu	Supply	*303*		*606*		*500*		*106*		*0*	
	Potential output	28	14	48	18	34	14	39	14	30	12
	Change	**275**	**289**	**558**	**588**	**466**	**486**	**67**	**92**	**–30**	**–12**
Ni	Supply	*18*		*38*		*13*		*25*		*0*	
	Potential output	12	7	15	6	12	5	11	5	10	6
	Change	**6**	**11**	**23**	**32**	**1**	**8**	**14**	**20**	**–10**	**–6**
Zn	Supply	*449*		*899*		*569*		*330*		*0*	
	Potential output	243	245	359	330	308	284	258	273	240	213
	Change	**206**	**204**	**540**	**569**	**261**	**285**	**72**	**57**	**–240**	**–213**

The mixtures of sludge and ash are applied to the willow coppice stands during the establishment phase and after every harvest – in other words, every three to seven years – in order to compensate for the removal of nutrients by harvesting. The maximum heavy metal amounts in the sludge and the maximum amounts of heavy metals supplied in the willow field are regulated by strict legislation. In practice, since the average Swedish sludge is of relative good quality terms of heavy metals, the amount applied is equivalent to about 22 to 35 kg of phosphorus per hectare per year (Swedish Environmental Protection Agency, 1994).

Research has shown that after application of sludge-ash mixtures, heavy metals in the soil-plant system are within the permitted limits (see Figure 1), and total concentrations of cadmium, which is considered one of the most hazardous metals for human health, are reduced (Klang-Westin & Eriksson, 2003; Dimitriou et *al.*, 2006). Cadmium and other heavy metals will remain in the different ash fractions after burning, and will need further attention in order not to be recycled back to arable land. It is technically relatively easy to clean ashes from heavy metals, but this environmental service is not being paid for today, and therefore heavy metal contaminated fly ashes are usually put on landfills.

CONCLUSION

Short-rotation willow coppice, when used for phytoremediation, offers advantages such as high biomass yields and removal of hazardous compounds through frequent harvests. The high evapotranspiration rate and root tolerance of willows allow the use of high irrigation rates. In addition, short-rotation willow coppice stands are capable of cleaning polluted sites by taking up substantial amounts of heavy metals such as cadmium, and they can retain large amounts of nutrients in the soil-plant system. Willows remove successfully hazardous compounds contained in the various wastes, and utilise the nutrients and water applied in the production of biomass. Large-scale systems provide ecologically sound and cheap alternative treatment solutions, while biomass production for energy purposes is increased.

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CLONAL REPLICATED PROVENANCES' PERFORMANCE AND CLONE SELECTION TECHNIQUES

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Abstract: *Only 20% of Italy's hardwood timber requirements are met from domestic markets. Noble hardwoods are very important for the Italian furniture industry, being one of the most advanced for design and technology in Europe. Approximately 150,000 ha have been planted with noble hardwoods since 1985 with 70% of these represented by wild cherry, walnuts and oaks. In the last ten years, the estimated turnover for seed supply alone exceeded 50 million €.*

Activities and results of improvement strategies of a model species, wild cherry (Prunus avium L.), are presented.

For wild cherry a survey was carried out between 1985 and 1990 within the forests of 10 regions in order to assess the most wide-spread populations. Wild cherry is distributed in sparse populations and relies on suckers for rapid re-colonisation concentrated.

Together with traditional provenance tests the selection of phenotypically superior trees was carried out within the main populations, and materials were stored in clone archives by grafting. Progeny and clonal (micro-propagation) tests have been planted in the most suitable environments in order to evaluate genotype x environment interaction. Preliminary studies have been undertaken to measure phenotypic variation, e.g. phenology traits, and neutral markers used to assess genetic variation. As for many forest tree species the individual genetic component was found to be higher than at the inter-population level. A multi-trait Selection Index method for clone evaluation was found to be very effective.

INTRODUCTION

Italy has approximately eight million hectares (ha) of mainly-mountain forest area (National Forest Inventory 1985). Only 20% of Italy's noble hardwoods timber requirements are met from domestic markets. Thirty percent of the country's annual production is used, whilst the remainder is accumulated in the forest stock. Fifty percent of national production is represented by poplars, about 150,000 ha, which is mainly concentrated in the Po Valley. Noble hardwoods, including wild cherry as one of the main species are very important for the Italian furniture industry.

Mixed temperate forests distributed from the Alps to Calabrian Appenines, the most suitable environment for those species, grow on the Prealps and at higher elevations on the Apennine range. Wood production from these forests is low, due to soil protection needs and very high manpower costs .Two main sources are therefore possible for supplying wood industries: importing wood, mainly from France and Balkan countries (3.5 million cubic metres are imported yearly (Assolegno, Private wood Industries association) and developing new plantation programmes. The Council for Research and Experimentation on Agriculture – Forest Research Centre[1] (CRA-SEL) has been undertaking the second strategy since the 1930s, originally for conifers, and since the early 1980s, for hardwoods. About 150,000 ha have been planted with noble hardwoods since that time and 70% of this future stock is represented by wild cherry and walnut. CRA – SEL (Ducci 2003) estimated the turnover for seed supply alone exceeded 50 million € during this period.

Problems concerning trading and certification of noble hardwood reproductive materials were initially severe, due to lack of knowledge about the distribution and genetic structure of the species in Italy and to the absence of regulations (Ducci 1989). Only recently have the latter problems been solved with the introduction of noble hardwoods within the list of species concerned in national laws (Ducci 1999, Camoriano and Ducci 2004) and European Directive 1999/105/CE.

This paper summarises a practical example of the improvement strategy followed for wild cherry. (Ducci 2008)

The example has focused on selection and evaluation of superior phenotypes to be used as *ortets* to establish clonal seed orchards or to produce clones.

[1] Formerly named the Istituto Sperimentale per la Selvicoltura di Arezzo, Italia.

Between 1985 and 1990 a survey of wild cherry (*Prunus avium* L.) genetic resources was carried out in the forests of the Alps and Apennines in order to record the most extended and valuable wild populations. In Italy intra-specific variation of wild cherry, recorded by neutral markers as isozymes as well as microsatellites, seems to be relatively low and homozygosis was noted to be quite high (Ducci and Proietti 1997, De Rogatis *et al.* in press). The individual component of general variation was very important and, as for walnut, phenotype selection could be of great interest for tree breeders. The gene pool appears to be discriminated by large regions such as North-western, North-eastern, central and southern areas (Ducci and Proietti 1997). Earlier studies on leaf shape of clones growing within the clonal archives of ISSA, showed higher differentiation among provenances but with similar results (Ducci *et al.* 1995).

After the preliminary provenance survey a phenotypic selection was also carried out. Its aim was to establish a genetic base for clone production (Ducci *et al.* 1988). Indeed, earlier pilot field tests showed promising results with the use of clones in several areas of Italy, one of them being from the Po Valley (Ducci *et al.* 1990, Nocetti *et al.* in press), where the mono-specific cultivation of poplar clones is practised widely. Cherry and other noble hardwoods are best planted where soil conditions are not suitable for poplars, generally far from rivers and closer to the Prealps.

Compared to other species, the biology of cherry is more complex. Indeed the species' habitats are mixed temperate forests (deciduous oak and beech mountain forests in Italy). Cherry adopts different reproductive strategies according to the ecological successional stages. When growing in almost homeostatic situations it is scattered as single or small groups of trees, originating from seed dispersal. However, when a rapid colonisation of bare ground is required, it is instead concentrated in small clumps originating from numerous root suckers. Studies have demonstrated that the genetic variation within clumps can be very low, indicating they originate from only one clone (Ducci and Santi 1997). This means that cherry plantations, even clonal ones, can be planted with more variation than natural populations.

The aim of this experiment, partially reported by Ducci *et al.* in 2005 and 2006, is to select *ortets* suitable for growing in the environment of the Po valley and to preserve variation suitable to local conditions.

MATERIALS AND METHOD

Exploring the Genetic variation among *ortets*

Neutral markers (isozymes)

The genetic variation of the material in the field tests was analysed and compared with that from earlier studies on Italian cherry populations using starch gel isozyme analysis according to methods described by Ducci and Proietti (1997). In total eight enzyme systems were analysed for 14 gene loci. The main genetic parameters were calculated via Biosys-1 (Swofford and Selander 1989) and Principal Component Analysis was carried out using NTSYS–1.80 (Rohlf 1994).

Adaptive markers (flowering phenology)

Flowering phenology was demonstrated to be a good marker of variation, being highly heritable among cherry genotypes (Ducci and Santi 1997). Early or late flowering is dependent on the amount of Chilling Units CU required by single genotypes to flower. For fruit cultivars the chilling requirement to induce flowering may vary between about 700 and 1340 hours, or 6.5-8 weeks, below 7°C (Seif and Gruppe 1984), these values corresponding to about 1100 and 1840 CU. Flowering time is important in establishing a seed orchard where pollen exchange among genotypes should ideally be synchronised. For this reason, flower time averaged over a three year period was adopted as a marker of adaptive variation for wild clones.

Flowering was recorded on each tree by the same researcher. Dates were scored from 1 to 10 between 27th March and 26th April and undertaken every three days between these dates. When at least half the of buds on a tree were flowering (almost half of the flowers open) this tree was scored at that day: higher scores represented later flowering. Data from approximately 250 clones of 11 provenances, from the Alps to Calabria, were recorded in two different clonal archives near Arezzo. The first was at 600 m a.s.l. and the second at 300 m with more frequent frosts in winter and early spring and a wider temperature range during the day. Clones, replicated 3-10 times, were grafted onto wild rootstocks of local provenances. No significant differences were observed within clones, with standard deviations varying between 0.00 and 0.07. Possible significant rootstock influences on flower induction were excluded. One clone (CT03) only showed a standard deviation of 4.24, probably due to the use of a commercial rootstock clone in contrast to the mix of wild seedling rootstocks.

Clones common to both sites were used to carry out an analysis of variance in order to assess possible clone x site interactions. The Duncan's multiple test between means was used to assess homogeneous groups and to rank them according to their precocity. Clones were also separated by provenance groups to help explain possible geographic trends.

Quantitative traits

Fifty-four clones from eight central and northern Italian provenances (Figure 1)

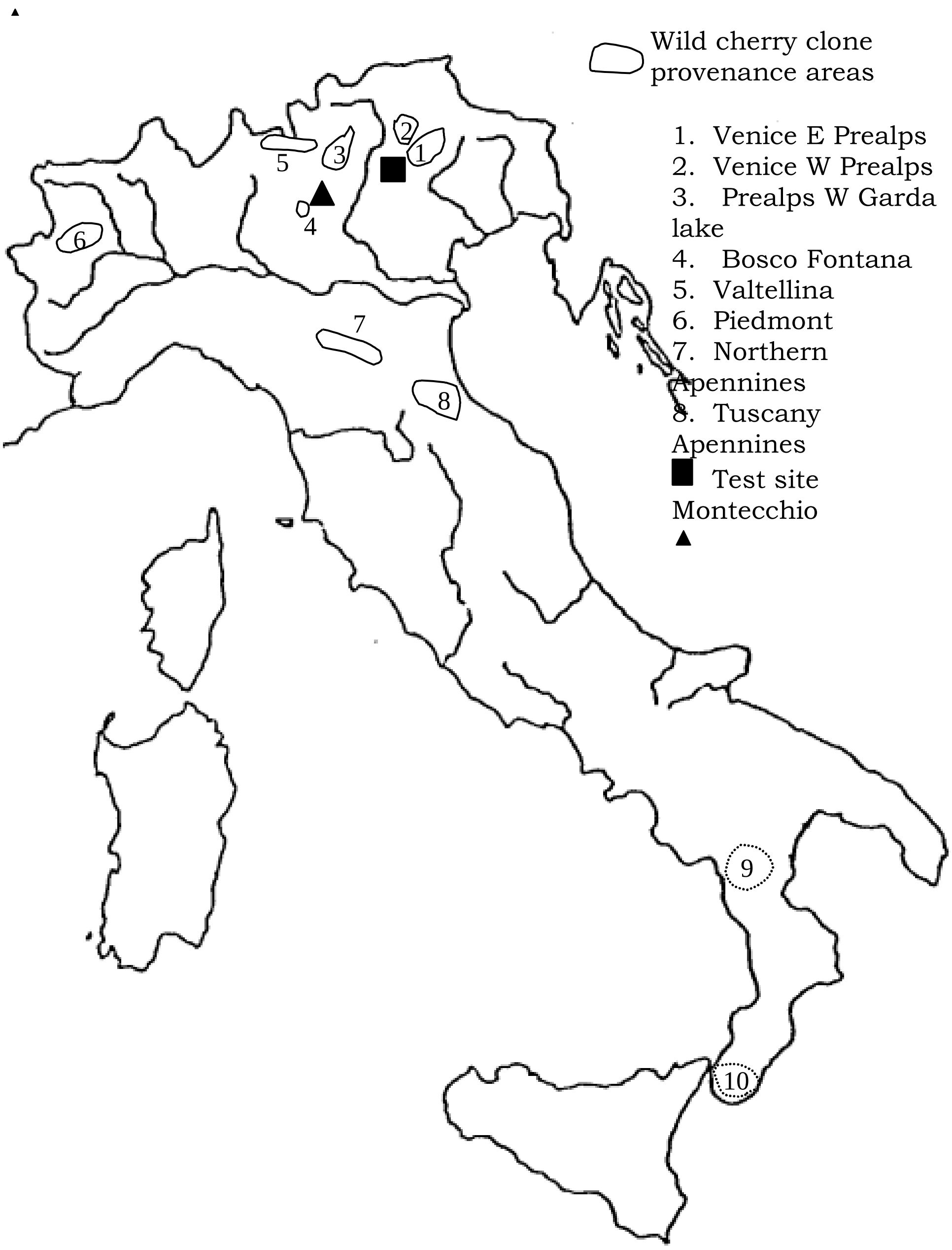

Figure 1. Wild cherry clone provenances and test sites

were planted during 1993 at two test sites: 1) Drugolo on the southern moraine hills of the Lake Garda, at 70 m; 2) Montecchio Precalcino near Vicenza close to the Venice Dolomites, on alluvial soils, at 40 m a.s.l.

The experiment was based on a seven randomised complete blocks, each block including one two-ramet individual plot per clone according to the method proposed by Muranty (1993).

Height (H) and basal diameters (Diam) for the first 4–5 years, and at 130 cm (dbh) after this were measured at planting. At ages two and nine, several other traits were measured on clones, including straightness (scored 1 crooked to 4 straight), branch angle averaged on the main branches in the fifth and sixth year whorls (scored 1 fastigiated to 7 horizontal), total number of branches at age six and mean number of branches per whorl. After this, four other traits were calculated: height growth H ($H_{92\text{-}93}$) and diameter growth ($dbh_{92\text{-}93}$) averaged over two years after planting, to obtain an idea of transplantating stress, and mean H and dbh increments, averaged between the third and ninth growing seasons.

Data were analysed following methods proposed by Ducci *et al.* (1990), Santi *et al.* (1998) and Muranty *et al.* (1998). As the differences between blocks within each site were significant, probably due to soil fertility differences of former agriculture fields, an intra-site factor analysis of variance was performed to evaluate the significance of clone x block interactions. Correlations were examined to investigate possible relationships between traits and geographic parameters of origin. Clonal broad sense heritability (Wright 1976, Santi *et al.* 1998) was estimated by site for each trait.

As for other valuable species, such as walnuts (*Juglans* spp.), several traits other than growth parameters were also considered as being important in clonal selection. These included stem form, dominance, number of branches per whorl, their size and angle. This last set of traits was considered meaningful for silviculture (*i.e.* planting spacings and pruning) and industrial purposes (*i.e.* knots and colour). Evaluating clones for each trait separately was practically impossible. It was therefore necessary to combine information on all traits of interest into a single index. A Selection Index (Zobel and Talbert 1984) was computed. Due the difficulty of establishing economic weights connected with each of traits, the index was synthesised (Ducci *et al.* 2006, Ducci 2008) from clone performances within each site as follows:

SI = *Perf.* Htot + *Perf.* Stem form * (1.30 *dom*) + [(*Perf.* Branch angle + Branch n. per whorl + $H_{92\text{-}93}$)/3];

where *Htot* is a production and adaptation indicator; *Stem form* is important for final products; as cherry clones are generally characterised by good *dominance*, this trait was included to improve the *Stem form* effect of 30%; traits enclosed in square brackets were weighted by about 30%, being important respectively for pruning and wood technology and ability to recover from planting stress. Clones showing a SI greater than 10% of the test site mean were selected.

RESULTS[2]

Variation among clones tested and their original populations

Genetic variation by neutral markers

The group of clones sampled to establish the experiment differed very little from the provenance populations assessed earlier by Ducci and Proietti (1997). The components of variance for cherry are similar to walnut, with 89 % of variance being dependent on the individual component (Fis = 0.108), whilst the Fit value at 0.216 confirms a scarcity of heterozygotes within populations and Fst 0.128 indicates low differentiation between populations. The UPGMA dendrogram (Figure 2) illustrates the genetic position of this artificial provenance compared to the natural populations where it is included within the northern group. It should be noted that West Venice, Asiago were included within a separate cluster.

Principal Component Analysis (PCA) indicated that the first three components explained 60.8 % of total variance, and the first five, 82.5 %. The most discriminating alleles were within 6Pgd1-b, Got3-b, Pgi2-a, Pgi2-b and Idh1-b.

Adaptive markers (flowering phenology) variation

[2] Specific data and detailed explanations, no showed here for editing reasons, can be requested also the corresponding Author.

An assessment of flowering precocity variation within the field trials was impossible because of long travelling distances from CRA SEL. For this reasons data were used from the grafted collections near Arezzo. The effect of different altitudes and aspects played a significant role on flowering periods. Indeed, the clone archive, located at 600 m with a western aspect, showed a mean flowering time 12 days later than the ISSA nursery.

Figure 2 – UPGMA clusters where the genetic position of the pooled population of clones selected in the test, is compared with the main Italian provenance areas (in *italic* provenances of clones). Numbers within brackets refer to fig. 1 legenda)

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                              Distance

.10       .08       .07       .05       .03       .02       .00
+----+----+----+----+----+----+----+----+----+----+----+----+

                                                            *  Piedmont (6)
                                                            *
                                                    *********  Bosco Fontana (4)
                                                    *       *
                                                    *       *  Tuscan Apenn.(8a)
                                                    *
                                                    *       *  W Garda Lake (3)
                                          ***********       *
                                          *         *      **  Valtellina (5)
                                          *         *      **  Clones pooled
                                          *         *
                                 **********         *****  **
                                 *        *             *   *  Tuscan Apenn.(8b)
                                 *        *             *
                                 *        *             *****  North. Apenn.(7)
                                 *        *
           ***********************        *               ***  N Calabria (9)
           *                     *        *****************
           *                     *                        ***  S Calabria (10)
           *                     *
           *                     *                         **  Venice Prealps(2)
           *                     ****************************
           *                                               **  Ven.Pr.W (1)
           *
           *
           ***************************************************  Other central Apennine pop.s
+----+----+----+----+----+----+----+----+----+----+----+----+
.10       .08       .07       .05       .03       .02       .00
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Figure 2. UPGMA dendrogram based on Nei's genetic distance (1978)

Clones, replicated 3 to 10 times, were grafted onto wild rootstocks of local provenances. No significant differences were observed within clones and possible significant rootstock influences on flower induction were excluded.

Differences between clones, provenances and clonal archives were significant but no interaction was evident among these factors. Therefore it was evident that clone behaviour was determined mainly by genotype. The highest percentage component of variance was due to the clones, confirming the importance of individual selection. Considering the total set of clones sub-divided by provenance areas, a clinal trend was evident. Only material from

Bosco Fontana, a mixed forest in the central Po valley near Mantua, was significantly different from all the other provenances (Figure 3). Groups of geographically neighbouring provenances were homogeneous but not consistently ranked according to a geographic gradient. The earliest bud breaking provenances were those from the central northern Apennines and the areas around the Lake Garda, the latest ones were from the Venice Prealps and southern Italy.

Amongst single clones the range of the flowering period was wide. Clone BF06, from Mantua, Po valley, was very early to flower (average score 1) whilst the latest were clones from all provenances between the Alps and Calabria and from altitudes ranging between 1320 m and 26 m a.s.l.. Scoring varied between 7 and 10, thus flowering occurred on average between 13 and 26 April. The difference between the earliest and the latest clone to flower was almost one month.

The latest flowering group was from areas between 44° and 46° N latitude, from the Po Valley. Three clones (AS07, ML07 and PVS01) were from the Venice Prealps, 500 to 700 m a.s.l., one from Piedmont (TO01), 300 m a.s.l., one (VC03) from the Apennines, 480 m, and two from the Lombardy Prealps (VF01 and VLN06), 600 m on average.

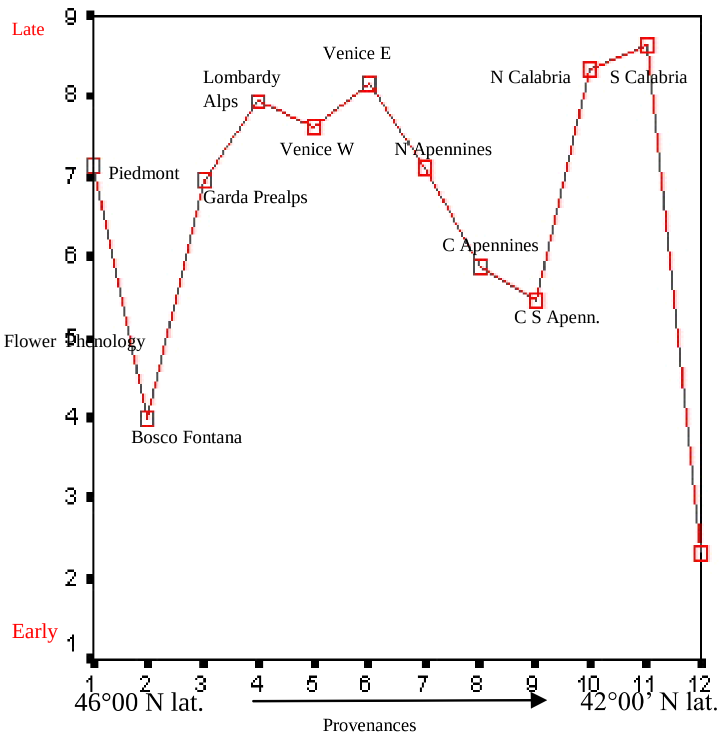

Figure 3. Flower phenology of wild cherry clone provenances according to the North-South gradient and altitude (n. 12 represents the out of range value by a central southern Apennines clone)

The earliest flowering clones were from a latitude range between 42° and 45° N, thus more southern than other material. The very earliest material to flower were clones BF03, BF06 and BF09, Mantua, 26 m. Their flowering was followed, after about one week, by 4 clones from the Apennines (VG01, VM01, VTN03, VTS01), between 700 and 200 m, and by clones from Piedmont (CT03), the Venice Prealps (AS05, AP03) and Lombardy (VLN05), from 500 to 700 m.

Quantitative trait variation (Ducci *et al.* 2006)

Broad sense heritability of traits by site confirmed the values found by Santi *et al.* (1998) for similar traits. They were very poor for Initial H increments, 0.45 for H increment 94-01, 0.48 for dbh increments, 0.34 for branch number, 0.50 for branch angle, poor for branch size, stem straightness, 0.60 for flowering. Generally heritability was lower in Drugolo, were the lower soil moisture (due to the morenic origin of the soils) is less suitable for trait expressions.

A) Site: Drugolo (Brescia, south of Lake Garda)

One-way analysis of variance of mean height increments (1994–2001), showed significant differences between clones of different provenances, but variation between blocks and provenance x block interaction were non significant.

Mean height increment of clone of different provenances varied between 54 and 67 cm per year during the assessed period. The lowest increments were observed, on average, among clones from the Prealps westwards to Lake Garda, whilst the best were among clones from Tuscany and the northern Apennines. Duncan's test showed a clinal variation among clones ranging between height increments of 42 and 75 cm per year.

The best 10 clones for height growth were relatively heterogeneous, being from several provenances: the Venice Prealps, Lombardy, Tuscany and Piedmont. Clonal increments varied, but not significantly, between 67 and 75 cm, with the overall mean being 60 cm. A local clone (VF01) was among the best. The best clones were PVN06 and AS06, respectively from the eastern and western Venice Prealps. Annual increments in height measured on both these clones averaged 20 cm more than the local provenance clones (Prealps westwards to Lake Garda) in Drugolo.

The worst clones were again from a mixture of several provenances: the Venice Prealps and Lombardy Prealps. No clones from central Italy or Piedmont were included in this clone set. As for height increments, the analysis of variance for diameter increments confirmed significance only for differences between provenances (*p* values between 0.05 and 0.01).

B) Site: Montecchio Precalcino, Vicenza, base of the Venice Prealps

Differences in height increment for both clones of different provenances and blocks were significant, but there was no significant *provenance x site interaction*.

Mean increment in height (1994 – 2001) was on average 61.8 cm per year, being very similar to the average in Drugolo (60.0 cm). Duncan's test on provenance ranking varied only for intermediate positions whilst outlying provenances showed low interactions between locations. Indeed provenances from the northern Apennines, the Lombardy Prealps and westwards to Lake Garda were respectively the best and the worst., but the latter was the only provenance to differ significantly from the others.

However, differences were evident for mean dbh increments. They were significant in the same way to height, but Duncan's test showed more structure within the material. The overall mean dbh increment was 11.1 mm. Again, the outlying provenances were the same as those observed for height increments. Ranking of other provenances varied notably, showing a possible variation in stem shape, intended as H/dbh ratio.

The best performing clonal provenances for annual dbh increments, were those within the northern Apennines, along with the Tuscan Apennines and the population from Bosco Fontana. Diameter increments among these groups varied between 14.3 and 12.3 mm. The poorest provenance, Lombardy Prealps westwards to Lake Garda, showed increments of only 9.8 mm.

The lack of a *clone x site interaction* was confirmed both for height and dbh increments, whilst differences between clones and blocks were significant (*p* values <0.05). Again, variation across ranks and Duncan's test revealed that homogeneous groups were clinal. Height increments ranged between 72.8 cm and 46.8 cm and dbh between 14.3 mm to 7.5 mm.

The fastest clone for height growth was ML06, collected from the western Venice Prealps at 600 m., followed by TO01, Piedmont, altitude 310 m. Both these clones were also noted as very late flowering. Other fast growing clones were from local provenances growing around the test site: western Venice Prealps (ML11, AS03, AS10, AS08), eastern Venice Prealps (PVN05, AP05), Tuscan Apennines (ACW01, VM01), Valtellina-Interior Lombardy Prealps (VLN09). The slowest clones were from the Lombardy Prealps westwards to Lake Garda, namely those from Valvestino (VV02, VV04, VV06, VV01, VV05) and from the eastern Venice Prealps (PVS03, PVS05).

For dbh increments there were no consistent relationships with height increment . Ranks also differed, though not significantly.

Another interesting set of traits was related to after-planting stress: where height and diameter increments were averaged over the two years after planting. In this case differences were probably caused by the effects of size and time of production in the laboratory, the materials being micro-propagated. In this case, differences between factors and their interactions were significant (*p* value <0.05). The mean initial increment varied between 51.5 cm and 148 cm, the average being 105.3 cm. Ranking was relatively different from more definitive data but the best materials showed within only the first years a good initial performance. The same situation was observed when the basal diameters increments were calculated. They varied between 9.8 and 17.7 mm and Duncan's test showed clinal variation of homogeneous groups.

Relationships among growth performances and the other traits (Table 1a and 1b)

Correlation analysis confirmed that traits connected to growth, such as height and diameter increments, are significantly related. It is worth noting that correlation between increments immediately after planting and increments in later years are low even though they are significant. Stem form is a trait independent others except for dominance, branch size and H_{94-01} increment. Branch size is positively correlated with dominance and negatively with dbh increment. Branch angle is poorly correlated with dbh increments.

Branch traits

Branch angle: This trait is relatively independent from blocks and sites, being non-significant for differences between blocks within sites and between sites. In general, clones showed low or no interaction with sites, therefore branch angle is dependent on single clone effects. As expected, no geographic trends were detected. No provenances had horizontal branches or fastigiated ones. Clone branch angle scores ranged on average between 1.9 (most fastigiated) and 3.8 (most horizontal). The most horizontally branched clones were significantly different (p value ≤ 0.05) from the others (VV04, ML11, PVN06, AP08).

Table 1.a. Relationships between main traits surveyed in Drugolo (Brescia)

	Incr. H 92-94	**Incr. H 94-01**	**Incr. dbase 92-94**	**Incr. dbase 98-94**	**Incr. Dbh 98-01**	**N. Branch/whorl**	**Total Branch at age VI**	**Stem form**	**Branch angle**
Incr. H 92-94	1	,192(**)	**,705**(**)	**,493**(**)	,131(**)	,186(**)	**,370**(**)	,147(**)	,151(**)
Incr. H 94-01		1	**,316**(**)	**,654**(**)	**,699**(**)	,015 (ns)	,285(**)	,204(**)	,010 (ns)
Incr. H 94-01			1	**,583**(**)	,213(**)	,190(**)	**,443**(**)	,090(*)	,131(**)
Incr. dbase 98-94				1	,5**98**(**)	,200(**)	**,564**(**)	,156(**)	,104(*)
Incr. Dbh 98-01					1	,096(*)	**,342**(**)	,080 (ns)	,034 (ns)
N. Branch/whorl						1	**,323**(**)	-,063 (ns)	**,310**(**)
Total Branch at age VI							1	,061 (ns)	**,300**(**)
Stem form								1	-,016 (ns)
Branch angle									1

Table 1.b. Relationships between main traits surveyed in Montecchio Prcalcino (Vicenza)

	Incr. H 94-01	**Incr. H 92-94**	**Branch angle**	**Incr. Dbh 94-01**	**Incr. Dbh 92-94**	**Branch size**	**Dominance**	**Stem Form**
Incr. H 94-01	1	-,231(**)	,071 (ns)	**,495**(**)	-,211(**)	,020 (ns)	,191(**)	,122(**)
Incr. H 92-94		1	,209(**)	,209(**)	**,736**(**)	-,097(*)	,060 (ns)	,055 (ns)
Branch angle			1	,129(**)	,138(**)	,046 (ns)	,089(*)	-,065 (ns)
Incr. Dbh 94-01				1	,202(**)	-,173(**)	,002 (ns)	-,011 (ns)
Incr. Dbh 92-94					1	-,170(**)	,010 (ns)	-,006 (ns)
Branch Size						1	**,307**(**)	**,301**(**)
Dominance							1	**,395**(**)
Stem Form								1

There is a significantly negative correlation between relative branch size and stem diameter at the same height. inmost of the clones tested had thin branches. The largest branch sizes were found in single genotypes, with no geographic trends being evident. Clones with small branch diameters were not necessarily the best in terms of growth, except for ML11.

Mean number of branches per whorl is another important trait for pruning and wood quality, due to the workability of the timber being directly related to knot occurrence. This trait was independent of clone x site interaction. Numbers of branches varied between about 2.0 to 4.5 per whorl and a positive and significant correlation was recorded with branch angle. Again, no geographic trends were detected, and no correlation was observed with growth traits. The highest numbers of branches per whorl were in ML11, VV04, VC04 and ACW08 which were all significantly different from the other clones. Clones with the lowest number of branches were AS03, AS07, TO01, VLS02, TO05 and PVN06.

Stem form

It is widely assumed that stem dominance is less important as a trait for cherry selection than stem form. Stem dominance was negatively correlated with growth in several clones: slow-growing clones have better stem form and are more common than the reverse. Some clones, characterised by a strong growth interaction with site, and can also change their behaviour for stem form. Other clones showed good stem characteristics everywhere, together with good growth performance: i. e. ML11 and VF01.

Multi-trait Selection index (Figure 4) (Ducci *et al.* 2006)

A group of 6 clones from several Alpine provenances showed a low genotype x site interaction and superiority of SI varying between 20 and 38% compared to the average of the clonal population. Other clones showed stronger interactions with each of the test sites. In Drugolo, with dryer soils and a more temperate climate, the best clones were from Piedmont, Apennines and the central Venice Prealps. In Montecchio were climatic conditions are colder and wetter, and soils heavier, clones from local provenances performed best. Other clones from relatively cold areas of the Apennines and Piedmont also showed adaptation to this site.

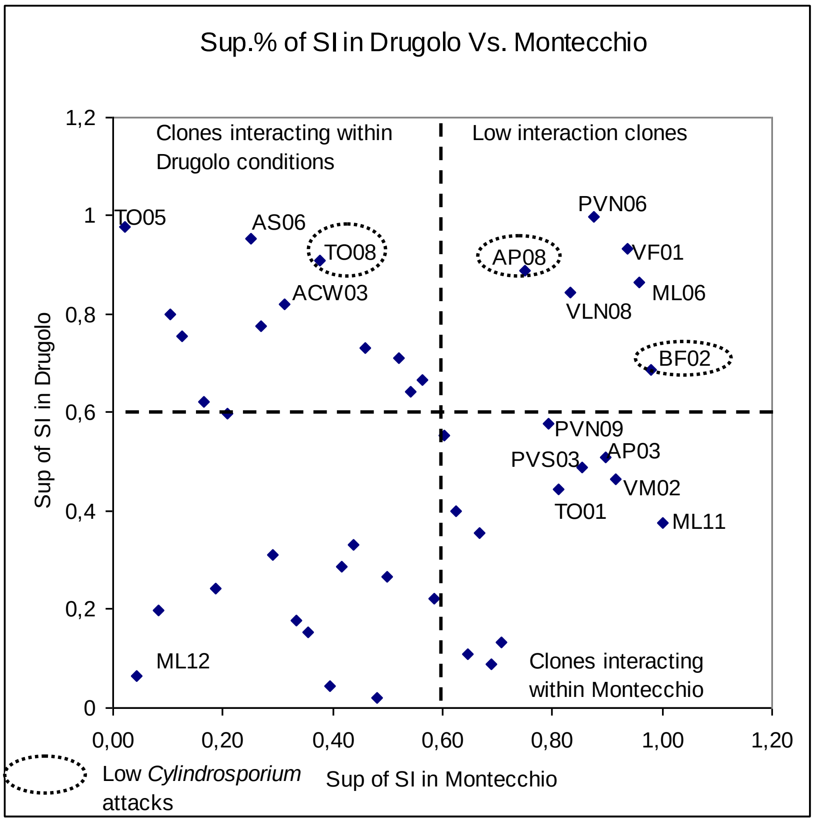

Figure 4. Clones were ranked according their multi-trait Selection Index, those showing SI higher 10% than the plantation average were selected

DISCUSSION AND CONCLUSIONS

Cherry is a species that is adaptable to wide range of environmental conditions, but to achieve good growth for wood production only sites with high fertility should be considered. This was observed in earlier preliminary studies (Ducci *et al.* 1990) and confirmed by Santi *et al.* (1998). In order to compare data, CRA SEL have used the same traits and research methods to those used by French researchers (Muranty 1993, Muranty *et al.* 1998) and the results presented here have confirmed similar findings, *i.e.* relationships between traits and broad sense heritability values (Ducci *et a.l.* 2006). As in French plantations, branch angle is independent from other traits and site conditions, suggesting the possibility of initiating improvement programmes by phenotypic selection.

This study is the first in Italy to be carried out on a large number of clones from several provenances. Results indicate that variation for growth parameters and flowering phenology is structured by provenance, whilst others traits such as stem and branch characteristics can interact with site conditions and vary according to single genotype characteristics. Provenance flowering was less clear, where probably several factors, such as geographic origin characteristics and other genetic traits affect adaptation of genotypes to chilling requirements. Latitude, altitude and aspect of origin can have similar effects on chilling requirements and often they can be reciprocally balanced.

Evaluating clones for each trait separately was practically impossible. It was therefore necessary to combine information into a single Selection Index (Zobel and Talbert 1986). This index still has to be improved upon because of the lack of knowledge relating to the economic weights of each single trait. Satisfactory results for selecting clones have been obtained by weighting traits for their supposed importance for cultivation (*e.g.* number of branches and their angle for pruning) or for wood technology (stem form and branch characteristics). Clones can be divided into those which interact with sites and those which do not, and be selected for characteristics in accordance with local site conditions. In view of a possible use of the selected clones to establishing seed orchards, the genetic variation assessed showed values similar to those in natural populations sampled throughout Italy.

Mixtures for improving levels of variation should be used containing low interaction clones and, prudently, clones that are 'specialised' or positively well-adapted to single site conditions. Within this experiment other traits are being improved through recurrent selection within seedling progenies obtained by crossing clones for resistance to *Cylindrosporium padi*, a fungus responsible for summer defoliation of cherry (Montecchio et al.). Three of the best clones were found resistant to this disease, endemic to the Prealps region due to the high air moisture in the summer.

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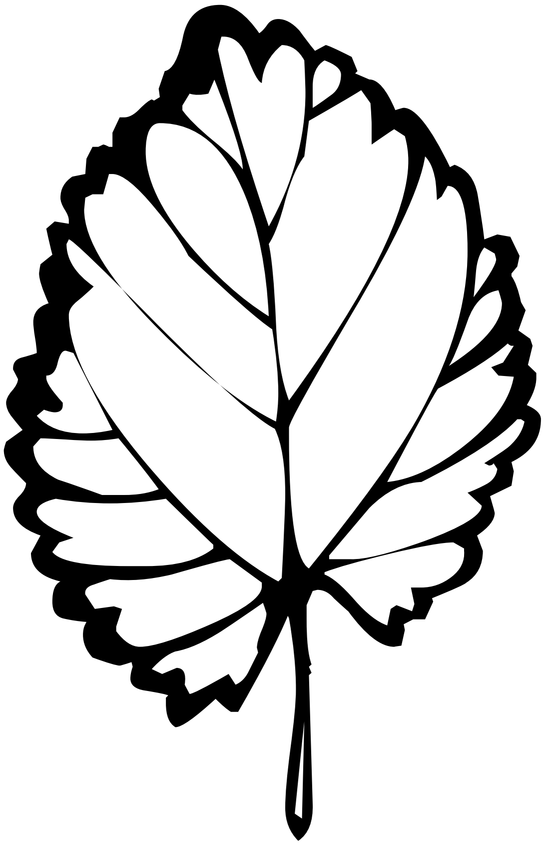

BIOMASS PRODUCTION WITH FAST GROWING WOODY PLANTS FOR ENERGY PURPOSES IN ITALY

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Abstract: *Short Rotation Forestry (including short rotation coppices –SRC) is an excellent renewable energy source. Recently the Italian Ministry of Agricultural, and Forestry Policy financed some research projects, which included study to test woody species in different sites of the country for the production of energy biomass. SRF plantations with poplar, willow, paulownia, black locust and eucalyptus were established in the period 2002-2008 in many different sites of the country utilizing old and new clones/provenance. At present the results obtained from the first six-year rotation are available. The mean annual yields achieved by the best willow and poplar clones were about 23 ODT ha-1 year-1. The best eucalyptus clone produced about 31 ODT ha-1 year-1. Good results were obtained also with black locust and paulownia. The selection tests highlight a good potential for genetic improvement for these species grown as SRF in Italy, especially for increasing the productivity*
Keywords: *Biomass production, woody species, Italy.*

INTRODUCTION

Italy imported fossil fuel to produce more than 80% of the total energy consumption which amounts to about 200 Mtoe (1 toe - ton of oil equivalent - = 41.868 GJ). Energy from renewable sources accounts for 7% of the total, and from biomasses for 2-3% only [1]. Since the years ’90s Italy adopted a Program and Action Plan to increase the contribution of bio-sources to 4-6% in the following years, in order to reduce greenhouse gases (GHG) according to the Kyoto Protocol (KP), ratified by the Italian government on 1st June 2002 by law n. 120. For Italy the goal is to reduce GHG emissions of 6.5%. Instead, an increase of 13.5% took place up today [1]. To reduce GHG emissions it is necessary to use a major part of renewable energies (RE) particularly bioenergy resources, such as forestry and agriculture crops, biomass residues and waste. Bioenergy offers cost-effective and sustainable opportunities with the potential to meet a high percentage of world energy demands during this century, while satisfying the requirement of reducing carbon emissions from fossil fuels. In the short period (2008-2010) the use of biomass residues, such as by-products from forestry utilization or from crop growing or crop processing, will allow to increase the bioenergy production in Italy up to 8-10 Mtoe per year, which corresponds to 4-5% of the total energy requirements, without reducing food production and maintaining soil fertility. In the long period (2020-2030) biomasses would contribute 15-20% of the total energy production according to European Union policy (EU). To obtain this result it will be necessary to implement energy crops, particularly Short Rotation Forestry (SRF) or Short Rotation Coppices (SRC) for energy purposes. During the last 6 years about 5000 hectares of SRF/SRC have been planted, mainly in the Lombardy Region where biomass thermoelectric power plants are been built or are under construction, and the former Program for Rural Development (PRD) included a series of financial incentives to support the establishment and maintenance of SRF/SRC plantations [2].

In the past, research on SRF was carried out in Italy by the Institute of the National Board for Cellulose and Paper (ENCC) on the cultivation of poplar, willow and eucalyptus with short rotations (5-6 years) aimed at the production of material for paper mills. In the last ten years of the past century, the former national electricity company (ENEL), now privatised, was a pioneer in this field and funded a research program [3], on a rather small scale, in order to study the potentialities of SRF for energy production. But a change in the Company's priorities brought this program to a premature end. The results obtained helped identify the species which are more suitable to SRF in Italy and to define a first cultural model. However, the real productive potentialities of these species in different environmental conditions were still to be investigated. The difference between the minimum and maximum productions observed was still too wide to make precise economic evaluations and to determine the actual profitability of the investments. Given the low quality of the material produced for energy purposes, the costs of production and transport heavily affected the economic balance of SRF plantations. For this reason the research carried out so far in the countries where this cultivation is widespread (Northern and Central Europe, USA) has been addressed towards the mechanization of harvesting operations in order to cut down costs with a reduction of labour [4]. On the contrary little attention has been devoted so far to the phase of establishment. The most interesting solutions on this topic have been found and applied in Sweden. However, since the species utilized (willows) and the environmental conditions (climate and soil) are quite different from the Italian ones, careful evaluation is necessary before these solutions can be implemented in Italy. In particular, it must be noted that the production costs of the propagation material and the costs of establishment have a considerable impact on the total production costs of the woody biomass because this type of cultivation is characterized by high investments in plants (from 1000 to 20000 per hectare).

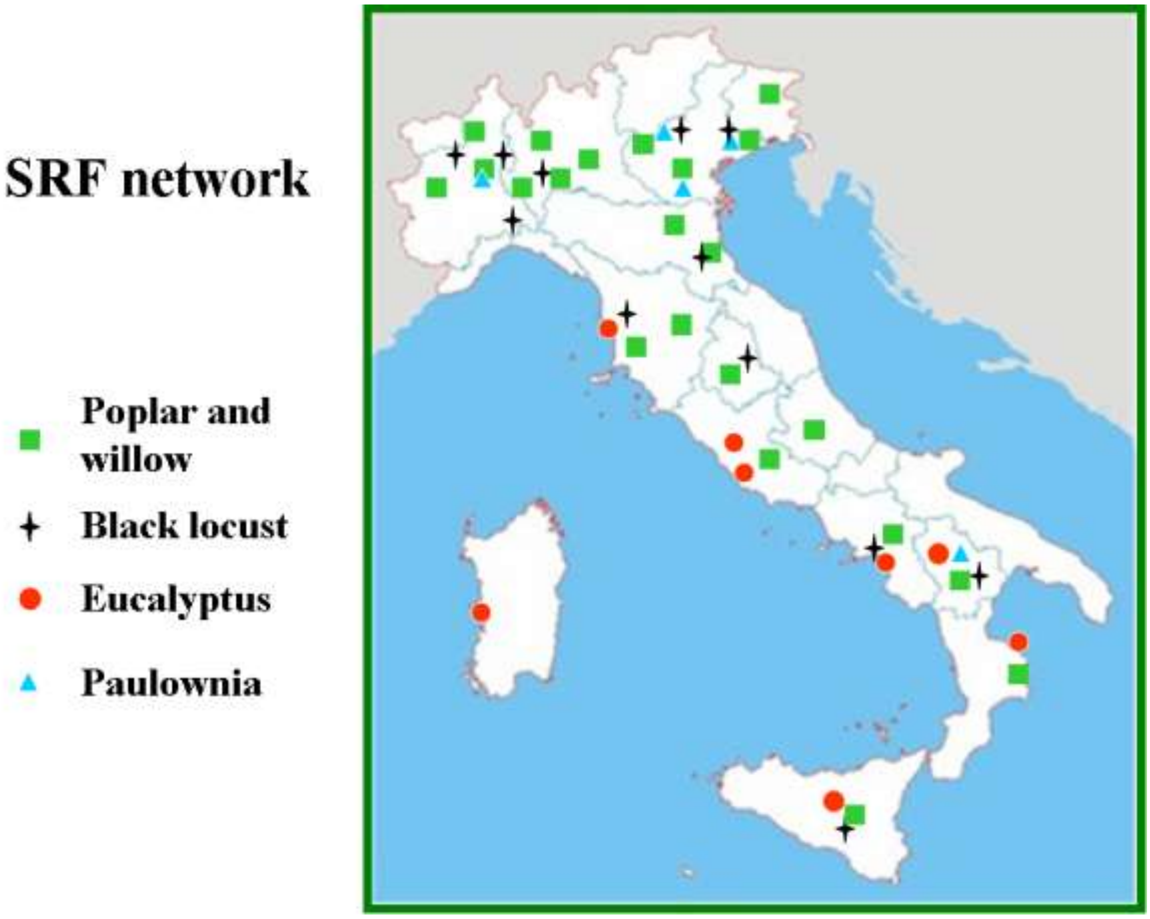

Figure 1. SRF network in Italy.

In 2001-2008 the Italian Ministry of Agricultural and Forestry Policy financed three research project (RI.SELV.ITALIA, BIOENERGIE, SUSCACE) which included some study on biomass supply chain, from crops to power plants, in order to give effective information to Italian farmers and energy-sector technicians and to help achieve the national objectives for renewable energy.

Particularly woody species and cultivars were studied in different sites of the country for the production of energy biomass. The low unitary value of woody chips made it necessary to reduce production costs in every phase, from planting to cultivation, to harvesting operations. Since the greater part of the production costs is related to the surface unit, a reduction of costs can only be obtained by increasing unitary productions. Cultivation techniques and genetic selection can influence the production significantly but the most important factor is the environment. Another uncertainty is represented by the effects of repeated coppicing on the survival of the stumps. In particular it is necessary to understand how much a progressive and inevitable decrease of the number of surviving stumps is compensated, in terms of production, by the larger space available for each of them.

Table 1. Biomass production (Oven Dry t·ha⁻¹·years⁻¹) obtained in Italy after the 1st (B1st) and the 2nd (B2nd) harvest

Species	Site	Trial	Density plant·ha⁻¹	Rotation years		B1st	B2nd
Poplar	Lombriasco (TO)	clonal test	5747	3	average	5.8	-
				3	best clone	6.7	-
	Casale Monferrato (AL)	clonal test	10000	2	average	12.5	7.4
					best clone	17.4	11.6
	Casale Monferrato (AL)	layout test	10000	2	single line	13.0	8.1
			10000	2	double line	11.5	6.9
	Motta dei Conti (VC)	species test	1333	5	mixed clones	16.2	14.7
	Ottobiano (PV)	fertilization test	1666	5	non fert.	5.0	-
				5	fert.	7.9	-
		demo plot	1666	5	average	11.1	-
	Cura Carpignano (PV)	plant density	4166	2	average	4.8	-
			13675	2	average	8.9	-
	Mantova (MN)	clonal test	8333	2	average	4.4	-
					best clone	5.9	-
	Osoppo (UD)	clonal test	6163	2	average	7.5	-
					best clone	8.8	-
	Pisa	demo plot	5100	2	average	7.0	9.2
	Bagni di Tivoli (RM)	clonal test	10000	3	average	8.3	-
					best clone	11.2	-
	Mirto Crosìa (CS)	clonal test	5500	2	average	11.1	11.8
					best clone	17.7	19.5
Willow	Casale Monferrato (AL)	clonal test	10000	2	average	12.9	10.8
					best clone	19.2	17.3
	Casale Monferrato (AL)	layout test	10000	2	single line	13.6	10.8
			10000	2	double line	12.2	10.7
	Motta dei Conti (VC)	species test	1333	5	mixed clones	16.2	20.3
	Lombriasco (TO)	clonal test	5747	3	average	6.7	-
				3	best clone	7.5	-
	Bagni di Tivoli (RM)	clonal test	10000	3	average	5.4	-
					best clone	6.2	-
	Osoppo (UD)	clonal test	6163	2	average	8.9	-
					best clone	10.5	-
Black locust	Casale Monferrato (AL)	plant density	8000	2	average	11.1	12.5
			12000	2	average	7.3	9.1
	Casale Monferrato (AL)	plant density	8333	3	average	7.5	
			1670	3	average	11.6	
	Trino (VC)	plant density	4000	2	average	2.3	5.3
			8000	2	average	2.1	8.8
	Fubine (AL)	demo Plot	1500	5	average	6.6	-
	Monterotondo (RM)	layout test	5926	4	single line	4.8	-
			8510	4	double line	6.6	-
Eucaliptus	Roma - Capocotta	clonal test	5500	1	average	11.3	-
					best clone	20.4	-
	Roma - Capocotta	clonal test	1600	1	average	3.2	-
					best clone	5.1	-
	Oristano	clonal test	5500	2	average	4.5	-
					best clone	6.0	-
	Mirto Crosìa (CS)	clonal test	5500	2	average	22.7	-
					best clone	31.5	-
	Mirto Crosìa (CS)	clonal test	1600	2	average	10.5	-
					best clone	16.4	-
Elm	Casale Monferrato	Demo plot	10000	2	average	7.0	-
Paulownia	Casale Monferrato	plant density	8333	3	average	10.3	-
			1670	3	average	7.6	-
	Vicenza	demo plot	3571	1	average	8.0	-
	Bressanvido (VI)	demo plot	8300	1	average	6.0	-

MATERIALS AND METHODS

The first experimental plots were established in 1994 in Pisa for the ENEL project, and new plantations were established in the period 2002-2008 in moreless sixty Italian locations to test the performance of experimental and commercial varieties for each species previously selected for energy purposes (fig. 1). Poplar and willow were planted in fertile soil all around the country, black locust in non irrigated soil particularly in the hills, paulownia in Northern Italy in sites with good water supply. Eucalyptus was tested only in Central and Southern Italy in warm climate conditions. This paper reports the results obtained in these SRF plantations. Trials were carried out to study simultaneously other cultural practices: fertilization, standing layout and planting densities.

The fields were previously ploughed at 30 cm depth and harrowed before planting. For poplar and willows unrooted cuttings, 20 cm long and 15÷23 mm in diameter, hydrated for two-three days before planting, were used [5]. For black locust, elm and paulownia one-year-old seedlings and for eucalyptus rooted cuttings or few-month-old seedlings were used. Planting was carried out with semi-automatic mechanical planters or by hand according to experimental plot dimensions.

Post-planting residual herbicides were applied to prevent germination of annual weeds for 30-40 days after planting.

Some trials were carried out to study the planting density and both the single and the twin rows layouts. The first planting layout is more suitable to cultivation, the latter makes harvesting suitable to mechanization. The planting density varied from 1600 to 10000 plants per hectare in single rows and from 10000 to 13675 plants per hectare in twin rows.

The rotation period varied from 1-2 to 5 years according to the density, one year in high and five years in low density. Coppicing was carried out with mechanical equipment.

A basic fertilization with P and K or with manure was performed during soil preparation, while 60 Kg per hectare of N was distributed in spring during the second year of each rotation. In two trials the N fertilization was studied: 60 and 100 Kg/ha in a 2-year rotation and 60, 90 and 120 Kg/ha in a 5-year rotation.

During the summer of the first year, three or four harrowings were carried out between the lines. Manual hoeing was done on the lines at the beginning of summer, only in a few poplar and willow trials. Repeated treatments against *Crisomela populi* (poplar and willow SRF) were necessary during spring and summer of the first year only in Northern Italy.

The cultural practices were intensive after establishment or coppicing. During the second and third year after establishment or coppicing cultivation practices were reduced; only weed control was done once by disc harrowing in late spring.

At the end of each vegetative season the following variables were recorded on all the trees/stumps:

- Rooting of cuttings and survival of plants/stumps
- Number of living shoots with height > 150 cm
- Diameters measured 130 cm above the ground
    - In each trial, a sample of 10-30 trees/stumps for all species/varieties was harvested and the following variables were recorded:
- Two orthogonal diameters measured 10 cm above the ground
- Two orthogonal diameters measured 130 cm above the ground
- Total height
- Total wet weight (stem and branches)

To obtain the dry weight the samples (stem and branches) were dried to constant weight in an oven at 105°C.

These data were used to estimate the production of oven dry biomass, expressed in tons (ODT) per hectare.

Analysis of variance (ANOVA) of the data recorded in each trial was performed.

RESULTS

The main result is the creation of a great net of plantations (fig. 1) all over Italy with the five species considered which, besides providing information on productivity in time, will serve as a base for giving precious technical and economic indications on this important new arboreal cultivation. These plantations could be used also for studies on growth models and carbon sinks.

At present the productivity results obtained from the first two rotation are available and are listed in Table 1. In general, the trials carried out in Italy showed productions between 2 and 31.5 Oven Dry tons (ODt) per hectare per year. In Northern Italy the mean annual yield achieved by the best willow clone (with a planting density of 10000 plants ha^{-1} and a 2-year rotation) was approximately 19 ODt ha^{-1} $year^{-1}$ and the yield of the best poplar clone with the same planting density and rotation period was about 17.4 ODt ha^{-1} $year^{-1}$. In Southern Italy the best poplar clone reaches 19.5 ODt ha^{-1} $year^{-1}$ during second rotation in soil with good water availability. In general the new clones of poplar are more productive than the old commercial ones.

Good results were obtained also with black locust (12.5 ODt ha^{-1} $year^{-1}$) and paulownia (8 ODt ha^{-1} $year^{-1}$). Black Locust is resistant to most pests, nitrogen fertilization and irrigation are not necessary; in general it requires fewer tending operations than poplar and willow.

In the Eucalyptus clonal test located in Calabria growth was excellent during both growing seasons: about 31.5 ODT ha^{-1} $year^{-1}$ were produced with a planting density of 5500 plants ha^{-1} and a 2-year rotation.

In Central and Southern Italy, eucalyptus and poplar seem to be more productive than willow, as the willow clones tested proved to be very sensitive to the summer droughts that characterize the Mediterranean climate in these areas. The selection tests for poplars, willows and eucalyptus highlight a good potential for genetic improvement for these species grown as SRF in Italy, especially for increasing the productivity on flat, fertile land with good water supply.

In general, production is proportional to the tree density with the same rotation period. A reduction of the density caused a decrease in production.

A slightly statistically significant difference was obtained between the two planting layouts: with the same density the production of poplar and willow cuttings planted in single rows is greater than the one in twin rows. The use of N fertilizer can improve production of 60% especially in poor sandy soil.

CONCLUSIONS

Even if most of the trials are still in course, the first results confirm the possibility to obtain satisfactory biomass productions when the best poplar, willow and Robinia clones are growth in suitable site conditions. SRF/SRC can be an excellent renewable energy source, policy conditions are good for its establishment and development. Although some good results were obtained there are big possibilities to improve this culture with research and development (R&D). R&D will play an important role especially to make new clones or varieties more suitable for biomass production. This implies the selection of clonal varieties that can increase more rapidly and have better disease and pests resistance/tolerance, and better ability to sprout after repeated coppicing.

The cultivation techniques of SRF are different from other crops and require the spreading of information in order to arise interest among the farmers, that generally consider SRF a high-risk culture for the duration of the cycle (12-15 years) compared to the annual traditional food crops. Initially, to encourage the farmers to establish plantations, SRF will require direct or indirect financial incentives. Such financial incentives are justified by the environmental benefits produced by SRF:

- fossil fuel substitution,
- carbon storage in stump and soil,
- reduction of heavy metals in the soil [6],
- reduction of N leaching,
- increase of biodiversity (compared with traditional crops).

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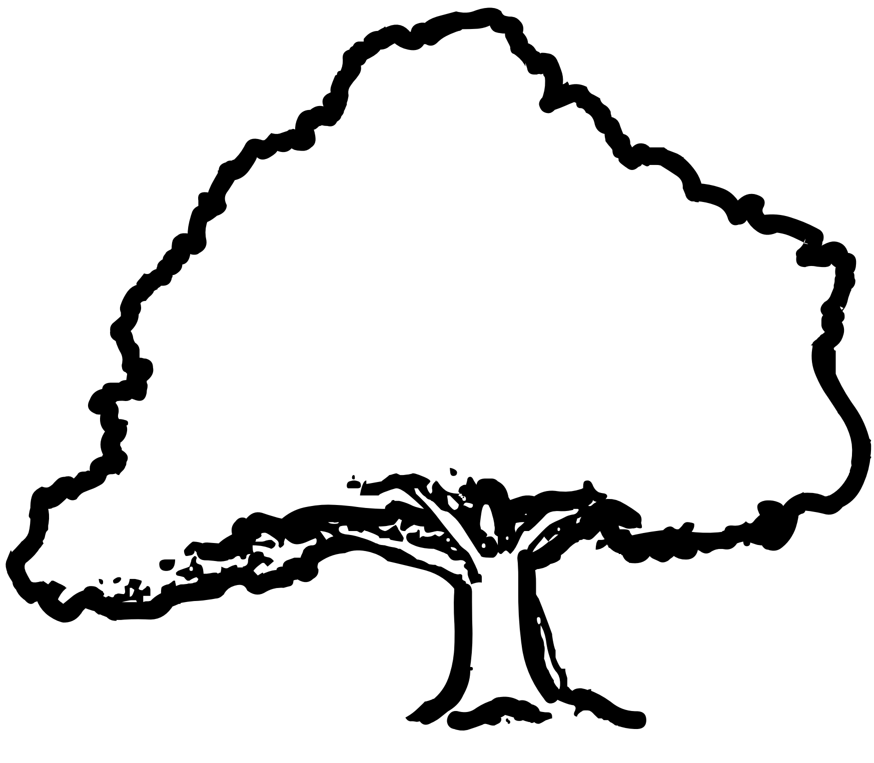

RESISTANCE OF DOUGLAS FIR (*Pseudotsuga menziesii* (Mirb.) Franco) TO *Rhabdocline* needle CAST IN BULGARIA

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Summary: *Fifty five provenances from the natural area of Douglas fir (Pseudotsuga menziesii (Mirb.) Franco) were examined in the period 2004–2007 for their resistance to needle cast disease caused by Rhabdocline pseudotsugae Syd. The survey was undertaken in four provenance experimental plantations. There were observed distinct differences between the provenances concerning their resistance. The provenances from the western and eastern areas of Cascade Range, from the Pacific Coast Range are the most resistant to Rhabdocline needle cast. The provenances from the area of northern interior – Greenwood (Washington), Keremeos (Washington), Whitefish (Montana), Bates (Oregon) and Canyon City (Oregon) are partly susceptible. Two provenances from the southern interior – Flagstaff (Arizona) and Alamogordo (New Mexico) are the most susceptible. The disease pathogenesis and intensity were studied in the most susceptible provenances. On the basis of the results obtained, a selection of resistant provenances for afforestation practice in Bulgaria is suggested.*
Key words: *Douglas-fir; Rhabdocline needle cast, resistance, differences between provenances*

INTRODUCTION

Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) is a valuable exotic forest tree species used in Bulgarian afforestation practice. Studies on the resistance of its different provenances to pathogenic organisms is one of the main problems in the future introduction and cultivation of the species in Bulgaria. In natural stands and in provenance experiments conducted in Europe, significant differance betwen the progenies resistance were investigated (Schober, 1954; Peace, 1962; Herrmann, 1973; Stephan, 1973, etc.).

Rhabdocline needle cast of Douglas fir is a serious disease caused by the ascomycetous fungus *Rhabdocline pseudotsugae* Syd. The disease is widespread in the natural range and in the countries where the species was cultivated (Boyce 1961; Stephan, 1973; Butin, 1995, etc.). In Bulgaria damages of *Rhabdocline* needle cast on Douglas fir were established for first time in 1977 (Rossnev, 1978). It has been found that each of the Douglas fir varieties – *menziesii, glauca* and *caesia,* suffer to different extend from this disease. *P. menziesii var. menziesii* was proved to be the most resistant variety. This was explained with the fact that the period of the new shoots development comes after the ascospores have been fully dispersed (Boyce, 1961; Georgieva, Rossnev, 2005).

The aim of the study is to determine the susceptibility of Douglas fir provenances to *Rhabdocline* needle cast and to suggest a selection of resistant provenances for afforestation practice in Bulgaria.

MATERIALS AND METHODS

The investigations were conducted during the period 2004–2007 in the experimental plantations of *Pseudotsugae menziesii* cultivated in Foresties Kostenets and Kyustendil, Training and Experimental Forest Range 'Petrohan' and Forest Research Institute – Sofia. The geographical and climatic data of the plantations are given in *Table 1*(Popov, Petkova, 2003).

The plantations were afforested during the period 1989–1990 and include 55 provenances from the natural range of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) as follows:

- 11 provenances from the continental areas – Washington (no. 1, 2); Montana (no. 3); Oregon (no. 21, 22, 23, 35, 36 and 37); Arizona (no. 54) and New Mexico (no. 55);
- 25 provenances from the West Cascade Range – Washington (no. 4, 5, 6, 7, 8 and 9); and Oregon (no. 24, 25, 26, 27, 28, 29, 30, 31, 39, 40, 42, 43, 44, 45, 49, 50 and 51);

- 13 provenances from the East Cascade Range – Washington (no. 13) and Oregon (no. 14, 15, 16, 17, 18, 19, 20, 32, 33, 38, 47 and 48);
- 6 provenances from the Coast Range – Washington (no. 10, 11, 12) and Oregon (no. 34, 52, 53).

Table 1. Geographical and climatic date for experimental plantations of Douglas fir (Pseudotsugae menziesii)

Region	Latitude (°N)	Longitude (°W)	Altitude (m)	Temperature, °C			Annual rainfall mm/m²
				Mean	Min.	Max.	
Forestry Kostenets	42°15’	23°58’	800	9.3	-25.0	37.8	747
Forestry Kjustendil	42°22’	22°54’	1000	11.2	-27.0	39.0	625
Forest Research Institute–Sofia	42°40’	23°18’	600	10.0	-31.2	38.8	620
Training and Experimental Forest Range ‘Petrohan’	43°14’	23°09’	600	10.2	-28.0	40.2	1004

The health condition of trees and the extent of damage were determined and analyzed by the methodology of the European project for forest ecosystems monitoring (ICP Forests Manual). The extent of damage indicated the quantity (%) of the needle area which was lost due to the action of the pathogen. Damage was assessed according 5–step scale from ‘0’= no attacked to ‘4’ = dead.

The differences in tree crowns defoliation intensity caused by the pathogen *R. pseudotsugae* for the periods 2004–2005 and 2004–2007, were assessed by Pearson criterion and its significance. Friedman nonparametric test was used.

RESULTS AND DISCUSSION

The results from the investigation showed that there are very conspicuous differences between the 55 Douglas fir provenances concerning their resistance to fungus pathogen *R. pseudotsugae*. Damages were identified in all four experimental plantations, but only on the continental provenances (no. 1, 2, 3, 21, 22, 23, 35, 36, 37, 54 and 55). The trees from the Coast Range (Washington and Oregon), and those from eastern and western areas of the Cascade Range (Washington and Oregon) were not attacked by the fungus.

Ascomycetous fungus *R. pseudotsugae* has caused very serious needle cast on the trees of continental provenances from the experimental plantation in Forestry Kostenets. The damages were appeared in preliminary cast of two and more years old needles, height and diameter growth decrease, which lead to physiological weakness and trees dead. The results of investigation show a strong variability in the susceptibility between the provenances (*Fig. 1*, *Table 3*).

In 2004, 88.6 % of all trees assessed had a needle loss more than 25.0 %. Average defoliation ranged from 20.0 % (no. 37 – Canyon City, Oregon) and 80.0 % (no. 54 – Flagstaff, Arizona) (*Table 3*). Without damages were 10.0 % of the trees of provenances no. 35 and no. 37 (Canyon City, Oregon) and 25.0 % of the trees of provenance 55 (Alamogordo, New Mexico) (*Fig. 1*).

Over the next years (2004–2005) the health condition of the trees from the continental areas has been mainly characterized by increasing of defoliation. In 2005 the share of trees with defoliation more than 25.0 %, increased to 97.7 % of all assessed trees (*Fig. 1, Table 3*). Most damaged trees were from provenance 54 (Flagstaff, Arizona), as 60,0 % of them were of extent ‘4’ (dead) and the rest – of extent "3"– heavily affected, with preserved only current year needles**.** The average assessment of crown defoliation for all continental provenances ranged between 55.0 % (no. 37 Canyon City, Oregon) and 100 % (no. 54 Flagstaff, Arizona) (*Table 3*).

For the period 2004-2007 the disease developed intensively as a percentage of dead trees increased from 2.3 % (2004) to 45.5 % (2007) (*Fig. 1*). For this period in all continental provenances statistically significant differences in the development process of trees defoliation with a level of importance ≤ 0.01 were proved (*Table 3*).

Table 3. Changes in crown defoliation intensity, caused by Rhabdocline pseudotsugae in the provenances from continental areas (CNT) in State Forest Kostenets during the period 2004–2007: Median (M);Range (R); Pearson criterion (x^2) Standard assessment of the differences (Z)

Number of provenance	**2004**		**2005**		**2007**		**x^2 (df; N; asimp. sig.)**	**Z**	
	M (%)	**R (%)**	**M (%)**	**R (%)**	**M (%)**	**R (%)**		**2004 2005**	**2004 2007**
CNT 1	60	30-80	70	50-90	90	80-100	35.4 (2; 20; 0.000)	-2.99^{ns}	-3.94**
CNT 2	40	30-70	70	60-100	100	80-100	36.7 (2; 20; 0.000)	-3.43**	-3.94**
CNT 3	70	40-100	80	70-100	100	90-100	29.9 (2; 20; 0.000)	-3.49**	-3.87**
CNT 21	70	50-80	80	70-100	80	80-100	24.4 (2; 20; 0.000)	-3.67**	-3.68**
CNT 22	40	30-80	70	50-100	90	70-100	32.9 (2; 20; 0.000)	-3.75**	-3.94**
CNT 23	65	40-80	80	40-100	90	80-100	33.2 (2; 20; 0.000)	-3.57**	-3.94**
CNT 35	40	10-70	80	70-100	100	90-100	35.8 (2; 20; 0.000)	-3.94**	-3.94**
CNT 36	40	30-80	70	50-100	90	80-100	31.7 (2; 20; 0.000)	-3.71**	-3.94**
CNT 37	20	10-70	55	20-100	90	70-100	28.6 (2; 20; 0.000)	- 2.32*	-3.70**
CNT 54	80	70-100	100	90-100	100	90-100	25.4 (2; 20; 0.000)	-3.39**	-3.70**
CNT 55	32.5	10-70	80	70-100	95	80-100	30.9 (2; 20; 0.000)	-3.62**	-3.93**

*) significances ≤ 0.05 **) significances ≤ 0.01 ns) not significant

Differences in the resistance of Douglas fir provenances from the different regions of North America to *R. pseudotsugae* justify the choice of origins for cultivating the species in Bulgaria. Many studies show that there is considerable variation regarding the sensitivity of origin Douglas fir from mainland areas to fungus *R. pseudotsugae*. The most sensitive origins mentioned are those of Arizona, New Mexico and Colorado (Merrill, Wenner, Gerhold, 1990).

Damages on needles of Douglas fir caused by the fungus *R. pseudotsugae* were established in the rest experimental plantations (Kyustendil; Petrohan and Sofia) where trees from continental areas are also severely affected with different intensity of development of the disease (*Table 4*). There were distinct differences between the provenances concerning their resistance. The assessments during the period 2004–2007 showed that damages by *R. pseudotsugae* increased, and no tree of the continental provenances was without damage in 2007.

As a result of the study of 55 Douglas fir provenances, three main groups can be differentiated concerning their resistance to *Rhabdocline* needle cast:

- resistance provenances – from the west areas of Cascade Range – Newhalem (Washington), Darrington (Washington), Monroe (Washington), Idanha (Oregon), Oakridge

(Oregon), Santiam Pass (Oregon), Crater Lake (Oregon), Medford (Oregon); from the east areas of Cascade Range – Ashford (Washington), Parkdale (Oregon), Warm Springs (Oregon), Santiam Pass (Oregon), Crescent (Oregon) and from the Pacific Coast Range – Bremerton and Moclips (Washington), Toledo and Brookings (Oregon);

- moderate to heavily susceptible provenances – from the area of northern interior – Greenwood (Washington), Keremeos (Washington), Whitefish (Montana), Bates (Oregon) and Canyon City (Oregon)
- heavy susceptible to *Rhabdocline* needle cast – two provenances from the southern interior – 54 (Flagstaff, Arizona) and 55 (Alamogordo, New Mexico)

Regarding the resistance to *Rhabdocline* needle cast and good growth of the provenances from Cascade Range (Washington and Oregon) and Coast Range (Washington and Oregon), they are of special interest to the future afforestation in Bulgaria.

Table 4. Differentiation of Douglas fir provenances according their resistance to Rhabdocline needle cast in the experimental plantations

Region	Resistant provenances	Moderate susceptible provenances	Heavy susceptible provenances
Kostenets	West Cascade Range East Cascade Range Coast Range	–	All continental provenances
Kjustendil	West Cascade Range East Cascade Range Coast Range	–	All continental provenances
Petrohan	West Cascade Range East Cascade Range Coast Range	Continental provenances: no. 1, 2, 3, 21, 22, 23	Continental provenances: no.35, 36, 37, 54, 55
Sofia	West Cascade Range East Cascade Range Coast Range	Continental provenances: no. 1, 2, 3, 21, 22, 23, 35, 36, 37	Continental provenances: no. 54, 55

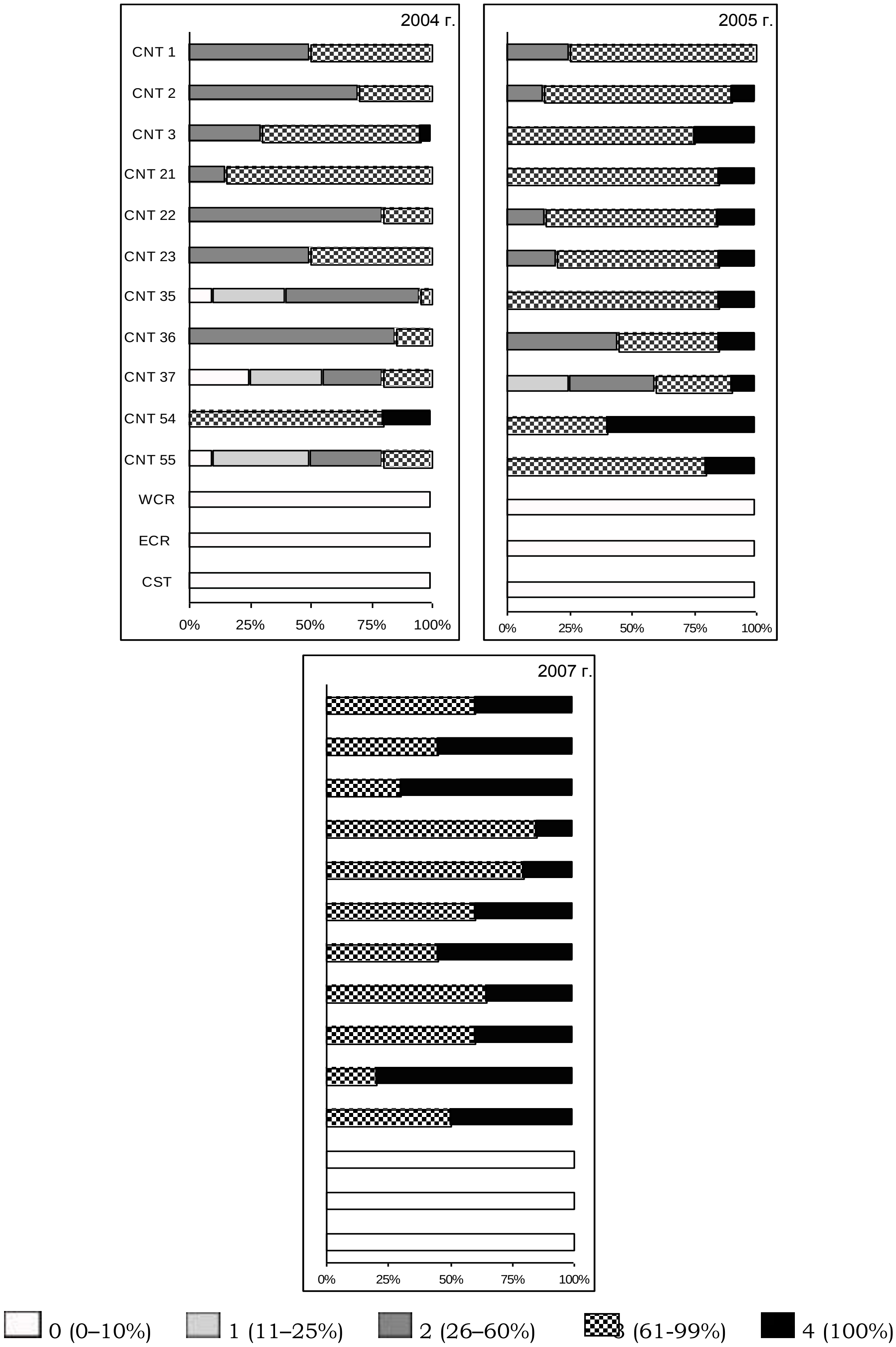

Figure 1. Distribution of assessed trees by extent of damage caused by Rhabdocline pseudotsugae Syd. depending on the origin: Continental areas (CNT), West Cascade Range (WCR), East Cascade Range (ECR) и Costal Range (CST) in Forestry Kostenets

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EFFECTS OF AEROPOLUTION ON STOMATAL DENSITY OF STUDIED WILD HORSE CHESTNUT (*Aesculus hippocastanum* L.) AND BIRCH (*Betula pendula Roth*) SPECIES IN THE AREA OF BANJALUKA

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Abstract: *The aim of this paper was to determine the effects of aeropolution in tested tree chestnut and birch species on the stomatal density under tested condition of aeropolution in the city of Banjaluka. Two locations in Banja Luka differing in aeropolution were tested: the first one was Student campus, where aeropolution was minimal, or none whatsoever, and the second one was the West transit, where the pollution was very intense due to enormous number of cars passing by. Degree of aeropolution between these two locations was very pronounced, and the aim of this paper was to see how the two locations offering such diverse conditions for growth of studied tree plants influenced the physiological processes such as transpiration and photosynthesis.*
Key words: *aeropolution, birch, wild horse chestnut, stomatal density*

INTRODUCTION

The technical-technology expansion, intensive traffic development, and sudden and fast urbanization (in regard to the tested city of Banjaluka) started causing environmental pollution at the end of the last and the beginning of this century, first of all due to anthropogenic factors. One country's pollution can, and often does, become another country's problem, so cross-boder pollution is a serious environmental problem of global proportion.

Plants are negatively influenced by air poluttion. Effects of air pollutants, such as SO_2 on plants are great, especially at high air humidity, high light intensity, and in the presence of other pollutants in the atmosphere. Dässler (1976) mentioned that SO_2 occurred as assimilation retardant disrupting photosynthesis in the presence of open stomata and greater amount of light. Functioning of stomatal apparatus is influenced by gaseous SO_2.

Concentrations of 0,1 – 0,5 ppm induced the opening of stomata. SO_2 caused opening of stomata in the dark, thus disrupting day-night rhythm of stomata opening and closure, water regime and exchange of materials with the environment. SO_2 and soot disrupted biochemical-physiological processes and anatomical-morphological structure of plant organs. Soot caused inhibition of photosynthesis by coating leaf and thus preventing light from reaching chloroplasts, which stopped chlorophyll synthesis. According to Caiazza-Ovinn data, cited by Dimitriva-Ninova (1994), the number of stoma (density) on leaf surface varies widely among different plant species and can be a good indicator of environmental pollution. Papers of Kosev-Čakalov (1992), and Kosev (1994), Jordanov-Gudeski (2002/2003) in which problem relating to structural changes of photosynthetic apparatus of wild horse chestnut (*A. hippocastanum*) in city polluted conditions was addressed, pointed out to anatomical-morphological structure of plants growing in the areas of urban environment.

BASIC BIOLOGICAL CHARACTERISTICS OF TESTED TREE SPECIES

Aesculus hippocastanum L. – wild horse chestnut

A deciduous tree species which under favourable conditions can reach the height of some 30 m, diameter at breast height of 1m, and can live to be over 200 years of age. The crown is broad and has dense branch structure, oval in shape with rare branches, and dense leaves.

The bark (approx. 1 cm thick) on young sprouts is smooth, greyish-brown in colour and with age it turns to black-greyish, finely cracked, and eventually flaking off into thin, narrow scales. The root system is well developed, superficial, with the main root being formed at young age, and the process continues in later years when the side roots are formed. Blossoming usually occurs immediately after leaf emergence in May. The flowers appear in tassel-like inflorescences 20-30cm long. Flowers are triple: some have seven stamens, and retarded pistil – male, others have 7 stamens and hemafrodit pistil, and some others have 7 stamens and a pistil, but the stamens fall off before the stigma of the same flower is receptive. The fruit is a flashy pod approx. 6 cm in diameter, covered with soft thorn-like spines. The pod contains 1-3 seeds, often flattened due to mutual pressure. The seed is large, round, and somewhat flattened, ripening in September-October. Its surface is covered with firm, brown, and shiny lupine, of an unpleasant, and a very bitter taste. It is favourite food of animals. It favours mostly deep, humus soils in gorges and river valleys, where it constitutes the composition of coastal forests. It proved to be very successful and is widely spread as a decorative species throughout Europe.

Image 1. Wild horse chestnut, Campus
May, 2008

Image 2. Wild horse chestnut, West transit
May, 2008

Betula pendula – birch

Deciduous tree up to 30 m high, with a diameter of 40-60 cm, and can live to be over 100 years of age. Habitus is characterized by the up right slender trunk stretching out almost to the tip of poor crown, and slim branches. Bark of the young tree is glossy white with periderm which is peeled off horizontally in tiny flakes, and later turns black and deeply cracked in the lower part. The root at a young age is a branched core which in time turns into superficial or heart shaped, very well branched root system depending on the habitat. The dates for beginning of leafing and soon after that the blooming are the end of March till May depending on the habitat. Leaves are tiny, rhomboid-egg to triangle shaped, long pointed, toothed at the base, sharp double serrated along the edge, 3-7 cm long on 2-3 cm long leaf stem, both naked. The fruit is a nut 2 mm long, oblong, with two remaining stigma, and two small wings on both sides. It is located in fertile hanging catkins on long tiny stem, brown in colour. It ripens in July or August.

Image 3. Birch, Campus, May, 2008

Image 4. Birch, West transit, May, 2008

Wide birch areal points out to its wide ecological amplitude. According to its areal it belongs to Euro-Siberian geographical floral element. It occurs in great part of Europe from north Spain, Sicily, Rodop and Kavkaz to Scandinavia, and in fareast along north Asia. It can be found at 600 m in Norvegia, and on Pirinei and Etna at approx. 2000 m. It can be grown on poor cambisol/podzolic soils, humid and acid, but on pretty dry soils too. It also appears on lime and silicate massifs. It is known as a pioneer species occupying clear cutting areas and forest fire areas, which spreads rapidly since its seed is desperse by the wind and can travel long distances.

MATERIAL AND METHOD OF WORK

Plant material of two tree species was used for this paper: wild horse chestnut (*Aesculus hippocastanum*) and birch (*Betula pendula*). The work consisted of field and laboratory parts. Field part consisting of plant material collection was accomplished during period from 19 to 30 May 2008 in Banja Luka.

Samples were taken from two locations with diverse aeropolution:

- the first location, the Student Campus, where aeropolution was minimal or none whatsoever,
- the second location was West transit, Krajiške brigade street, where aeropolution was great, due to numerous cars using this transit. Daily flow of cars at the access to Krajiške brigade and Ranka Šipke crossroad was 23750.[1]

Data on aeropolution for May 2008 are[2]:

CO - 0,526 mg/m^3,
SO_2 – 22,718µg/m^3,
NO – 8,765 µg/m^3,
NO_2 – 15,302 µg/m^3,
Soot – 16,246 µg/m^3,
Dust – 23,444 µg/m^3.

Leaf samples were taken from two positions on the tree:

- first position was from uouter parts of the crown (light) with leaves fully exposed to light
- the second position was from the inner part of the crown (shadow) with less light

Material and tool: plant material, wild horse chestnut leaves, microscopis glass plates, colourless nail polish, transparent sticky tape, light microscope, ocular micometer, objective micrometer.

Stomata samples were taken from the leaf sheat of wild horse chestnut and birch (10 leaves x 5 replications – preparates) in such a way that small amount of nail polish was deposited on the leaf sheat. The nail polish was then left to dry, and sticky tape was glued onto the nail polish, and then abruptly snapped off with leaf epidermis print left on it.

Preparation for microscopic observation was prepared by gluing the sticking tape with the stoma print in dried glue onto the objective lenses. Preparation obtained in this manner can be kept and used for several years. Preparations were observed under light microscope, where there was certain number of stomata in the field of view, and area of field of view was determined. On the basis of the number of stomata counted in the field of view, and determined area of the field of view the stomatal density in tested tree species from different ecology habitat was estimated. The obtained results were statistically processed, and significance of the interaction relationships between modalities were analyzed using multifactorial analysis according to model 2 x 2 x 2 (Hadživuković, 1977).

RESULTS OF INVESTIGATION

Results of investigation, i.e. determination of stomatal density in tested wild horse chestnut and birch leaves in diverse aeropolution is given in two parts:

- tabular presentation of tested indicators,
- graphic presentation of observed and tested indicators.

[1] Administrative service of Banjaluka city, department for traffic bussines
[2] Bureau for ecology and environment protection, Banjaluka

Table 1. Stomatal density measured in leaves of tested wild horse chestnut and birch species *(mm²)*

Species (A)	Horse chestnut				Birch			
Place (B)	Campus		Transit		Campus		Transit	
Position (C)	light	darkness	light	darkness	light	darkness	light	darkness
1	293,94	190,91	321,21	218,18	109,09	121,21	233,33	127,27
2	372,73	169,70	284,85	193,94	133,33	133,33	239,39	130,30
3	324,24	230,30	236,36	187,88	124,24	121,21	151,52	151,52

First-order interaction

Species	
Horse chestnut	282,58
Birch	117,42

Place	
Campus	285.35
Transit	114.65

Position	
light	197.98
darkness	152.78

Second-order interaction

AB	Horse chestnut	Birch
Campus	404,04	166,67
Transit	161,11	68,18

AC	Horse chestnut	Birch
Light	265,15	130,81
Darkness	201,51	104,04

BC	Campus	Transit
Light	227,78	168,18
Darkness	244,44	61,11

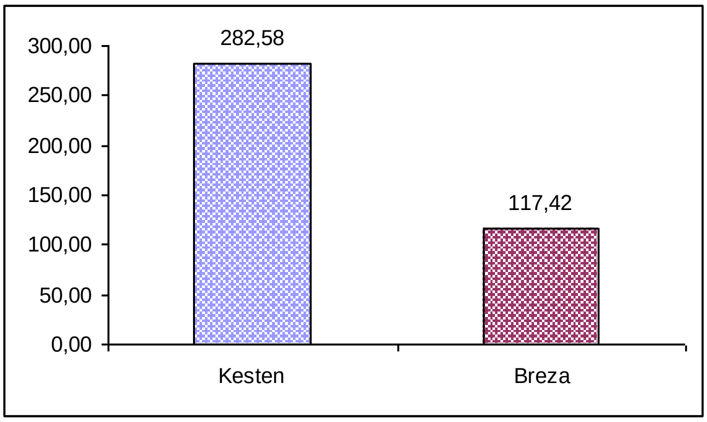
Analysis of variance for stomatal density of tested horse chestnut and birch species

Sources of variation	Number of degrees of freedom	Square mean	F - test	F - tabulary 0.05	F - tabulary 0.01	F - signif.
Blocks	2	552,07	0,57	3,44	5,72	NS
Species (A)	1	64948,01	66,62	4,30	7,94	**
Place (B)	1	956,60	0,98	4,30	7,94	NS
Position (C)	1	29996,60	30,77	4,30	7,94	**
AB	1	7715,64	7,91	4,30	7,94	*
AC	1	7933,75	8,14	4,30	7,94	**
BC	1	185,15	0,19	4,30	7,94	NS
ABC	1	6074,44	6,23	4,30	7,94	*
Error	14	974,90				
Total:	23					

It can be seen from the table 1 that the least stomatal density was found for birch in Campus, in the light (109.09), while the greatest one was found for horse chestnut in Campus, in the light (372.73) which was in accordance with the results of Jordanov-Gudeski (2002/2003).

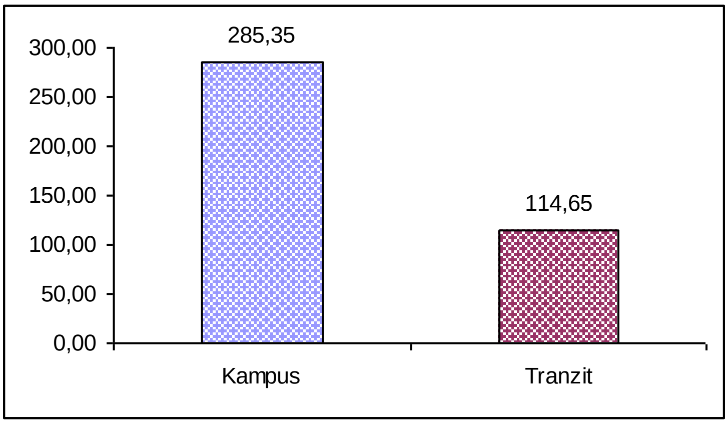
Analysis of variance for stomatal density of tested wild horse chestnut and birch species revealed statistically highly significant influence of tested species and stomata position, while that of place was not statistically significant.

Effect of interaction in relation to species x position revealed highly significant statistical influence, while interaction relations between species x place, and species x place x position revealed that expressed differences in interactions were accidental.



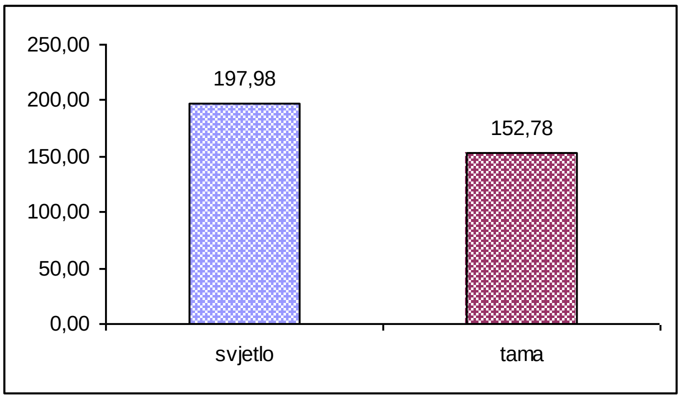
Graphic 1. *Relationship between stomatal density in leaves of tested horse chestnut and birch species*

It can be seen from the relationship interaction between tested wild horse chestnut and birch species that (Graph. 1) stomatal density in horse chestnut (282.58) was greater in relation to birch, regardless of the place and position of leaf on the tree.



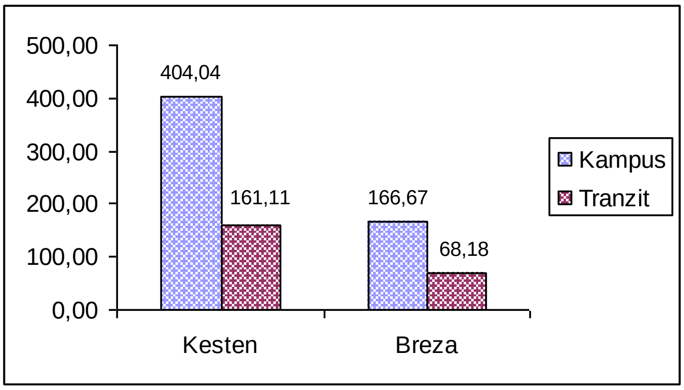
Graphic 2. *Relationship between stomatal density in leaves of tested species found in Student campus and West transit*

If relationship interaction was observed depending on geographical position of studied species (Graph 2) it could be seen that greater stomatal density was observed in Campus in relation to transit, regardless of studied species and position of leaf on the tree.



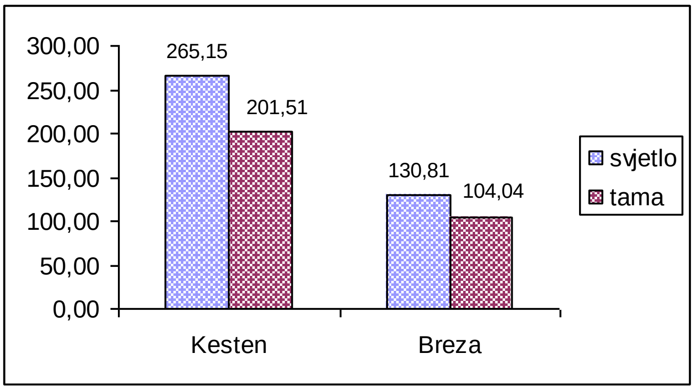
Graph 3. *Relationship between stomatal density in leaves of tested species in light and dark*

If relationship interaction was observed depending on leaf position on the tree (Graph 3) it could be seen that greater stomatal density was measured in the full light (197.98) in relation to darkness (152.78), regardless of species and geographical position of tested species.



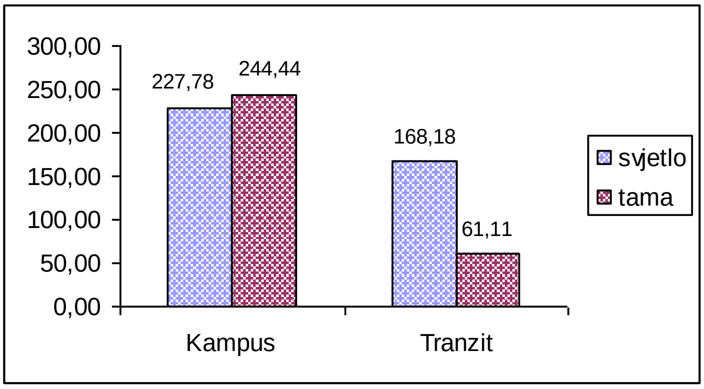
Graphic 4. *Relationship between stomatal density in leaves of tested horse chestnut and birch species found in Student campus and West transit*

It can be seen from the relationship interaction between tested wild horse chestnut and birch species and geographical position (graph 4) that greater stomatal density was measured in horse chestnut in Student campus and West transit (404.04 and 161.11) in relation to birch (166.67 and 68.18), regardless of leaf position on the tree.



Graphic 5. *Relationship between stomatal density in leaves of tested horse chestnut and birch species found in full light and shadow*

From the relationship interaction between the tested species and leaf position on the tree (Graph 5) it can be seen that the greatest stomatal density was measured in horse chestnut in full light and shadow (265.15 and 201.51) in relation to birch (130.81 and 104.04), regardless of geographical position of tested species.



Graphic 6. *Relationship between stomatal density in leaves of tested species in full light and in shadow found in Student campus and West transit*

It can be concluded from the relationship interaction between geographical position and position of leaf on the tree that stomatal density (Graph 6) was greater both in shadow (244.44) and in light (227.78) in Student campus in relation to West transit (168.18 and 61.11), regardless of tested species.

CONCLUSION

On the basis of investigation of behaviour of studied wild horse chestnut and birch species in polluted city of Banjaluka the following can be concluded:
- stomatal density of both tested species (wild horse chestnut and birch) revealed highly significant dependence on aeropolution;
- stomatal density of tested tree species (wild horse chestnut and birch) in different geographical position (Campus, west transit) revealed highly significant dependence on aeropolution;
- Influence of leaf position on the tree (light and shadow) on stomatal density of studied tree species (wild horse chestnut and birch) revealed highly significant dependence on aeropolution;
- Relationship interaction between species x position revealed highy significant stomatal density dependence on aeropolution;
- Relationship interaction between species x position revealed significant stomatal density dependence on aeropolution

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Dothistroma septosporum AND *Lecanosticta acicola* NEW THREATS OF PINES IN CENTRAL AND NORTHERN EUROPE?

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Abstract: *Dothistroma septosporum, D. pini and Lecanosticta acicola are quarantine pest according to EPPO and European law. Dothistroma septosporum is common all over the world on many species of coniferous trees, especially pines. Together with D. pini, it is reported from more than 80 hosts. To date, in the Czech Republic, it has been identified on 21 species of pine, 4 species of spruce and on Douglas fir. Large Scots pine plantations infested by Dothistroma needle blight were registered in Southern Bohemia, Czech Republic, in March 2008. Large infected areas in natural Scots pine stands in Central Finland were found in spring and autumn 2008. Situation has in some areas epidemic character, infected are mostly stands in age 5-15 years, infection was also observed in 60-80 years old stands of Scots pine as well. Reasons of this sudden outbreak are discussed now, one of the most important phenomenon could be extremely warm winter time 2007/2008 which allowed full development of symptoms on Pinus sylvestris.*
Key words: *Dothistroma needle blight, Brown spot needle blight, Dothistroma septosporum, Dothistroma pini, Lecanosticta acicola, pines, Pinus*

INTRODUCTION

Dothistroma needle blight is caused by *Dothistroma septosporum* (Dorog.) Morelet and *Dothistroma pini* Hulbary, resp. theirs teleomorphic stage *Mycosphaerella pini* E. Rostr. (syn. *Scirrhia pini* Funk & Parker, *Eruptio pini* (Rostr. apud Munk) M. E. Barr.) and brown spot needle blight is caused by *Lecanosticta acicola* (Thüm.) Syd. with teleomorphic stage *Mycosphaerella dearnessii* M.E. Barr (syn. *Scirrhia acicola* (Dearn.) Sigg).

Mycosphaerella pini was described from Europe, more exactly from Russia as *Cytosporina septosporum* Dorog (Doroguine 1911). Saccardo (1920) described the Dothistroma needle blight fungus found on *P. ponderosa* in Idaho as *Actinothyrium marginatum* Sacc. Later, anamorphic stage was described as *Dothistroma pini* Hulbary (Hulbary 1941). The connection between the American and European pathogen was given when Gremmen (1968) and Morelet (1968) realized that the fungus described in Europe as *C. septosporum* was the same as *D. pini* causing Dothistroma needle blight in the United States. Morelet (1968) found that it referred to the same fungus and created a new combination *Dothistroma septosporum* (Dorog.) Morelet. Both names are commonly used. Some papers note differences between these two anamorphs. Eg. Barnes et. al (2004) found on the bases of phylogenetic studies, that *D. septosporum* and *D. pini* make up two distinct phylogenetic lineages. *Dothistroma septosporum* has a worldwide distribution and it is the causal agent of the disease that has severely damaged plantations of *P. radiata*, grown as an exotic in the Southern Hemisphere. In contrast, *D. pini* is a serious pathogen of pines that currently appears to be restricted in distribution to the North Central United States; however it was confirmed also from Ukraine recently (Barnes 2007).

Lecanosticta acicola (Thüm.) Syd. anamorph of *Mycosphaerella dearnessii* M.E. Barr, is a causal agent of brown spot needle blight. It is reported from Asia (China, Georgia), Republic of South Africa, North America (Canada, USA) and Central America (eg. Belize, Costa Rica, Cuba, Guatemala, Honduras, Mexico). *Lecanosticta acicola* is listed as an A2 quarantine EPPO pest and also in EU Annex designation as II/A1.

Aim of this paper is to evaluate contemporary situation on distribution and host spectrum of *Dothistroma septosporum*, *D. pini* and *Lecanosticta acicola* in Europe with evaluation of risk of spreading into new areas in Europe.

MATERIAL AND METHODS

Records from the Czech Republic are based on monitoring carried out in 2000 – 2008. Pine and also spruce and Douglas fir needle samples were examined, mainly from regions of southern and central Moravia, Silesia, Eastern and Central Bohemia.

Samples from Central Finland were collected in 36 plots around Pieksamaki, Ahtari, Toysä, Jyväskylä, Tuomarniemi, national park Koli etc. in April - June 2008.

The presence of the pathogen was always investigated according to characteristic symptoms such as red bands, dying tips of needles or the occurrence of subepidermal sporocarps, acervuli. A precise identification was proved on the basis of microscopic analyses of conidia.

Isolation of culture was made on MEA 3 containing malt extract 30 g/l, pepton 5 g/l, agar 15 g/l, without addition of any antibiotics. Pieces of needles with acervuli 3 - 5 mm long were on surface sterilized by sodium hypochlorite 7%, subsequently by ethanol 96% and washed by sterilized water and put on malt extract agar. After 3 days of incubation, when new conidia occurred on fruiting bodies, conidia were inoculated into new medium.

Herbarium specimens are deposited at Herbarium of Faculty of Forestry and Wood Technology (BRNL), some isolated strains are deposited in culture collections in the Czech Republic (CCM).

RESULTS AND DISCUSSION

Dothistroma needle blight is widespread in most European countries with different impact on forest stands and plantations. Dothistroma needle blight is known from most of European countries (see fig. 1), eg. France, Italy, Portugal, Spain, Georgia (Ivory 1994), Slovenia (Macek 1975), Croatia (Novak-Agbaba, Halambek, M. 1997; EPPO 2005), Monte Negro and Serbia (Karadzic 1989, 2004), Romania (Gremmen 1968) etc. From Central Europe it was reported from Austria (Petrak 1961), Slovenia (Macek 1975), Germany (Butin, Richter 1983) and Poland (Kowalski, Jankowiak 1998) where it was found in May 1990, Slovakia (Kunca, Foffová 2000), Hungary (Koltay 1997), Czech Republic (Jankovský et al. 2000, 2004). It is known from UK (Murray and Batko, 1962), where it was found in 1954. Recent findings are from Netherlands (EPPO 2007), Belgium (EPPO 2008a). Up to 2008 there was no report on Dothistroma from Scandinavia, actually there are reports from Estonia (Hanso, Drenkhan 2008), Finland (EPPO 2008b), Sweden (Stenlid J, oral communication; DNA isolated sequence from needle, no symptoms), it is also reported from Lithuania. European strains belong mostly to *Dothistroma septosporum*, however Barnes et al. (2004, 2007) recorded also *D. pini* from samples from Ukraine. However, distribution of *D. pini* could be much more extensive, as shows recent research from France (Marcais, B. 2008, oral communication). Further studies in Europe on this subject are required.

More than 80 host species of Dothistroma needle blight are mentioned from all continents (Bednářová et al. 2006). To date, it has been identified on 21 species of pine, 4 species of spruce and also on Douglas fir in the CR: *Pinus aristata* Engelm., *P. attenuata* Lemon, *Pinus banksiana* Lamb., *Pinus cembra* L. var. *sibirica* (Du Tour) G. Don, *Pinus contorta* Douglas ex Loudon, Pinus x digenea Beck (=*P. rotundata x P. sylvestris*), *Pinus heldreichii* H. Christ, *Pinus heldreichii* H. Christ var. *leucodermis* (Antoine) Markgraf ex Fitschen, syn. *Pinus leucodermis* Ant., *Pinus jeffreyi* Grev. et Balf, *Pinus mugo* Turra, *Pinus nigra* Arnold, *Pinus ponderosa* Douglas ex Lawson, *Pinus pungens* Lambert, *Pinus rigida* Miller, *Pinus rotundata* Link = *Pinus mugo* nothosubsp. *rotundata* (Link) Janchen & Neumayer, *Pinus strobus* L. var. *sibirica*, *Pinus sylvestris* L., *Pinus tabuliformis* Hort. ex Carrière, *Pinus taeda* L., *Pinus thunbergii* Parlatore, syn. *Pinus thunbergiana* Franco, *Pinus wallichiana* A. B. Jackson, *Picea abies* L. Karst., *Picea pungens* Engelm., *Picea omorika* (Pančić) Purkyně, *Picea schrenkiana* Fisch. & C. A. Mey, *Pseudotsuga menziesii.* Austrian pine *Pinus nigra* Arnold, mountain pine *Pinus mugo* Turra, *Pinus ponderosa* Douglas ex Lawson, *Pinus jeffreyi* Grev. are the most frequent and most susceptible hosts. As for species of other genera *Picea pungens* Engelm., *Picea abies* L. Karst., *Picea omorika* Purkyně and *Picea schrenkiana* Fisch. & C. A. Mey were noted as hosts. *Dothistroma septosporum* was also isolated from needles of *Pseudotsuga menziesii* from Řícmanice arboretum close to Brno. Symptoms on needles of Douglas fir were not so clear, acervuli were observed only exceptionally.

Records on Scots pine were exceptionally rare in the CR and also in Europe up to spring 2008. Risk of Dothistroma needle blight for Scots pine in Europe is noted eg. by Lang and Karadzic (1987). According to Gadgil (1984), *Pinus sylvestris* is highly susceptible. Contrary, according to data from Great Britain, Peterson (1982), mentions that the attack occurs very rarely. However several hectares of Scots pine plantations, infested by *Dothistroma septosporum*, about 10 years old, were registered in Southern Bohemia, in Forest district Nove Hrady, Trebon area, in March, 2008. Some other large infestations were registered also on Scots pine in natural reserve in peat bog Sobeslavska blata in Southern Bohemia. Surrounding commercial forest was not affected.

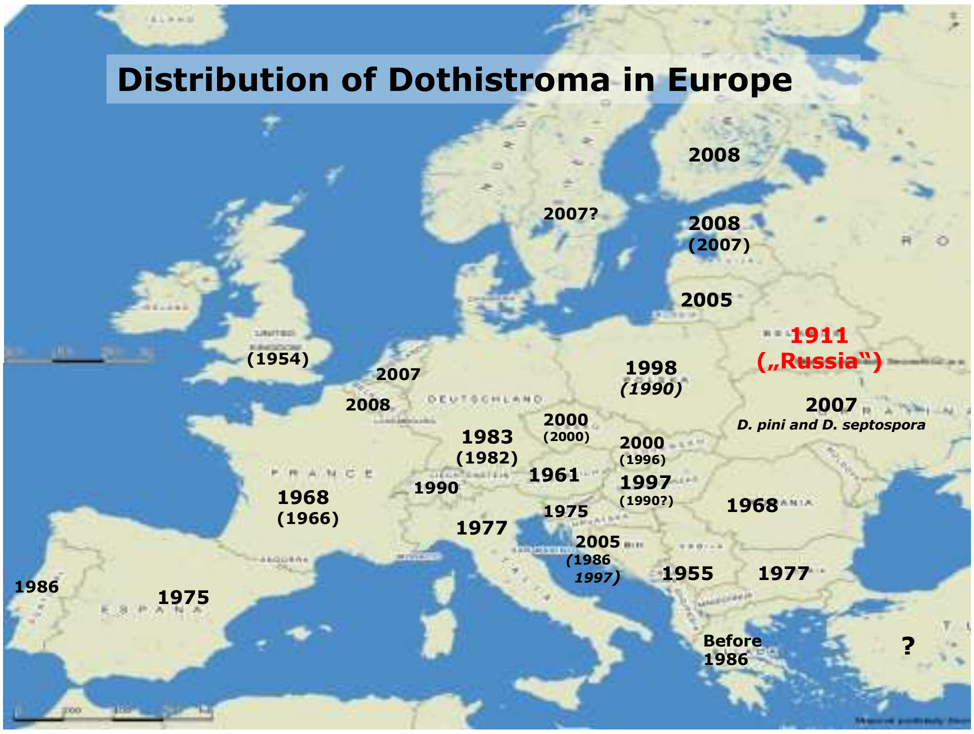

Figure 1. Distribution of Dothistroma needle blight in Europe. Years mean first published report, in parentheses are years of findings, if differ from year of publishing.

In the same time, *Dothistroma septosporum* outbreak was observed in large areas in natural Scots pine stands across the Central Finland in spring 2008 (EPPO 2008). Situation has in some areas epidemic character, infected are mostly stands 5-15 years old, infection was also observed in 60-80 years old stands of Scots pine. Typical symptoms as red bands and acervuli were found in all visited (36) plots around Pieksamaki, Ahtari, Toysä, Jyväskylä, Tuomarniemi, national park Koli etc. in June 2008. Typical symptoms like red bands were observed in most localities. The magnitude of the outbreak is such that eradication of the disease is considered impossible, and therefore no eradication measures were taken.

Reasons of these sudden outbreaks on Scots pine are discussed now, one of the most important phenomenon could be very warm winter time 2007/2008 which allowed full development of symptoms on *Pinus sylvestris.* Needles of Scots pine dropped generally before development of full symptoms and ripening of acervuli. Most of symptomatic needles were noted on flash dried needles sticking in tips of dead twigs. Symptoms on Scots pine were almost the same in the Czech Republic and Finland. We suppose that the infection of Scots pines by Dothistroma, both in the Czech Republic and Finland is more extent, than it was reported up to date. Large area of distribution shows, that the disease was spread into area many years ago. Despite intensive studies on pine needle casts in area, it was not observed yet (Muller M, 2008, oral communication).

With respect to actual epidemic situation in some countries, it is necessary to discus the role of climatic factors (Woods et al. 2005) in Europe and trade with plant material as main risk factors for spreading of both diseases.

Lecanosticta acicola is in Europe (see fig. 2) reported from Austria (doubt Petrak 1961, Brandstetter, Cech 1999, 2003, Kirisits, Cech 2006), France (Chandalier et al 1993), Italy (Porta, Capretti 2000), Germany (Butin et Richter 1983, Pehl 1995), Switzerland (Holdenrieder, Sieber 1995), Bulgaria and formerly Yugoslavia (Holdenrieder, Sieber 1995), Serbia (Milanovic and Karadzic, oral communication), from 1979 it is reported from Croatia (Novak-Agbaba, Halambek 1997, EPPO 2007). Some new records are from Estonia (Cech T. 2008, oral communication), Lithuania, Slovenia (Jurc D., in press). This species could be still neglected and confused with Dothistroma needle blight in some areas.

Brown spot needle blight caused by *Lecanosticta acicola* was for the first time reported in the Czech Republic on June 10, 2007. The first record is from the peat bog

National Nature Reserve Červená Blata in South Bohemia, close to town Třeboň; coord. N 48°51'37.06", E 14°48'44.09". The disease was observed on 10 – 40 years old trees of *Pinus rotundata*, (Jankovský et al. 2008). The new record in the CR is from the same host species, 10-60 years old in National Nature Reserve Borkovická blata, near town Soběslav (coord. N 49°14'16.3" E 14°37'54.2") on August 7, 2008. (Jankovský et al., in press). Both places are very strictly protected areas. Typical symptoms (according to EPPO 2005) were observed in current year needles declined from tips in middle of July and in August. Brown spots with apparent yellow separation were present on green needles as well. Visible yellow belts were present between dead tissues of killed tips and green tissues. Studied conidia were subhyalinne, even dark olive green, surface of conidia echinulate to verrucose or tuberculate, straight to curved, with one to five septae, fusiform to cylindrical, size 3 - 5 µm × 21 - 44 µm. On 3% MEA medium, the fungus produced grayish green olive to olive black stromatic colonies, producing slime with conidias. DNA sequences of the ITS region of the mycelium from isolated strain (GenBank Acc. No. EU117117) showed 99-100% similarities with other species of *M. dearnessii* held in GenBank (Jankovský et al. 2008).

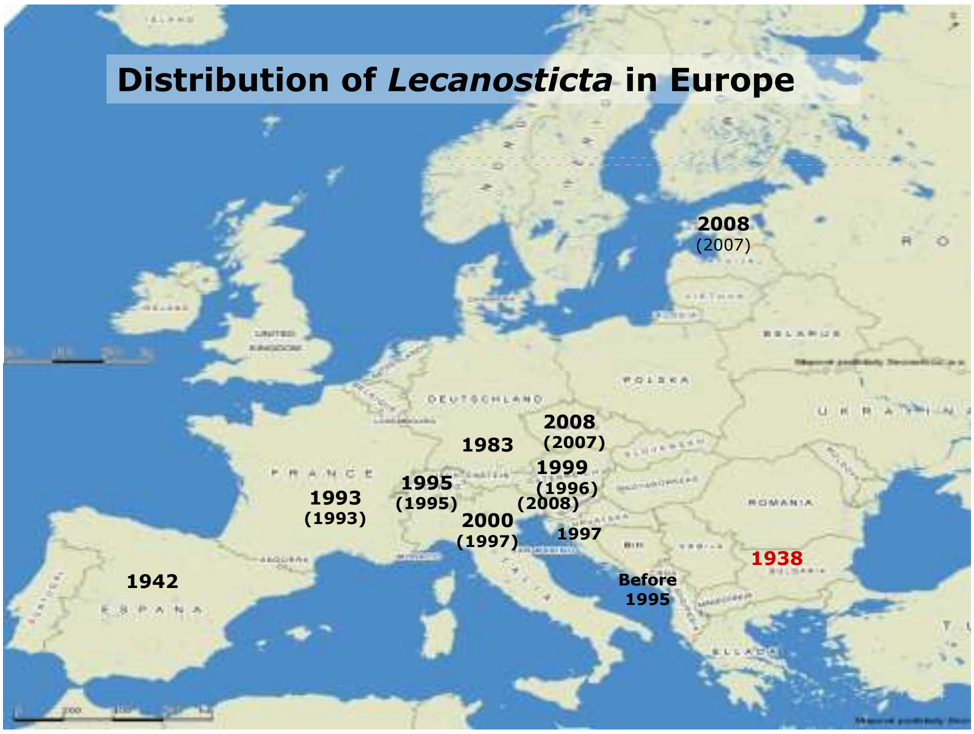

Figure 2. Distribution of *Lecanosticta acicola* in Europe. Years mean first report, in parentheses are years of findings, if differ from year of publishing.

Lecanosticta acicola coincides in both localities with causal agent of red band needle blight *Dothistroma septosporum* on Scots pine *Pinus sylvestris*, bog pine *Pinus rotundata* and their hybrid *P. × digenea.* Nevertheless, the threat of the disease spreading to Scots pines frequently planted in the region remains unclear yet. While bog pines inside the nature reserves display remarkable needle defoliation, Scots pines in surrounding managed stands are without visible symptoms of infection by *Lecanosticta acicola.*

CONLUSIONS

Dothistroma needle blight and brown spot needle blight are relatively quickly spreading needle casts in Central and also in Northern Europe. Within past 15 years, Dothistroma was reported from many new areas. Spreading of these diseases should be considered as result of climatic extremes and also one of exhibitions of climatic changes (eg. Woods et al. 2005), although reasons of this spreading are still not sure. We cannot exclude also some other factors as trade with plant material. Reasons of occurrence of disease of strictly protected areas without any human interventions fir many decades are not sure. Control of these needlecasts is problematic due distribution in large infested areas. Dothistroma needle blight is established in most areas across Europe now and seems to be problem in Central and North Europe now due very quick spreading and adaptation for climatic and natural conditions in new areas.

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VIRGIN FORESTS ON THE BALKANS

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Summary: *Due to the mountainous character of Balkan Peninsula and expression of specific climate areas it presents the largest forest biodiversity of the Old Continent with valuable genetic resources. The relatively low affect of the Ice Age on the Balkans remained them as one of the refugia for European forest tree species. Ancient Greek, as well as Roman, Byzantine and Ottoman Empire civilizations influenced significantly Balkan nature, in particular the most important component – forests. Cattle-breeding, farming and forest fires, on one hand, and ore melting and shipbuilding, on the other, contributed to significant decrease of forest land. Felling of forests affected not only the river banks of Danube, Sava, Morava, Matitsa and Vardar but Dinar Mountains, Pind, Thracian-Macedonian Massive, Balkan Range and other smaller mountains. Particularly intensive was the deforestation in XVIII and XIX century, when the areas for pasture and agriculture increased drastically and large autochthonous forests were destroyed, and virgin forests were significantly fragmentased. Broaden investigations on virgin forests in Romania and Bulgaria were initialized by Royal Dutch Society of Nature Protection few years ago but entire picture of their inventory is still uncompleted. This requires international cooperation of investigations on the virgin forests fragments on the Balkans, which status is an indicator for influence of the expected climate changes on forest populations.*
Key words: *virgin forests, biodiversity, refugia*

Nowadays the virgin forests in Europe are mainly in the Nordic areas of Russia, in Scandinavia, in the Alps, Carpathians and Balkans. Out of a total 38 countries of the Old continent, for which there is information, only in 12 the undisturbed by man forests exceed 1% from the area (TBFRA, 2000). Among them are Sweden, Finland, European Russia, Poland, Slovakia, Romania, as well as some Balkan countries. The high mountains of the Balkans as Rila, Pirin, Olympus, Pind, Shar mountain, Dinar Mountains, Durmitor, Balkan range, the Rhodopes, etc. form diverse climate: from moderate continental in north to Mediterranean in south, with transitional climatic zones between them. This combination of horizontal with vertical zonality determines high diversity of the vegetation: from middle-european to sub-tropic, from evergreen forests to sub-arctic in the high mountains, from steppe formations in Dobrudzha to hydrophytes formations along the rivers and water basins. During the glaciation the Balkans turned into one of the refugia of the European forest tree species, which after the Ice age started migration and restored the forest vegetation in Central and North Europe.

The civilizations of Ancient Greece, of Roman, Byzantine and Ottoman Empire influenced negatively on the forests in South-East Europe. The forest areas are reduced as a result of extensive agriculture and cattle breeding and as a sequence by the development of metallurgy and ship building. Forest felling was realized not only along Danube, Sava, Drava, Vardar, Maritsa rivers, etc. but also in the mountains as Dinars, Pind, Thracian-Macedonian Massive, Balkan Range, Strandzha, etc. this leaded to significant decrease of the autochthonous forests and fragmentation of the virgin forests.

There are reliable historical data about presence of venerable forests in the Balkan countries since the XIV century. The fires and the fellings of forests for pasture and agriculture, for wood and charcoal decreases the forest cover and the area of virgin forests. The fast development of industry, agriculture, transport and trade during the second half of XIX century and beginning of XX demand increased request of wood and forced usage of forests. The Balkan war (1912-1913), the Interallied war (1913) and the First World War (1914-1918) leaded to economical crisis, out-of-regulations usage of forests and significant extend of forest fires. The Second World War (1941-1945), along with the economical disaster, provoked significant changes in the forest ownership, intensive fellings and usage of inaccessible forest basins. This leaded to reduction of autochthonous and venerable virgin forests.

After the year 1990 most of the Balkan countries belonged to the counties in transition from centralized to market economy. During the change of the ownership corruption practices appeared which reflected negatively on the forest status, including the virgin ones.

The term ‘virgin forest’ is near to the term ‘natural forest’, ‘native forest’, ‘ancient or old forest’ and ‘primary forest’ (Peterken, 1997). The common between these names is that they are related to forests undisturbed by man.

The virgin forest is characterized by: complex spatial structure of local species with wide age range and presence of dead and decaying trees. According some experts from the Royal Dutch Society for Nature Protection: ‘virgin forest is a natural woodland where trees and shrub species are in various stages of their life cycle (seedlings, young growth, advanced growth, naturally and old growth) and as dead wood (standing and laying) in various stages of decay, thus resulting in a more or less complex vertical and horizontal structure. This is the expression on continuous existence without limit of time’ (Rossnev et *al.*, 2002).

During the realization of the project ‘Inventory and Strategy for Sustainable Management and Protection of Virgin Forests’ in Bulgaria were accepted the following criteria for selection of such forests (Rossnev et *al.*, 2002):

- natural origin of the tree, shrub and grass vegetation;
- lack of human activity or weak traces of such since 50 years;
- presence of trees of different age class, including old individuals the maximum age being 100-120.
- presence of typical endemic vegetation;
- good health status of the forest;
- presence of local mammals and birds;
- well distinguished natural boarders such as ridges, rivers, ravines, etc.
- presence of buffer zones of 50-100 m, especially in the lower and middle forest vegetation zone;
- minimum area for coniferous virgin forest should be 20 ha, for deciduous – 30 ha and for mixed – 25 ha.

These criteria could have mostly regional importance for mountain terrains while for the plains they should be précised.

One of the first inventories of natural forests in Europe was realized in Austria (Grabheer, 1997), applying 11 criteria among which more significant are forest tree and grass composition, presence of determined quantity of dead wood, intensity of usage, etc.

It should be noted that the term ‘virgin forest’ in Europe is with slightly lowered criteria than those in other continents and countries where the human influence on the nature and forests was more insignificant. The area of the true virgin forests in Europe is less than the officially registered but in reality few countries did scientifically based inventory of such forests. The mentioned 4% undisturbed by man forests in Europe (UN, 2000) and nearly to them results by Bucking et al. (2000) are probably a bit exaggerated due to more liberal methodical approach and lack of common criteria with those from other investigators. Some authors tend to ally the forests in protected territories to virgin ones which is methodically unsustainable as up to now they do not have the necessary characteristics.

The virgin forests on the Balkans are divided by area absolutely irregularly, the highest percentage (8.56) reached in Albania, where they cover 84 800 ha (PHARE, 1999). Bulgaria occupies second place with 2.91% which correspond to 103 354 ha (Veen et *al.*, 2006). According to some approximate but non-official data Croatia is on the third place with about 1.5%, followed by Macedonia (FYROM) – 1.16% on the base of 10 500 ha and Serbia, also non-official data, with approximately 1%. At a descending rate are ordered 3 countries with relatively few virgin forests: Slovenia – 3500 ha (0.32%), Bosnia and Herzegovina – 2200 ha (0.1%), (PHARE, 1999) and Greece – approximately 1500 (0.04%). On the Balkan Peninsula which forest area is about 17 million ha the virgin forests are within the range of 1.5 – 2% - an indicator for well expressed anthropogenic influence in the forests, especially in Greece. This could be expected having in mind the goat breeding having been practiced in this country for many centuries and is an undisputable destroyer of forests.

The virgin forests of the Balkans are mainly belonged to the following biomes:

Deciduous forests with shedding leaves of the moderate zone, coniferous forests of the moderate zone end ever-green sclerophilic forests and shrubs (Udvardy, 1975; Nedialkov, 1998). From the sub-biomes among them of special scientific interest are the south-euxinic or kolhidic forests in Strandzha, where typical species are *Fagus orientalis* Lypsky, *Quercus polycarpa* Schur., with undergrowth from *Rhododendron ponticum* L., as well as flood plan forests – longoz on some river banks with participation of representatives of genus: *Fraxinus*, *Ulmus*, *Quercus*, *Populus*, *Salix*, etc. with a complex of lianas. Longoz forests are unique for Europe but they are conservated only in isolated areas, sometimes boarded in reserves.

Main forest tree species which form virgin forests in Bulgaria are mainly *Fagus silvatica* L., *Quercus petraea* Liebl., *Quercus robur* L., *Pinus mugo* Turra, *Picea abies* (L.) Karst., *Pinus silvestris* L., *Pinus nigra* Arn., *Pinus peuce* Grisb., *Pinus heldreichi* Christ., *Abies alba* Mill., etc.

Balkan virgin forests include many endemic, sub-endemic and relict species as: *Picea omorica* (Panc.) Purk., *Aesculus hippocastanum* L., *Pinus heldreichi* Christ., *Pinus peuce* Grisb., *Juniperus excelsa* M.B., *Ostrya carpinifolia* Scop., *Abies cephalonica* Lond., *Quercus hartwissiana* Stev., *Acer heldreichi* Orch., etc.

The location of virgin forests is mainly in the mountain parts where anthropogenic influence was more limited. The virgin forests on the Balkans are with well-expressed media formation functions – hydrological, soil protective, climate regulative, closely related to other ecological, social and economic functions. With the realized during the last two decades economic changes in most of the Balkan countries serious challenges appeared, even threats for the future of the virgin forests. The construction of high ways, especially in the mountains, of large tourist centers with numerous ski tracks and funiculars, of gas and petrol pipes, as well as other anthropogenic interactions are among them. Significant damages on the virgin forests during the last years did the forests fires. The conflagrations destroyed hundreds of hectares in the National Park Rila and National Park Pirin, as well as in same Nature parks. Renting specialized aircrafts from Russia and France to fight the forest fires in Bulgaria in 2008 resulted well but the losses of virgin forests are irreversible.

The problem with the forest fires becomes too actual about all Balkan countries with the expected climate changes and especially serious consequences are expressed in Greece. Other reason contributed to decreasing of virgin forests is the removing of the boarder equipment, including fences and other obstacles which conserved the natural forests.

Protection of Balkan virgin forests could be realized via respective specialized programmes at national and international level. Some of the necessary activities in this direction include:

- Précising of the access to closed forest areas which are significant resources for virgin but not protected forests;
- For part of the water supply zones, including virgin forests, is necessary to combine the hydrological with conservation aims in order to ensure their better protection;
- Some border forest massifs of Balkan countries is desirable to obtain a status of nature parks in which the present virgin forests to be differentiated in reserves;
- The monitoring of the virgin forests on Balkan Peninsula has to be implemented without delay.

All these activities require cooperation in research and management of virgin forests fragments which status is an indicator for influence of climate changes on forest populations as well as containers of valuable primary gene pool.

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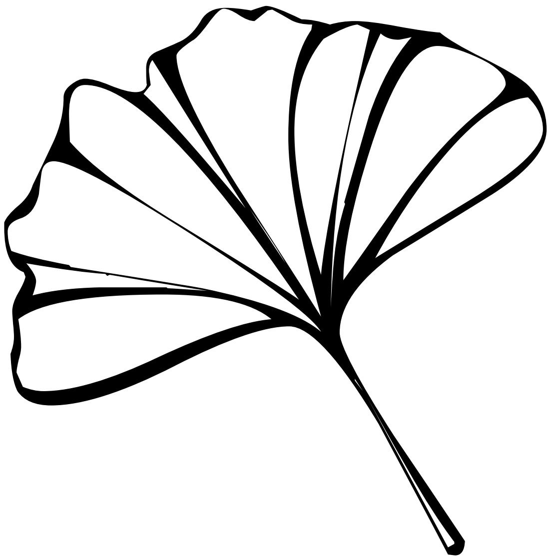

AN INNOVATIVE APPROACH FOR LARGE-SCALE PRODUCTION OF *Robinia pseudoacacia*, *Cupressus sempervirens*, *Pinus brutia* AND *Pinus nigra* PLANTING STOCK MATERIAL

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Summary: *This study was conducted within the PRE-FOREST project (CRAFT - 6th Framework Programme). One of the main objectives of the project was to study the interaction among production technology, mini-plug container design and rooting media, in order to develop cultivation protocols for forest planting stock material of special economic and ecological importance for each participating country. Initially, basic technological, economical and biological studies were conducted. The results of these studies are currently being applied for the production of forest planting stock in an innovative prototype unit. This unit is based on large-scale seedling production adapted to pre-cultivation in mini-plugs and is independent of environmental conditions. Objective of our work was to identify the suitable growing conditions for the production of high quality forest planting stock of black locust (Robinia pseudoacacia L.), Italian cypress (Cupressus sempervirens L.), brutia pine (Pinus brutia Ten.) and black pine (Pinus nigra Arn.). Seeds of the above species were sown in mini-plug plastic trays filled with either enriched peat or stabilized peat at four densities: 3500/m2 (volume 3 cc), 1820/m2 (9 cc), 1460/m2 (13 cc) and 975/m2 (18 cc). The seedlings were cultivated under controlled conditions in growth chambers. At the end of the cultivation period, seedling survival, root and shoot growth were measured, while physiological (shoot electrolyte leakage) and performance tests (root growth potential) were applied. The optimum cultivation period was found to be five weeks for Italian cypress while for the rest species a four week one. The stabilized medium proved to be the best substrate for the cultivation of all species tested. In Italian cypress, enriched peat also resulted in good quality seedlings, and is therefore recommended as a more cost efficient material. Black locust and brutia pine seemed to perform better when grown in intermediate densities (1460-1820 mini-plugs/m2). For Italian cypress the use of the lowest densities (975-1460 mini-plugs/m2) is suggested. Black pine was found to produce higher quality seedlings in densities less than 2000 mini-plugs/m2.*
Keywords: *mini-plugs, density, growing medium, root growth potential*

INTRODUCTION

Containerized transplants grown in mini-plugs are a new establishment system with potential for reducing post-planting stress. In the ornamental and vegetable industry, plants have been grown in small plug containers for many years; however, this practice is relatively new for forest trees and other indigenous plants. The published literature is also rather sparse; only a few articles have been published about mini-plugs in forest and conservation nurseries (Riley & Steinfield, 2005). Small cell size offers the nursery the advantage of more plants in the greenhouse space and, consequently, lower cost compared to larger cell volumes (Vavrina, 1995). Mini-plugs are very small container plants grown in predominately stabilized medium in containers less than 33 cm^3 in volume, produced in a variety of shapes, volumes and densities. In contrast to standard container seedling culture, mini-plug seedlings are grown with the objective of producing a fully extractable seedling with a dimensionally stable root plug that will tolerate transplanting within a relatively short growing period (3 to 4 months) (Landis, 1990). Mini-plugs are used in two distinct stocktypes: container-to-bareroot transplants and container-to-container transplants (Riley & Steinfield, 2005). The advantages of mini-plugs are the almost 100% yield, resulting in few culls, the highest plant density per production area, the maximum use efficiency of seeds or cuttings, the shorter crop rotation and the improved stock quality, as plants with large stem diameter and fibrous root systems are produced (Landis, 2007).

In the Forest Research Institute (NAGREF) in Greece basic technological, economical and biological studies were conducted, concerning some forest species. Objective of our work was to identify the suitable growing conditions for the production of high quality forest planting stock of special interest for Greece, such as black locust, Italian cypress, brutia pine and black pine. The produced cultivation protocols could then be applied for the production of forest planting stock material in the prototype unit that was developed under the frame of PRE-FOREST project (Radoglou *et al.*, 2009).

MATERIALS AND METHODS

Experiments were carried out at the Forest Research Institute (NAGREF) in Loutra Thermis, about 20 Km NE of the city of Thessaloniki, Central Macedonia, Greece (40° 35' N and 22° 58' E). Seeds of black locust (*Robinia pseudoacacia* L.) were collected in 2006 from a small seed orchard, which was established near Thessaloniki (40° 46' 09'' N, 22° 21' 03'' E, altitude 10 m a.s.l.). Orchard seedlings were raised by seeds, provided by the Forest Research Institute of Budapest, from three different seed crop stands of Hungary. Brutia (*Pinus brutia* Ten.) and black pine (*Pinus nigra* Arnold) seeds were provided by the Directorate of Reforestation & Watershed Management (Section of Forest Nurseries & Seed Production) (year of collection: 2004 and 2006 respectively). Italian cypress (*Cupressus sempervirens* L.) seeds were provided by the Forest Service of Cyprus (year of collection: 2006). Before sowing seed germination capacity was determined for each species (black locust: 86%, brutia pine: 75%, black pine: 78%, Italian cypress: 40%) (Spyroglou *et al.*, 2009).

Seeds were sown from March to June 2007 in mini-plug plastic trays designed for pre-cultivation of horticulture crops (QuickPot®, Herkuplast-Kubern GmbH, Ering, Germany) at four densities: 3500 mini-plugs/m^2 (volume 3 cm^3, depth 30 mm), 1820 mini-plugs/m^2 (9 cm^3, depth 40 mm), 1460 mini-plugs/m^2 (13 cm^3, depth 37 mm) and 975 mini-plugs/m^2 (18 cm^3, depth 37 mm). The dimensions of all trays were 310 mm x 530 mm. Two growing media were used: enriched peat (Klassmann TS1, Klassmann-Deilmann GmbH, Geeste, Germany) and stabilized medium (Preforma PP01, Jiffy Products International AS, Stange, Norway). Trays filled with enriched peat were fully hydrated prior to sowing. The trays with the stabilized medium were hydrated from the manufacturer. Only one seed was placed in each mini-plug. The depth of sowing was not more than three times the seed's diameter (Hartman & Kester, 1983). Five replications of 40 seedlings per mini-plug density and growing medium were employed.

After sowing, mini-plug trays were placed in environmentally controlled growth chambers of 400 lt (KB8000FL, Termaks AS, Bergen, Norway). The growing conditions were 14h photoperiod, 250 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD), 80±10% relative humidity (RH) and 20/15°C day/night temperature. Watering was applied every second day. After irrigation, trays were rotated (inwards-outwards, left-right) to ensure uniform conditions.

At the end of the cultivation period in the chambers (4-5 weeks) 15 pre-cultivated seedlings per species, density and growing medium were randomly selected. The growth parameters measured were: and the following growth parameters were measured: root length (cm), shoot height (cm), leaf area (including the shoot) (cm^2), root dry weight (mg) and shoot dry weight (mg). Shoot height and root length were measured as the distance from the root collar to the upper and lower end respectively of a seedling. Leaf area was measured using an area meter (AM100, ADC Bioscientific Ltd., Herts, UK). Root and shoot dry weight were assessed after oven drying at 70°C for 48h. The root: shoot ratio was calculated on a dry weight basis. Seedling vitality was assessed by the end of the cultivation period in the chambers by applying the Shoot Electrolyte Leakage (SEL, %) test using five shoot samples per treatment (McKay, 1992; Radoglou & Raftoyannis, 2002).

At the end of the cultivation period in the chambers 16 seedlings per species, mini-plug density and growing media were randomly selected and transplanted into mini-plug containers of each density. The mini-plug containers were placed into stainless boxes (35 x 26 x 8 cm) filled with equal volumes of peat (Klassmann Base Substrate 250I, Klassmann-Delmann GmbH, Geeste, Germany) and sand. The trays were immersed in a stainless water bath, according to the standardized Root Growth Potential (RGP) technique described by Mattsson (1986), in a completely randomized experimental design with four replications of four plants. During the 21 day test period, water and air temperature were maintained at 21 ± 2°C, relative humidity at 40% ± 10% and the photoperiod was set to 14 hours, with a PPFD at plant level of 300 μmol $m^{-2}s^{-1}$. Seedlings were watered every second day in order to keep the growing medium at the point of runoff. Excess water was sucked out from each RGP box one hour after watering. No fertilizer was added during the test period. On the 21st day, all roots that had developed outside the mini-plug containers were washed in order to remove peat and sand, and cut off. RGP was assessed as the root dry weight (in mg) after oven drying at 70°C for 48 hours (Brønnum, 2005).

Seedlings of all species were transplanted to containers of 240 plants/m² (330 cm³), where they remained for one growing season. The second year the seedlings were transplanted to the field in a completely randomized block design and their field performance is being monitored.

Statistical analysis of the data was conducted using the univariate analysis procedure carried out with SPSS® statistical software v.15.0 (SPSS, 2006). Duncan's multiple range tests at 0.05 probability were employed in order to rank significant means.

RESULTS

Black locust

The optimum cultivation period of black locust seedlings in mini-plugs was found to be four weeks. After that period seedling survival was more or less stable (data not shown). The type of substrate and the interaction between substrate and density significantly affected seedling survival at the end of the cultivation period (Table 1). Seedlings grown in peat had lower survival (56%) compared to the ones grown in the stabilised medium (71%). SEL of all treatments had values well below 10%, indicating that no stress effect was imposed on seedlings during the cultivation period in the mini-plugs.

The substrate was found to affect significantly almost all morphological traits of black locust (Table 1). Plants grown in the stabilised medium had higher root length (6.32 cm), shoot height (8.76 cm), leaf area (8.98 cm²), root (6.77 mg) and shoot dry weight (19.19 mg) compared to seedlings grown in peat (4.61 cm, 7.86 cm, 6.79 cm², 4.38 mg and 13.97 mg respectively). On the contrary, root growth potential was higher in plants grown in peat (26.49 mg) in relation to the ones grown in the stabilised medium (19.95 mg).

Mini-plug density significantly affected only the root characteristics of black locust, namely root length, root dry weight and root growth potential (Table 1). The lowest density (975/m²) produced seedlings with the highest root length (6.17 cm), dry weight (6.77 mg) and root growth potential (28.39 mg), while the highest density (3500/m²) produced seedlings with the lowest values of these parameters (4.59 cm, 4.62 mg and 18.16 mg respectively). The intermediate densities (1460 and 1820/m²) had more or less similar values to those of the low density (975/m²).

Table 1. Univariate analysis of variance of the effect of susbstrate (S), density (D) and their interaction (SxD) on growth traits of black locust and Italian cypress. Data represent the F value. * P<0.05, ** P<0.01, *** P<0.001, ns: not significant

	Black locust			**Italian cypress**		
	S	**D**	**S x D**	**S**	**D**	**S x D**
Survival	75.21***	1.65ns	5.47**	0.22ns	0.40ns	1.12ns
Root length	43.98***	7.80***	3.22*	0.40ns	5.31**	0.21ns
Shoot height	9.05**	1.88ns	1.26ns	0.14ns	2.15ns	1.02ns
Leaf area	14.83***	0.33ns	0.97ns	45.76***	3.13*	0.64ns
Root dry weight	25.97***	2.91*	2.52ns	37.37***	3.34*	0.89ns
Shoot dry weight	22.11***	0.67ns	0.99ns	14.49***	2.62ns	0.97ns
Root:shoot ratio	0.02ns	1.72ns	1.21ns	2.64ns	0.78ns	0.38ns
SEL	13.71**	12.28** [1]	11.45** [1]	3.74ns	6.01**	3.09*
RGP	13.05***	6.75***	6.85***	2.97ns	10.79***	2.19ns

[1] two densities (3500 and 975 mini-plugs/m²) were tested

Italian cypress

In order to pre-cultivate cypress seedlings in mini-plugs a minimum period of five weeks is needed. This is due to the fact that seedling emergence from the seed takes place later compared to the other species tested. No effect of substrate or density on seedling survival five weeks after sowing was observed (Table 1); the values of survival in all treatments ranged between 90.3 and 93.6%. SEL values in all treatments were below 5%.

The type of substrate exerted a significant effect on leaf area and root and shoot dry weight (Table 1). Seedlings grown in peat had higher values of leaf area (1.96 cm^2), root (1.74 mg) and shoot dry weight (5.44 mg) compared to seedlings established in stabilised medium (1.36 cm^2, 1.13 mg and 4.15 mg respectively).

Mini-plug density significantly affected root length, leaf area, root dry weight and root growth potential (Table 1). The highest density (3500/m^2) showed the lowest values of root length, root dry weight and leaf area, while the other three densities formed a more or less uniform group of similar values. Interestingly, the highest density showed the highest root growth potential (4.81 mg), followed by the lowest density (3.58 mg) and the intermediate ones (2.53 and 2.48 mg for 1460 and 1820 mini-plugs/m^2 respectively).

Brutia pine

The optimum cultivation period for brutia pine was four weeks, as indicated by seedling survival. A significant effect of density and of the interaction between substrate and density on seedling survival four weeks after sowing was observed (Table 2). The highest survival was found in the intermediate densities 1820/m^2 (92%) and 1460/m^2 (84%), and the lowest in the low (75%) and the high density (73%). SEL values of less than 5 % were recorded in all treatments.

The type of substrate used influenced the shoot height, root: shoot ratio and root growth potential of brutia pine seedlings (Table 2). More specifically, shoot length was found to be lower in peat (6.68 cm) compared to the stabilised medium (7.45 cm). In addition, a higher biomass allocation in the roots of plants grown in peat was observed, as indicated by the higher root: shoot ratio found (0.26) in relation to the stabilised medium (0.23). Plants grown in the stabilised medium had almost double root growth potential (6.30 mg) compared to the ones established in peat (3.77 mg).

Density was found to affect root length, shoot height, leaf area and root growth potential of brutia pine seedlings (Table 2). The intermediate densities (1820 and 1460/m^2) showed significantly higher values of root length (5.68 cm and 5.33 cm respectively), shoot height (7.76 cm and 7.11 cm respectively) and leaf area (3.78 cm^2 and 3.64 cm^2 respectively). The lowest values were observed in the highest density 3500/m^2 (root length: 4.01 cm, shoot height: 6.44 cm and leaf area: 3.27 cm^2) and in the lowest density 975/m^2 (root length: 4.74 cm, shoot height: 6.95 cm and leaf area: 3.33 cm^2). On the other hand, the lowest and highest densities resulted in the highest root growth potential (7.61 mg and 6.22 mg respectively).

Table 2. Univariate analysis of variance of the effect of susbstrate (S), density (D) and their interaction (SxD) on growth traits of brutia and black pine. Data represent the F value. *P<0.05, ** P<0.01, *** P<0.001, ns: not significant

	Brutia pine			Black pine		
	S	**D**	**S x D**	**S**	**D**	**S x D**
Survival	0.77^{ns}	4.80**	10.44***	200.18***	2.27^{ns}	2.11^{ns}
Root length	0.00^{ns}	9.02***	4.20**	14.11***	9.54***	0.69^{ns}
Shoot height	27.71***	13.78***	1.52^{ns}	5.86*	3.09*	0.41^{ns}
Leaf area	1.31^{ns}	2.80*	1.16^{ns}	15.28***	6.53***	0.78^{ns}
Root dry weight	0.33^{ns}	2.02^{ns}	4.01**	1.75^{ns}	8.11***	0.62^{ns}
Shoot dry weight	2.82^{ns}	1.59^{ns}	1.16^{ns}	20.96***	3.84*	0.69^{ns}
Root:shoot ratio	4.68*	0.20^{ns}	0.59^{ns}	12.04**	4.67**	1.47^{ns}
SEL	0.09^{ns}	0.17^{ns}	1.17^{ns}	8.67**	3.61*	3.71*
RGP	17.99***	14.33***	5.26**	6.17*	7.96***	0.51^{ns}

Black pine

For cultivation of black pine seedlings in mini-plugs a period of 4 weeks is needed. Seedling survival at that period was found to be significantly affected by the type of substrate used (Table 2). The use of peat resulted in low seedling survival (55%); the use of the stabilised medium instead led to higher seedling survival (95%). Root length, shoot height, leaf area, shoot dry weight and root: shoot ratio differed in relation to the type of substrate used (Table 2). Shoot height (8.87 cm), leaf area (3.30 cm^2) and shoot dry weight (15.99 mg) were higher in peat, compared to the stabilised medium (8.45 cm, 2.97 cm^2 and 13.75 mg for shoot height, leaf area and shoot dry weight respectively). On the contrary, root length and root: shoot ratio were higher in the stabilised medium, having values of 3.84 cm and 0.14 (compared to 2.99 cm and 0.11 respectively in peat). The root growth potential was also significantly higher in the stabilised medium (9.52 mg) in relation to the value of the same variable in peat (7.21 mg). SEL values were found to be less than 3%, indicating unstressed plants.

Seedling density exerted a significant effect in all variables measured (Table 2). The lowest values were observed in the highest density (3500/m^2) for root length (2.41 cm), shoot height (8.21 cm), leaf area (2.83 cm^2), root dry weight (1.37 mg), shoot dry weight (13.68 mg), as well as, for root: shoot ratio (0.10). The other three densities (975-1820/m^2) formed a more or less solid group with similar values in all morphological parameters tested. Root growth potential showed the highest values in the low density 975/m^2 (10.93 mg); however, also the high density resulted in similar values of root growth potential (9.67 mg). The lowest root growth potential was observed in the density of 1820 mini-plugs/m^2.

DISCUSSION

Subjecting young plants to ideal growing conditions during germination and early growth gives the seedlings momentum that carries forward in the crop cycle. The first few weeks in the life cycle of the plant are the most critical. Establishing strong top growth and strong root development is critical for transplanting success (Pelton, 2003). Mini-plug transplants have been found to perform as well as or in some cases even better than other conventional stock types in a number of papers (Tanaka *et al.*, 1988; Scarratt, 1989; Généré 1998). Our results show that the species tested were able to produce a firm root plug after only four to five weeks of precultivation in mini-plugs under optimal conditions. After that period seedlings are ready to be transplanted to bigger containers or to nursery beds. Seedling survival of all species in most cases was relatively high and was analogous to the seed germination capacities determined for the seedlots used (Spyroglou *et al.*, 2008). In addition, shoot electrolyte leakage (SEL) values indicated healthy, unstressed seedlings. Shoot electrolyte leakage (SEL) is a physiological index that has been correlated with field performance in many cases (Maki & Colombo, 2001; Radoglou & Raftoyannis, 2001; Percival, 2004; Brønnum, 2005).

Substrate was found to play an important role in seedling survival and morphological traits of all species tested. The physical and chemical properties of the substrate (e.g. porosity, water retention etc.) are considered to have an important influence in seedling root development during nursery phase; in some cases this influence can be maintained during the early period after plantation (Costa *et al.*, 2004). Most forest and conservation nurseries that produce mini-plug seedlings use stabilized growing media - defined as any growing medium that holds the root system together when removed from the container, regardless of root development. Stabilized media allow mini-plugs to be extracted from their containers before a firm root plug has formed. This allows mini-plugs to be transplanted weeks before the seedling root system would have formed a firm plug and is one of the system's primary advantages. Furthermore, roots in stabilized plugs do not develop the typical deformities that often lead to structural defects in the transplants (Riley & Steinfeld, 2005). Our results showed that the use of the stabilized medium clearly favored survival and growth of black locust and survival and root characteristics of black pine.

Root growth potential (RGP) is considered to be a physiological indicator of stock quality because it is correlated with seedling stress resistance and is related to increased survival under moisture stress conditions (Simpson & Ritchie, 1996). RGP can be described as the capacity of a seedling to increase the size of its root system at a certain time and in a controlled test environment through the elongation of roots already present and/or the initiation of new roots and their elongation (Mattsson, 1986). A certain difficulty was faced in measuring RGP of black locust seedlings since this species develops a very aggressive root system, hard to be separated from the growing medium. Root growth potential of black locust was higher when peat was used, while RGP of the pine species was higher when the seedlings were established in the stabilized medium. This result could be attributed to the different properties between the two substrates (electrical conductivity, pH, porosity etc.). On the contrary, cypress seedlings performed well in the peat substrate as well. In this case, it is recommended to use this type of substrate for this species due to its lower price in the market, especially in combination to an intermediate or high density where the plug is small, more uniform and easier to transplant.

Density seemed to play an important role in black locust seedling production. It is clear from our results that as density decreased, root characteristics, as expressed by root length, root dry weight and root growth potential, were improving. When higher densities are used there is higher seedling competition for light, which results in fewer carbohydrates available for root elongation and growth (Barnett, 1980). In cypress, although the highest density resulted in shorter and lighter roots, when the seedlings of this density were transplanted to the RGP test facilities high values of root growth potential were observed. It is possible that the small size and low depth (30 mm) of the cavities of this container type favor the development of new roots, since roots reach faster the cavity walls and the cavity bottom. At the drainage hole roots are air pruned, resulting in the formation of secondary roots at the top of the plug (Ortega *et al.*, 2006). The formation of new roots occurs primarily on lateral roots; seedlings, therefore, with more lateral roots will have more sites available for new root growth (South *et al.*, 1990). The same was also observed in the two pine species.

These results lead to the assumption that density may not be the most important factor in this early stage of growth, especially for the conifer species that do not form a very dense aboveground biomass, limiting seedling competition for light. Thus, the differences among densities observed in the conifer species tested in the present study may be a result of a rooting volume effect and not of a density effect. It has been previously shown that the effect of density depends on the length of the growing season (Landis, 1990); as longer seedling culture periods are used, density becomes more important (Barnett, 1980). Thus, in this early stage, light competition and, consequently, density effect, might be minimal and rooting volume could be the most crucial factor for new root development.

CONCLUSIONS

One of the main benefits of the new technology developed by PRE-FOREST project is the possibility of environmental friendly production of a large number of seedlings per square meter, leading to a cost efficient result. For black locust, brutia and black pine four weeks is the optimum cultivation period in mini-plugs,, while for Italian cypress a five week one. The stabilized medium proved to be the best substrate for the cultivation of all species tested. In Italian cypress enriched peat also resulted in good quality seedlings, and is therefore recommended as a more cost efficient material. Black locust and brutia pine seemed to perform better when grown in intermediate densities (1460-1820 mini-plugs/m^2). For Italian cypress the use of the lowest densities (975-1460 mini-plugs/m^2) is suggested. Black pine was found to produce higher quality seedlings in densities less than 2000 mini-plugs/m^2.

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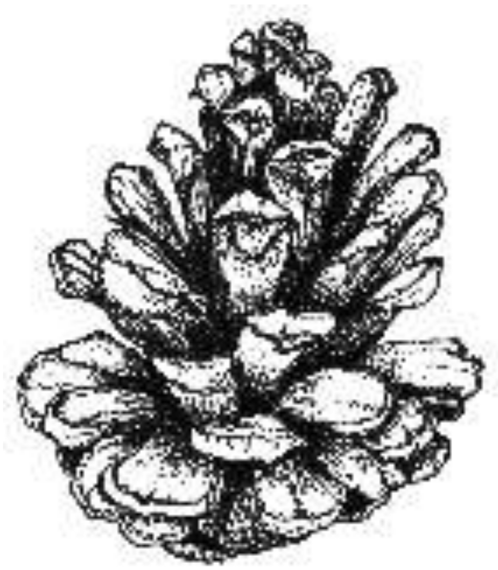

SCIENTIFIC STUDIES IN SHELTER BELTS FORESTRY IN BULGARIA

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Abstract: *First forest shelter belts in Bulgaria were established in the 1930s as artificial forest ecosystems, constructed by several tree and shrub species. Their positive influence is expressed through decreasing of the power of predominating winds, more even distribution of snowfalls and soil moisture, which brings to increasing of agricultural crops and to formation of habitats and refugia for wild animals and new flora and fauna. The present work makes short analysis of conclusions, recommendations and aims, connected with establishment of forest shelter belts in Bulgaria and some other European countries. Their present status is assessed and trends for future scientific studies and decisions are given in the light of framework conventions for environmental improvement.*
Key words: *forest shelter belts, biodiversity, current structure of tree stands, efficiency of forest shelter belt.*

INTRODUCTION

Studies on the influence of forest shelter belts on microclimate and agricultural sciences have begun as early as with the formation of field-protecting forestry. In 1873, Lomikovski published results in Russia, which confirm the favourable influence of trees planted around agricultural lands. Shatilov (1893) drew the conclusion that forest shelter belts should not be too wide and dense.

First forest shelter belts in Bulgaria have been established in 1928 near the town of Knezha (North-West Bulgaria) and later (1937) in Southern Dobrudzha, by Bulgarian and Romanian foresters (Marinov et *al.*, 2003).

In Bulgaria, Zahariev (1948), in his article "Forest vegetation and drought control" recommends to carry out studies on the influence of microclimate on agricultural crops in forest shelter belts near the village Karvuna. Later on, Prof. Nedyalkov (1955) studied the growth of main tree species in these belts. G. Georgiev & Y. Georgieva (1956) indicated the absence of oaks as main disadvantage of these belts, and the Canadian poplar and black locust – as not suitable to be used as main tree species.

Ganchev & Stefanov (1953) wrote in their article "Method for fast establishment of forest shelter belts" that "… *Gleditsia triacanthos* L. and the Przewalski poplar, as fast-growing and drought-resistant species, could be used as additional and even as main tree species". Serafimov (1960) had similar point of view, concerning the poplar of Przewalski. Based on his own observations, he also recommended common oak as drought-resistant species.

In 1960 Georgiev drew the conclusion that climatic and soil conditions in Dobrudzha are favourable for growing of oaks, ashes, *Gleditsia triacanthos* L., walnut tree, *Ulmus pumila* L., black locust and red oak as main tree species, and as accompanying species – *Tilia tomentosa* Moench, *Acer campestre* L. and *A. platanoides* L.

In 1951, experimental field on agromeliorations was established on the land of Kavarna village. Czechoslovak-Bulgarian expedition began research activities on wind-protective and snow-retentive function of shelter belts and their influence on microclimate of neighbouring fields. Results of these studies were published in numerous articles of Dimitrov (1972), Kukularov et *al.* (1972), Peev (1989).

Since 1980 forest shelter belts became exclusive state ownership and were transferred to the state forest fund. They are managed on the basis and recommendations given by forest management plans. Both regional and national meetings have been organised on status and problems of forest shelter belts in Dobrudzha (2002, 2006) with participation of researchers and specialists from practice.

Actuality of the scientific problem in Europe

Natural conditions are sophisticated complex of limiting factors, that's why different forestry-meliorating regimes are accepted in various European countries.

In Denmark, forest shelter belts are established to protect lands from north-western sea winds, in Germany – for erosion control of soils. Similar experiments have been carried out in Sweden and Belgium.

In England it is also relied on forest shelter belts with aerated construction and triangular vertical section.

In Italy and Spain forest shelter belts are used in private farms not only for improvement of growing conditions but to provide timber for building needs, as well.

Poland, the Czech Republic and Hungary also have significant experience in shelter belts forestry with basic aim protection of crops from dry and other winds.

First afforestations in Romania and Serbia have been carried out to protect and reinforce sands along the river Danube current and date back as early as in the middle of the 19-th century.

State of forest shelter belts in Bulgaria

During the inventory, carried out in 2006 (Marinov & Genova, 2006), it was established that:

- Forest shelter belts have been established unevenly on the territory of North Bulgaria. In its western and central parts belts are situated singly, have disturbed structure and are not connected in a shelter belt system;
- In the eastern part of the region (Silistra and Dobrich districts) forest shelter belts cover the whole territory (about 8000 ha) and are entirely state-owned (Simeonov et *al.*, 2007);
- Forest shelter belts with main tree species *Quercus cerris* L. or *Gleditsia triacanthos* L. are in best status. *Quercus cerris* L. shows good growth in relatively more dry and severe climate of Dobrudzha, where other species suffer from frost and ice, and *Gleditsia triacanthos* L. is more resistant to climatic anomalies in central and western regions of North Bulgaria (Marinov et *al.*, 2002).

No tending activities have been carried out regularly in a big part of forest shelter belts. The main tree species is depressed and its participation in the composition is limited. The absence of thinnings has brought to formation of belts with non-aerated construction, i.e. their efficiency and protection have been decreased.

The non-carried out sylvicultural activities for the period until the end of the 1970s could be explained by the fact that forestry administration has not been the owner of forest shelter belts and has not maintained these belts.

Today, big part of shelter belts with *Robinia pseudoacacia* L. are too old, trees are with big diameter, as well as with worsened timber quality as result of pathogenic diseases.

The non-regulated fellings, grazing and climatic anomalies have considerable negative influence and contribute to the change of structure of tree stands and in forest shelter belts.

CONCLUSION

To meet obligations of the Republic of Bulgaria within the UN Framework Convention on Climate Change, the Concept of Biological Diversity and the Convention for Combat Desertification, for improvement of forest shelter belt network in North Bulgaria and increasing its efficiency, the following recommendations could be outlined:

- To continue restoration and completion of forest shelter belt network in western and central part of North Bulgaria, which has been destroyed for one reason or another.
- During future afforestations, 5-row forest shelter belts with a width 10-11 m should be established, with main tree species in three single rows, separated by two rows of accompanying species.
- Regular maintenance activities should be carried out to keep the optimal aeration structure and stability of forest shelter belts.
- Scientific studies should be directed to the establishment of contemporary structure of tree stand, biometric parameters and characteristics of tree crowns, their spatial position and hierarchy, with the aim optimisation of parameters of each single tree in the stand for better ecological effect on agricultural lands and quality of production.
- Most suitable main tree species for forest shelter belts in North Bulgaria are oaks (*Quercus robur* L., *Q. cerris* L., *Q. frainetto* Ten., Q. *rubra* L.) and *Gleditsia*

triacanthos L., while *Tilia tomentosa* Moench., *Acer campestre* L., *Acer tataricum* L. are good to be used as accompanying speces.

These are part of the problems, which make the question about establishment, growing and management of forest shelter belts actual, controversial and demanding complete scientific observation as soon as possible.

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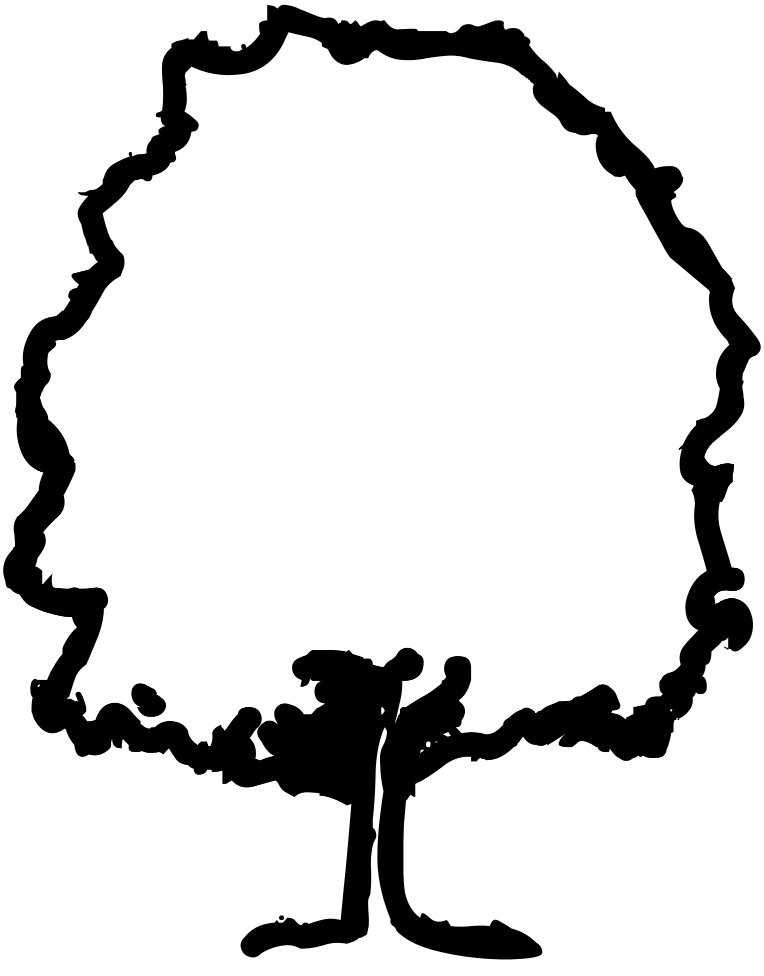

GENTIC VARIATION OF *Quercus robur* (L.) PROVENANCES IN MULTISITE OAK PROVENANCE TRIALS

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Summary: *This paper is referring to the researches performed in four pedunculate oak provenance trials established in 1977, where there are tested 8 Romanian oak provenances. There were made observations and measurements for many traits, and the results are presented for total height (m), average volume/ tree (dm*^{3}*), trunk form (index) and survival (%) at 29 years after establishment of the plantation.*

The results of the analysis of variance suggest: highly significant differences ($p<0.001$*) among provenances for total height, trunk form and survival traits in all trials; for average volume/tree there were non significant differences among provenances in one trial, highly significant differences (*$p<0.01$ *and* $p< 0.001$*) in two trials and only significant differences (*$p< 0.05$*) in one trial. The traits variation was continuous, characteristic for the polygene-controlled quantitative characters. Examination of the phenotypic correlations between the analyzed traits, on one hand, and between traits and geographic parameters of the areas of origin, on the other hand, has revealed the existence of significant correlations of different intensities.*

In the four testing sites there were established the most valuable provenances from the silvo - economic point of view and the provenances best adapted to the site conditions. These provenances will be designated as "Tested sources" in the region of provenance where they were tested and six provenances were designated as the best provenances.

The ne-optimality of local provenances hypothesis has been confirmed, because in only one of the four provenance trials, the local provenance was the best.

Keywords: *Quercus robur***,** *provenance, genetic variance, quantitative and adaptive traits, phenotypic correlation*

INTRODUCTION

In Romania the genus *Quercus* is represented by 5-9 species depending on the taxonomic ranking of different authors, but only 5 species are easily distinguishable and widely accepted among botanists: *Quercus robur* L., *Quercus petraea* (Matt.) Liebl., *Q. pubescens* Willd., *Quercus frainetto* Ten., and *Quercus cerris* L. (Curtu, 2006).

The surface covered by *Quercus genus* is around 1 077 000 hectares (according to Romanian National Institute of Statistics, 2008) and the pedunculate oak represent about 2% from the total surface of Romanian forests. In the 20th century it has been registered a great decrease of the pedunculate oak stands surface, from 600 000 hectares in 1922 to 130 000 in 1974 (Giurgiu, 1974).

Pedunculate oak prefers nutrient-rich and wetter soils, which can be subjected to flooding for a short period of time (Stănescu et *al*, 1977).

As an expression of its intrapopulational variability, the oak is a highly polymorphic species, and so in its Romanian areal, there have been identified many varieties and forms determined by their crown, leaf or acorn characteristics (Dumitriu-Tătăranu, 1960); also, the varieties *tardiflora* and *praecox,* which are spread mostly in the western part of the country, have 2-3 weeks difference in vegetation starting, according to the vegetation starting decalogue (Stănescu, 1984).

In 1974-1982 period there was put in place a large breeding program for the main broadleaved species when more pedunculate oak series of trials were established, as it follows: 9 trials were established, of which 4 in 1976 (Dispositive 1) and 5 in 1977 (Dispositive 2) where 16 provenances are tested, and 3 trials established in 1982 where other 9 provenances are tested (Contescu et *al.*, 1993).

The research results were published as it follows: for the juvenile stage (in the nursery) by Contescu et *al.* (1983), and at the age of 10 and, respectively, 15 years after the establishment of the plantation by Smîntînă (1991 and 1995).

This paper presents the results obtained in four peduculate oak provenance trials established in the year 1977.

MATERIALS AND METHODS

The study material consists of 9 *Quercus robur* populations (provenances) (whose origin is presented in Table 1, which are tested in 4 comparative trials; their general description and icluding the site conditions are presented in the Table 2.

Table 1. *Quercus robur* provenances tested in provenance trials

Provenance Number	Provenance Localization, County - FOREST DISTRICT, Management unit, parcel	Latitude N	Longitude E	Altitude (m)	Year of the Seed collection	Region of provenance
6	Dr. Tr Severin- STREHAIA II, 89 d, 90 a	44°35’	23°12’	130	1974	C 270
9	Bucureşti – SNAGOV I, 8 a	44°35’	26°10’	100	1975	J 160
10	Tg. Mureş – REGHIN I, 47	46°47’	24°42’	450	1975	F 160
12	Satu Mare – LIVADA IV, 24, 25	47°56’	23°05’	130	1975	K 290
13	Tg. Jiu – TISMANA VI, 55, 56	44o45’	22°55’	250	1975	C 250
14	Târgovişte – GĂEŞTI IX, 67, 68 b	44°35’	25°25’	160	1975	J 170
15	Slobozia – SLOBOZIA VIII, 25	44°34’	27°22’	27	1975	H 28A
16	Timişoara – LUNCA TIMIŞULUI Pădurea Verde	45°46’	21°15’	91	1975	K 190

The geographic localization of the provenances and trials it is presented on the map of pedunculate oak regions of provenances elaborated by Pârnuță & Lorenț. (2007).

The experimental design used is balanced square grid: 3x3 with 2 replications. The observations and measurements of many traits were made, and the results are presented for total height (m), average volume/tree (dm^3), trunk form (index) and survival (%) evaluated at 29 years after the establishment of the plantation.

For investigating the interpopulational genetic variability of the studied traits, it has been used a simple analysis of variance with two tests of significance (F test and "multiple t" test). To reveal the phenotypic correlations between the studied traits and between them and the geographic gradients of populations' origin place, the simple correlation coefficients (Pearson) were calculated. The data obtained from field observation and measurements was processed and statistically interpreted using Microsoft Office Excel and SPSS softwares.

Table 2. Localization of the *Quercus robur* provenance trials

Trial ID	Administrative localization County - FOREST DISTRICT Management unit, parcel	Surface (ha)	Year of plantation	Geographical coordinates Latitude (N) Longitude(E) Altitude (m)	Region of provenance	Site conditions Altitudinal plant layer, relief, soil, slope, aspect.
PVqro76A01 PVqro77B01	Focşani- GUGEŞTI VII, 7 c	0,74	1976 1977	45°30' 27°07' 90	H280	Plain forest zone, Cambic Chernozem, plain terrain.
PVqro76A02 PVqro77B03	Iaşi – PODUL ILOAIEI VI, 18 b	1,00	1976 1977	47°10' 27°15' 240	G 160	Hilly, argillic soil, easy slope, partially sunny
PVqro77A01 PVqro77B02	Timişoara – LUNCA TIMIŞULUI I, 68 b	0,80	1976 1977	45°40' 21°29' 100	K 190	Plain forest zone, plain meadow, gleyed alluvial soil,
PVqro77A02 PVqro77B04	Satu Mare- SATU MARE I, 5 c	0,80	1976 1977	47°49' 22°52' 60	K 290	Plain forest zone, brown podzolic pseudogleyed soil, plain meadow

RESULTS

Interpopulational genetic variability

The results of the analysis of variance within the four trials are presented in table 3. The differences among provenances are from significant ($p<0.05$) to highly significant ($p<0.001$) for all studied traits in all trials, except Lunca Timişului trial where the differences are non significant for average volume/tree and survival traits.

Total height (m).

The analysis of variance for total height within the tested provenances in Gugeşti, Podul Iloaiei, Lunca Timişului and Satu Mare trials, at the age of 29 years after the establishment of the plantation, reveals significant differences between the provenances tested in Satu Mare trial and highly significant differences between the provenances tested in the other three trials. The multiple test "t" (figure 2) presents in the first variation class the provenances 14- Găeşti, 6-Strehaia, 9- Snagov and 12- Livada (in Gugeşti trial) and provenances 6- Strehaia, 12- Livada and 14- Găeşti (in Podul Iloaiei trial). On the last places we find the provenances 10- Reghin, and 13- Tismana (in Gugeşti trial) and 9-Snagov, 15- Slobozia and 16- Lunca Timişului (in Podul Iloaiei trial).

We can observe the different position of provenance 9- Snagov which situates in the first variation class in Gugeşti trial and in the last variation class in Podul Iloaiei trial, beside provenances 15- Slobozia (from South) and 16- Lunca Timişului (from West). These results allow establishing the correct groundwork for the transfer of different provenances on regions of provenances.

The total height variation for the provenances tested in Lunca Timişului and Satu Mare trials presents in the first variation class the provenances 14- Găeşti, 9- Snagov and 16 Lunca Timişului (in the first trial) and provenances 6- Strehaia, 16- Lunca Timişului and 14- Găeşti in Satu Mare trial. In these two trials, the provenance 10- Reghin situates on the last place together with 6- Strehaia (in Lunca Timişului trial) and provenances 15- Slobozia and 12- Livada (in Satu Mare trial).

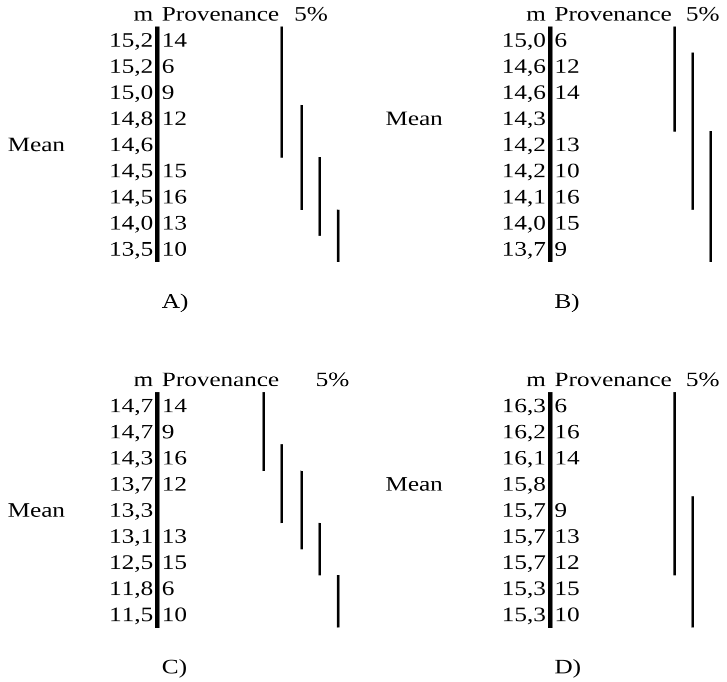

Figure 2. Variation of total height H(m) for the Oak provenances tested in the comparative trials A) Gugeşti, B) Podul Iloaiei, C) Lunca Timişului and D) Satu Mare -2006

Average volume/tree (dm³)

Within the tested provenances there are significant differences in Satu Mare trial, highly significant in Gugeşti trial, very significant in Podul Iloaiei trial and non significant in Lunca Timişului trial.

The biggest values for the variation of average volume/tree are registered in Satu Mare trial, where the leading provenances (Fig 3) are 16- Lunca Timişului, 12- Livada and 9- Snagov, provenance 6- Strehaia in Podul Iloaiei trial, and a 6- Strehaia and 9- Snagov in Gugeşti trial. The lowest average volume/tree was registered by provenances 10- Reghin (Gugeşti trial), 15- Slobozia and 9- Snagov (in Podul Iloaiei trial), 13- Tismana and 15- Slobozia in Satu Mare trial.

The value for average volume/tree of the provenance from the first place was 75% higher then the one of the provenance from the last place of the classification in Gugeşti trial, 81% higher in Podul Iloaiei trial, and 57 higher in Satu Mare trial.

Table 3. Analysis of variance for studied traits in the Oak provenances trials, at 29 years after establishment of the plantation

Variation source	D.F.	Total height			Average volume/tree			Trunk form			Survival		
		S.P.A.	s^2	F	S.P.A.	s^2	F	S.P.A.	s^2	F	S.P.A.	s^2	F
Gugeşti trial													
Provenances	7	52,893	7,556	12,932***	223447,177	31921,025	2,813**	11,005	1,572	2,726*	1148,650	164,093	23,825***
Replications	1	2,485	1,485		383,681	383,681		0,048	0,048		125,195	125,195	
Error	144	84,138	0,584		1633961,456	11346,955		83,057	0,577		275,497	6,887	
Total		139,516			1857792,314			94,110			1549,342		
Podul Iloaiei trial													
Provenances	7	23,829	3,404	4,569***	235335,437	33619,348	4,237***	28,749	4,107	10,337***	1491,992	213,142	13,096***
Replications	1	2,012	2,012		618,966	618,966		0,436	0,436		0,302	0,302	
Error	144	107,281	0,745		1142599,699	7934,72		57,213	0,397		621,03	16,276	
Total		133,122						86,398			2113,324		
Lunca Timişului trial													
Provenances	7	222,765	31,824	31,642***	117570,456	16795,779	1,96	8,196	1,171	4,257***	298,875	42,696	1,809
Replications	1	13,551	13,551		7457,24	7457,24		0,093	0,093		34,935	34,935	
Error	144	144,826	1,006		1234126,228	8570,321		39,437	0,274		944,041	23,601	
Total		381,142			1359153,924			47,726			1277,851		
Satu Mare trial													
Provenances	7	16,145	2,306	2,632*	418531,58	59790,226	2,325*	12,869	1,838	4,155***	620,724	88,675	6,722***
Replications	1	4,935	4,935		102367,861	102367,86		0,085	0,085		60,48	60,48	
Error	112	98,165	0,876		2880097,551	25715,157		49,562	0,443		527,668	13,192	
Total		119,245			3400996,992			62,516			1208,872		

	dm^3	Provenance
	265,42	6
	262,01	9
	237,82	12
	214,29	14
Mean	212,84	
	199,38	15
	186,98	16
	185,18	13
	151,61	10

A)

	dm^3	Provenance
	290,82	6
	215,90	16
	199,65	14
Mean	199,07	
	192,48	10
	188,04	12
	174,65	13
	170,59	9
	160,43	15

B)

	dm^3	Provenance
	423,60	16
	396,74	12
	370,08	9
Mean	325,90	
	296,76	10
	289,69	6
	289,43	14
	270,71	15
	270,18	13

C)

Figure 3. Variation of average volume/tree (dm3) for the Oak provenances tested in the comparative trials A) Gugeti, B) Podul Iloaiei and C) Satu Mare 2006

Trunk form (index).

The analysis of variance shows significant differences between the provenances tested in Gugeşti trial and very significant within the other three trials.

The provenances with superior quality for the trunk form were 14- Găeşti, 12- Livada and 13- Tismana in Lunca Timişului and Satu Mare trials. The provenance 10- Reghin was in all four trials in the variation class of the indexes which express a low quality of the trunk form, together with provenance 16- Lunca Timişului (in Gugeşti and Satu Mare trials), with 9- Snagov (in podul Iloaiei trial) and with 6- Strehaia (in Lunca Timişului trial) – Fig. 4.

	Index	Provenance
	2,85	16
	2,65	10
	2,40	9
	2,40	12
	2,40	13
Mean	2,38	
	2,20	6
	2,10	15
	2,00	14

A)

	Index	Provenance
	2,63	9
	2,33	10
	2,26	16
Mean	1,91	
	1,79	13
	1,75	12
	1,65	14
	1,58	15
	1,27	6

B)

	Index	Provenance
	2,43	10
	2,43	6
	2,41	16
	2,28	9
	2,22	13
Mean	2,19	
	1,98	15
	1,95	12
	1,82	14

C)

	Index	Provenance
	2,25	10
	2,17	16
	2,10	9
	1,85	6
Mean	1,81	
	1,75	15
	1,51	14
	1,47	13
	1,38	12

D)

Figure 4. Variation of trunk form (index) for the Oak provenances tested in the comparative trials A) Gugeşti, B) Podul Iloaiei, C) Lunca Timişului and D) Satu Mare -2006

Survival (%)

At the age of 29 years after the establishment of the plantation, the analysis of variance for this trait reveals very significant differences between the provenances tested in 3 of 4 trials, by these three meaning Gugeşti, Podul Iloaiei şi Satu Mare, and non significant differences in Lunca Timişului trial.

The provenances 6- Lunca Timişului (in Gugeşti trial), 12- Livada and 14- Găeşti (in Podul Iloaiei trial) and 13- Tismana (in Satu Mare trial) have proven to be the provenances best adapted to the site conditions. The figure 5 shows that the least adapted provenances were 10-Reghin in Gugeşti and Satu Mare trial and 9- Snagov in Podul Iloaiei trial.

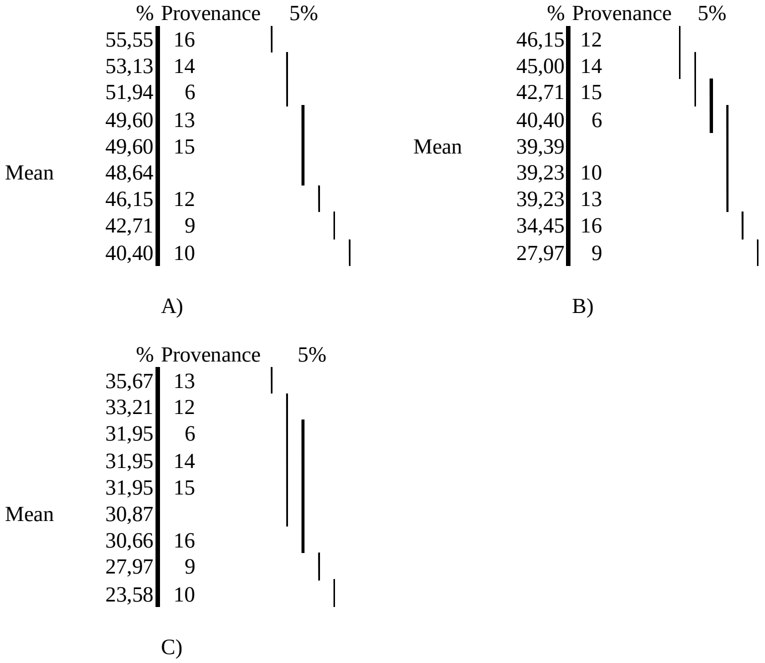

Figure 5.Variation of survival (%) for the Oak provenances tested in the comparative trials A) Gugeşti, B) Podul Iloaiei and C) Satu Mare -2006)

PHENOTYPIC CORRELATIONS BETWEEN THE STUDIED TRAITS AND BETWEEN THESE AND THE ORIGIN OF PROVENANCES GEOGRAPHICAL GRADIENTS

Tables 4 and 5 present the matrixes of correlation coefficients between the studied traits for the tested provenances in the four trials.

The total height (m) correlates positively, and in significant way with the average volume/tree in Podul Iloaiei trial and highly significant in Gugeşti trial where a same strong correlation exists between the total height and the D.B.H. (the diameter at 1,30 m). In Gugeşti trial, there is also a negative and highly significant correlation between the total height and the original altitude of the tested provenances. In Podul Iloaiei trial, the total height correlates highly significant positively with the survival and negatively with the trunk form, which means that the provenances with active height growth have superior qualities for the trunk form and that they are better adapted in the north-eastern part of the country. In the Lunca Timişului trial there is a strong and direct connection between total and pruning height, while in the Satu Mare trial there is a positive correlation between total the height and the branches width.

The average volume/tree (dm^3) has a strong and direct correlation with the branches width in Gugeşti trial, and less strong in Satu Mare trial (Table 5). In the Gugeşti trial there is a positive and significant correlation with the corrected latitude, meaning that the northern provenances of the areal have registered a significant higher average volume /tree than the southern ones.

The pruning height (m) correlates positive and significant with survival only in Podul Iloaiei trial.

The trunk form (index) correlates significant and negative with survival in Podul Iloaiei trial, and in the same direction with the branches width in Satu Mare trial.

The survival (%) correlates significant and negative with the stem form in Satu Mare trial, which means that the provenances with a superior stem form are better adapted to the site conditions from de north-western part of the country.

DISCUSSION

Taking into account the observations and measurements made, the statistical data analysis and the interpretation of the obtained results for the provenances tested in the trials installed in 1977, at the age of 29 years after the establishment of the plantation, we can conclude the following:

- There is a large interpopulational variability among the provenances of different origin (genetic variability geographically determined) for the main growth, form and adaptive traits in the studied trials. The traits variability was in general continuous, characteristic to the quantitative polygene controlled traits.
- The study of the phenotypic correlations between analyzed traits, on one hand, and between these and the geographic gradients of the origin of provenances, by the other hand, revealed the existence of different intensities correlations statistically assured. On these bases we can proceed further to the indirect selection of the provenances considering the correlated traits which are important for the oak breeding program.
- For each trial there can be designated the tested provenances which have registered the highest growth performances, the best qualities for trunk and stem form, and the best adapted to the testing site conditions and which can be recommended for utilisation and transfer in the region of provenances (Rof P) according to the testing site, as it follows:

Trial	Rof P	The best provenances from (R of P)
Lunca Timişului	K190	9 Snagov (J160)
		14 Găeşti (J170)
		16 Lunca Timişului (K190)
		12 Livada (K290)
Satu Mare	K290	12 Livada (K290) (local provenance)
		14 Găeşti (J170)
		13 Tismana (C250)
		16 Lunca Timişului (K190)
Gugeşti	H280	6 Strehaia (C270)
		9 Snagov (J160)
		15 Slobozia (H28A)
		16 Lunca Timişului (K190)
Podul Iloaiei	G160	6 Strehaia (C270)
		12 Livada (K290)
		14 Găeşti (J170)
		15Slobozia (H28A)

These provenances will be designated as “Tested sources” in the regions of provenances where they were tested. The non-optimality of local provenance hypothesis has been confirmed, because the local provenance was the best only in one of four provenance trials.

Table 4. Matrix of correlation coefficients and their significance for Oak traits tested in Gugeşti (at numerator) and Podul Iloaiei (at denominator) comparative trials (1977 Series) - 2006

Traits / Geographical Gradients	Average volume/tree	D.B.H.	Pruning height	Branches Width	Stem Form	Trunk form	Survival	Corrected Latitude	Longitude	Altitude
Total height	0,689** / 0,595*	0,670** / 0,491	0,133 / 0,203	0,259 / 0,206	-0,376 / -0,291	-0,459 / -0,690**	0,248 / 0,629**	0,365 / 0,133	0,150 / -0,293	-0,637** / 0,025
Average volume/tree		0,987** / 0,985**	0,448 / -0,475	0,665** / 0,344	-0,280 / -0,269	-0,384 / -0,454	0,005 / 0,030	0,607* / 0,481	0,115 / -0,447	-0,477 / -0,026
D.B.H.			0,515* / -0,553*	0,650** / 0,395	-0,247 / -0,164	-0,386 / -0,341	0,031 / -0,058	0,564* / 0,486	0,151 / -0,451	-0,492 / 0,011
Pruning height				0,413 / -0,062	-0,025 / -0,336	0,147 / -0,290	0,123 / 0,540*	0,469 / -0,297	0,006 / 0,075	-0,445 / -0,127
Branches Width					-0,337 / 0,087	-0,263 / 0,318	0,078 / -,254	0,405 / 0,550*	0,031 / -0,371	-0,233 / 0,164
Stem Form						0,578* / 0,600*	-0,082 / -0,045	-0,228 / -0,251	-0,335 / -0,009	0,053 / 0,334
Trunk form							0,053 / -0,610*	0,035 / 0,125	-0,438 / 0,029	0,230 / 0,251
Survival								-0,308 / -0,367	-0,349 / 0,003	-0,495 / 0,056
Corrected Latitude									-0,015 / -0,015	-0,091 / -0,091
Longitude										-0,155 / -0,155

Table 5. Matrix of correlation coefficients and their significance for Oak traits tested in Lunca Timişului (at numerator) and Satu Mare (at denominator) comparative trials (1977 Series)- 2006

Traits / Geographical Gradients	Average volume/tree	D.B.H	Pruning height	Branches Width	Stem Form	Trunk form	Survival	Corrected Latitude	Longitude	Altitude
Total height	0,489 / 0,462	-0,001 / 0,354	0,844** / 0,459	-0,278 / 0,547*	-0,041 / 0,112	-0,457 / -0,216	0,402 / -0,019	-0,105 / 0,148	-0,018 / -0,352	-0,433 / -0,264
Average volume/tree		0,861** / 0,975**	0,161 / 0,144	0,222 / 0,573*	0,483 / 0,469	0,094 / 0,106	-0,142 / -0,167	0,100 / 0,210	0,002 / -0,341	-0,023 / -0,243
D.B.H			-0,299 / 0,138	0,468 / 0,549*	0,520* / 0,576*	0,353 / 0,132	-0,437 / -0,259	0,097 / 0,235	0,051 / -0,245	0,228 / -0,131
Pruning height				-0,419 / 0,225	-0,071 / 0,131	-0,443 / -0,006	0,339 / -0,050	-0,145 / 0,028	-0,070 / 0,016	-0,312 / 0,107
Branches Width					-0,208 / 0,201	-0,180 / -0,564*	-0,277 / -0,018	-0,266 / 0,018	0,310 / -0,132	0,225 / -0,411
Stem Form						0,606* / 0,435	-0,244 / -0,587*	0,398 / 0,612*	-0,438 / 0,237	0,531* / -0,053
Trunk form							-0,450 / -0,409	0,388 / 0,274	-0,378 / 0,013	0,297 / 0,209
Survival								0,029 / -0,180	0,048 / -0,196	-0,118 / -0,399
Corrected Latitude									-0,015 / -0,015	-0,091 / -0,091
Longitude										-0,155 / -0,155

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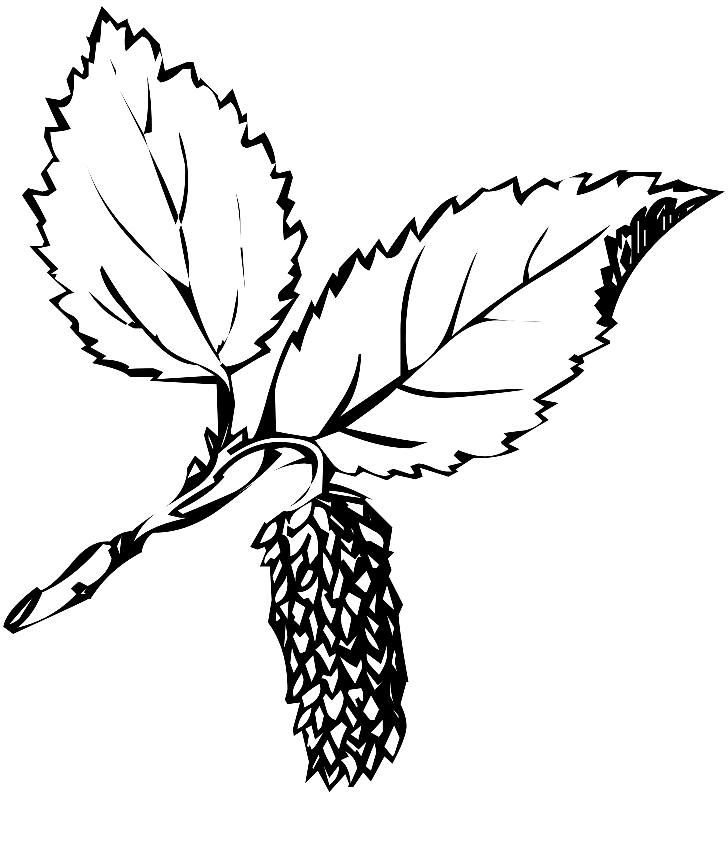

FOREST ECOSYSTEMS HEALTH STATUS IN WEST REGION OF MEDIUM-HIGH PLATEAUX OF NORTHEAST BULGARIA

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Summary: *The study was realized during 2007-2008 on the base of 17 sample plots in West region of Medium-high plateaux of Northeast Bulgaria in natural stands of Quercus cerris, Q. frainetto, Q. robur, Q. petraea, Tillia cordata, Carpinus betulus and plantations of Pinus nigra. During the study were established 19 species of pathogens: 6 on C. betulus, 5 on P. nigra and P. sylvestris, 3 on T. cordata, 3 on Betula pendula, 2 on Q. cerris and 1 on Q. petraea. There were also established 22 insect pest species from 10 families. There is potential danger of calamitily developed Lymantria dispar and Euproctis chysorrhoea which are in phase 'increasing in number'. Q. petraea and Q. frainetto are in non-satisfying condition. The drying trees are 23% with clearly expressed tracheomycotic disease, influenced by long droughts during the last years. Lime forests are in good health condition. There are established partial damages on the leaves and rotting in the above-ground parts of the trees. Carpinus ecosystems are in non-satisfying health status with significant number of dry and drying trees and damages on the stems by necroses and canker, leading to dieback and drying. Betulus plantations are in bad conditions in their large part with massive ice damages and snow damages, and rotting of the stem timber. A cause for these massive damages is lack of silvicultural activities in these plantations. Coniferous plantations are in relatively good status for the region. Their development is accompanied with damages by pathogens, causing needles cast, which reflects on their growth and development. The studied ecosystems are influenced to different degree by pathologic, entomologic and abiotic actions and need to be treated differently by silvicultural and protecting activities.*
Key words: *forest ecosystems, pathogens, insect pests, Northeast Bulgaria*

INTRODUCTION

The forests in Northeast Bulgaria fall in the lower or plain-hilly and fore-mountain belt of oaks and *Pinus nigra* Arn. with territories at an altitude between 0 and 600-800 m a.s.l. the region covers two geographic districts – Ludogorie and South Dobrudzha where there are significant differences in the landscape, climate and vegetation even within their own borders (Dontchev, 1964). In both districts the author distinguishes 5 climatic regions where Razgrad belongs to the West region of Medium-high plateaux. This region is characterized with cold winter, dry and warm summer. The mean annual minimum and maximum temperatures are – 10.5 and 11 ^{o}C, with amplitude of 26 ^{o}C which is one of the highest in the country. The annual precipitations are 570-630 mm from which 210-230 mm during the summer period. The forest vegetation is mainly of coppice origin. Most distributed are *Quercus cerris* L., *Q. frainetto* Ten., *Q. petraea* Liebl., *Tilia* spp., and *Carpinus betulus* L. At many places there are plantation of *Pinus nigra* Arn., mixed *P. nigra* and *Pinus sylvestris* L., and mosaically *Betula pendula* Roth. The predominating part of the natural stands are aged 60-80, and of the plantations – about 30 years.

This investigation is realized in order to establish main factors influencing on the health status of the forests in the region of Razgrad and outline activities for their improvement.

MATERIAL AND METHODS

The health status of the plantations and factors determining them were investigated in 2007 and 2008 in 17 sample plots (SP) on the territory of Municipality Forestry Razgrad. All basic forest ecosystems in the region were covered – broadleaved natural and coppice plantations (*Tilia cordata*, C. *betulus*, *Q. cerris*, *Q. frainetto*, *Q. petraea*) and plantations of *P. nigra*. The age of SP is from 20 to 86 years. The diversity of ecological conditions is not great: altitude from 300 to 400 m. plain to hilly landscape, soils - тъмносиви, песъкливо-глинести, deep, dry to fresh, rich. In each SP were observed 40 – 100 trees. An evaluation was made of the defoliation degree and leaf color, drying/withering of branches and trees and damages on them according the Methodic of ICP 'Forests'.

Pathogens and insect pests were studied according standard phytopathological and entomological methods. During the analysis of the factors influencing on the health status of the forest sin the region were used data from Forest Protection Station – Varna about the silvicutural activities realized during the period 1990 – 2006.

RESULTS AND DISCUSSION

During the period 1990 – 2006 in the region of Razdrad were realised forest protection activities on an area of 8561.3 ha (Table 1). They are related basically with 3 main groups of problems – defoliations by insects, damages by fungal and bacterial pathogens and withering of different tree species.

The control of leaf-grazing insects takes 80.6% of all forest protection activities, 76.3% of which being related to *Lymantria dispar* L. and the rest – to the complex Geometridae and Tortricidae.

Table 1. Forest protection problems during the period 1990-2006

Forest protection problem	Activities	
	Area, ha	Percentage
Lymantria dispar	6537.2	76.3
Drying of *Carpinus betulus*	675.5	7.9
Oak tracheomicotics	386.3	4.5
Geometridae and Tortricidae	367.2	4.3
Bacteriosis and anthracnosis	232.5	2.7
Drying of *Quercus cerris*	162.6	1.9
Drying of *Juglans regia*	165.6	1.9
Drying of *Quercus* spp.	24.4	0.3
Drying of *Tilia* spp.	4.0	0.1
Drying of *Pinus nigra*	6.0	0.1
Total	**8561.3**	**100.0**

The problems related to phytopathogenic fungi and bacteria (tracheomicosis on oak, bacteriosis and anthracnosis on *J. regia*) make 7.2% from the forest protection activities and the rest (12.4 %) – with drying of the tree species.

The status of the tree species and plantations observed, and the damages on them in SP are showed in Table 2. On some trees were established 19 species causing diseases (Table 3). In part of the plantations withering of the trees was observed up to 5-40% as well as presence of different damages – necroses, cancer, frost cracks, mechanical damages, etc. The results from the investigation showed that *T. cordata* is in a very good health condition. On the leaves were observed single damages by *P. tilliae* and *C. microspora*, which do not have economic significance. The necroses on the bark are few.

Damages on *C. betulus* are of pathological character manifested by necrosis of the bark (up to 40% from the trees), canker, dieback, etc. causing agents are fungi *A. mellea* – complex, *S. rugosum*, *T. versicolor, Nectria* sp., etc.

The condition of the plantation by *B. pendula* is bad because of ice and snow damages. On the stems, mainly in the ground part fungi were established as follows: *P. betulinus*, *F. igniarius*, *T. confragosa.* They accelerate the destructive processes of damaged trees.

Participation of *Q. petraea* is with singletrees in the plantations and its condition is satisfactory. On dying and dead trees are observed damages of tracheomicotic type, caused by the fungus *C. roboris*.

Quercus frainetto is in relatively good health condition for about 60.0 % from the trees observed. In the rest drying, starting from the upper crown part and gradually spread on middle and low parts was observed, and it leads to total dying of the trees. The causes for the drying are not fully clarified and are of complex character (so called 'oak decline').

In *Q. cerris* plantations observed up to 55% of the trees are damaged by hypoxylon cancer, caused by the fungi *H. mediterraneum* and *D. mutila*, with wet spots on the bark, epicormic shoots and withering of some branches and trees. In a SP was established drying of 5% from the trees. This data differs significantly from the results of other investigations in the lower parts of Northeast Bulgaria (Russe, Varna and Shumen), where the drying in some plantations reaches 35-43 % (Rossnev et *al.*, 2006).

Table 2. Condition of the trees in the sample plots observed

Experimental plot	Tree species	Age, years	Condition of the trees			Type of the damages
			Healthy	With damages	Dry	
1	*Tilia cordata*	86	92.5	7.5	0.0	Necrosis, mech. damages
2	*Carpinus betulus*	56	37.5	62.5	0.0	Necrosis, mech. damages, cancer
3	*Carpinus betulus*	56	40.0	60.0	0.0	Necrosis, mech. damages, cancer
4	*Carpinus betulus*	86	40.0	57.5	2.5	Necrosis, mech. damages, cancer
5	*Quercus frainetto*	71	77.5	2.5	20.0	Tracheomicosis
6	*Tilia cordata*	66	45.0	45.0	10.0	Necrosis, mech. damages
7	*Pinus nigra, Pinus sylvestris*	36	95.0	5.0	0.0	Needles diseases
8	*Betula pendula*	20	10.0	50.0	40.0	Snow crack, wood destroying fungi
9	*Pinus nigra*	41	0.0	100	0.0	Needles diseases
10	*Carpinus betulus*	66	30.0	70.0	0.0	Necrosis, cancer
11	*Pinus nigra*	36	37.5	62.5	0.0	Needles diseases
12	*Carpinus betulus*	61	17.5	82.5	0.0	Necrosis, mech. damages
13	*Quercus frainetto*	71	15.0	65.0	20.0	Tracheomicosis
14	*Quercus frainetto*	71	45.0	45.0	10.0	Tracheomicosis
15	*Quercus cerris*	26	15.0	80.0	5.0	Hypoxylon cancer, mech. damages
16	*Quercus cerris*	23	55.0	45.0	0.0	Hypoxylon cancer, mech. damages, frost cracks
17	*Quercus cerris*	56	42.5	57.5	0.0	Hypoxylon cancer, mech. damages, frost cracks

In the plantations of *P. nigra* it was observed significant tree crown defoliation of the crowns as a result from fungi development of *D. pini*, *L. seditiosum* and *S. sapinea*.

During the entomological investigations were established 22 insect species from 10 families – 12 species xyloppages (on stems, branches and stumps), 1 phloemophague (on phloem tissues) and 9 species phylophagues (on leaves and buds), Table 4.

Table 3. Parasitic fungi on tree species in SP

N	**Name**	**Host**	**Organs damaged**	**Established in SP N**	**Importance**	
					At the moment	**Potential**
1	*Armillaria mellea* - complex	*C. betulus*	Roots	13	+	++
2	*Ceratocystis roboris* (Georg. et Teod.) Potl.	*Q. petraea*	Stem	13	++	+++
3	*Cytospora decipiens* Sacc.	*C. betulus*	Stem	10	+	+
4	*Diplodia mutila* (Fr.) Mont.	*Q. cerris*	Stem	15, 16, 17	++	+++
5	*Dothistroma pini* Hulbary	*P. nigra*	Needles	9, 11	++	++
6	*Fomes igniarius* (L.) Gillet	*B. pendula*	Stem	8	+	+
7	*Hypoxylon mediterraneum* (de Not.) Mill.	*Q. cerris*	Stem	15, 16, 17	+	+
8	*Lophodermium pinastri* (Schrad.) Chev.	*P. nigra*	Needles	7, 9, 11	+	+
9	*Lophodermium seditiosum* Minter, Stally & Millar	*P. nigra*	Needles	7, 9	+	++
10	*Cercospora microsora* Sacc.	*T. cordata*	Leaves	1, 6	+	+
11	*Naemacyclus niveus* (Pers.:Fr.) Saccardo	*P. nigra*	Needles	7, 9, 11	+	+
12	*Nectria* spp.	*T.a cordata* *C. betulus*	Stem	повсемест но	+ ++	+ ++
13	*Pezicula carpinea* (Pers.) Tul. ex Fuckel	*C. betulus*	Stem	6	+	+++
14	*Phyllosticta tilliae* Sacc. et Spergazini	*T. cordata*	Leaves	1, 6	+	+
15	*Polyporus betulinus* (Bull.:Fr.) Fr.)	*B. pendula*	Stem	8	+	++
16	*Sphaeropsis sapinea* (Fr.) Dyko & Sutton	*P. nigra*, *P. sylvestris*	Needles, shoots, cones	7,9	++	+++
17	*Stereum rugosum* (Pers.: Fr.) Fr.	*C. betulus*	Stem	10	+	+
18	*Trametes confragosa* (Bolton) Jorst	*B. pendula*	Stem	8	+	+
19	*Trametes versicolor* (L.: Fries) Pilat	*C. betulus*	Stem	10	+	+

Legend: + low importance; ++ moderate importance; +++ significant importance

Table 4. Insect pests on forest trees vegetation

Insect species	Host plant	Location	Experimental plot	Importance	
				At the moment	Potential
Kermestidae					
Kermes quercus (Linnaeus, 1758)	*Q. cerris*	stems and branches	15, 16	+	++
Buprestidae					
Dicerca aenea (Linnaeus, 1766)	*P. tremula*	stems	2, 3	+	+
Agrilus biguttatus (Fabricius, 1777)	*Q. cerris, Q. petraea*	stems and branches	5	++	+++
Agrilus viridis Linnaeus, 1758	*P. tremula, Q. petraea, Q. frainetto*	stems and branches	2, 3, 13, 14	+	++
Cerambycidae					
Phymatodes testaceus (Linnaeus, 1758)	*Q. petraea, Q. frainetto*	stems	13, 14	+	+
Plagionotus detritus (Linnaeus, 1758)	*B. pendula*	stems	8, 9	+	+
Rhagium inquisitor (Linnaeus, 1758)	*P. nigra*	stems	11	+	++
Spondylis buprestoides (Linnaeus, 1758)	*P. nigra*	stumps	8, 9	+	+
Monochamus galloprovincialis pistor (Germar, 1818)	*P. nigra*	stems	7	+	+
Scolytidae					
Scolytus carpini (Ratzeburg, 1837)	*C. betulus*	stems and branches	4	+	+
Scolytus intricatus (Ratzeburg, 1837)	*Q. petraea, Q. frainetto*	stems and branches	13, 14	+	++
Tomicus minor (Hartig 1834)	*P. nigra*	stems	8, 9	+	++
Tomicus piniperda (Linnaeus, 1758)	*P. nigra*	stems	7, 8, 9	+	++
Nepticulidae					
Stigmella betulicola (Stainton, 1856)	*B. pendula*	leaves	8, 9	+	+
Pyralidae					
Acrobasis consociella (Hübner, 1813)	*Q. cerris*	leaves	17	+	+
Tortricidae					
Archips xylosteana (Linnaeus, 1758)	*Q. petraea, Q. frainetto*	leaves	13, 14	+	+++
Tortrix viridana Linnaeus, 1758	*Q. petraea, Q. frainetto*	leaves	13, 14	+	+++
Geometridae					
Ennomos quercinaria (Hufnagel, 1767)	*Q. petraea, Q. frainetto*	leaves	13, 14	+	+
Erannis defoliaria (Clerck, 1759)	*Q. petraea, Q. frainetto*	leaves	13, 14	+	+++
Operophtera brumata (Linnaeus, 1758)	*C. betulus. Q. petraea, Q. frainetto*	leaves	10, 13, 14	+	+++
Cynipidae					
Andricus kollari (Hartig, 1843)	*Q. cerris*	leaves	16	+	+
Cecidomyiidae					
Dryomyia circinans (Giraud 1861)	*Q. cerris*	buds	17	+	+

Legend: + low importance; ++ moderate importance; +++ significant importance

The number of pests is relatively low. Defoliations are with low values – do not exceed 5-10% and do not cause damages of the host plants. In the group of phylophages from fam. Geometridae and Tortricidae (with exception of *E. quercinaria*) there is a gradual type of population dynamics and could form calamities. At the moment they are in the phase of 'depression' but in the next years their number will increase inevitably. The control could be realsied with high-effective bacterial preparations on the base of *Bacillus thuringiensis* var. *kurstaki* – Foray, D-Stop, etc. (Tabakovich-Tosic, 2008).

The damages by xylophages are concentrated on single weak, withering and dry trees. Potential threat for oak plantations in the region are *A. bigutattus*, *A. viridis* and *S. intricatus*. Both species form genus *Agrilus* attack the branches and top parts of the stems of live plants. They damage phloem tissues and cause diebacks and tree physiological weakness, which become vulnerable towards attacks by other insects and diseases. *S. intricatus* is the main vector of tracheomicotic diseases on oaks.

In *P. nigra* plantations potential pests could be *R. inquisitor*, *T. piniperda* and *T. minor*.

Apart from insects, on the leaves of lime were established a pest from Arachnida – *Eriophyes tiliae* (Pagenstecher 1857) (Prostigmata: Eriophyidae). Its number is also low and the damages by it are insignificant.

The most dangerous leafgrazing pests in deciduos forests in Bulgaria - *Lymantria dispar* (L.) and *Euproctis chrysorrhoea* (L.) (Lepidoptera: Lymantriidae) – were not established during these investigations. In Bulgaria *L. dispar* is now entering the phase 'increasing of the number' and it is not impossible in the near 2-3 years to form calamities. It should be reminded that the control of the pest covers about ¾ form all the forest protection activities in the region of Razgrad (Table 1). Concerning *E. chysorrhoea*, the species is one of the best expressed allergens among insects and its appearance in forests with recreational functions is not desirable. In case of necessity the control of both species could be successfully realized by bacterial preparations on the base of *Bacillus thuringiensis* var. *kurstaki* or *Saccharopolyspora spinosa*.

The health status of the deciduous forests in the region of Razgrad could be improved via increasing the role of the limiting complex of *L. dispar* introducing the entomopathogenic fungus *Entomophaga maimaiga* Humber, Shimauzu & Soper (Entomophtorales: Entomophtoraceae). The natural area of the species covers Japan, east parts of China and Far East of Russia (Hajek et al., 2005). The pathogen is species specific – in natural conditions develops on *L. dispar* only. It is introduced in North America and at the moment is distributed in 17 states of USA (Hoover, 2000; Balser, Baumgard, 2001; Hajek et al., 2005; etc.) and a province of Canada (Ontario), (Howse, Scarr, 2002). In the places where the pathogen is present the damages by *L. dispar* decrease significantly due to infestment of large part of the larvae up to 61-98 % (Hajek, 1997). During the period 1996–2001 *E. maimaiga* was introduced in Bulgaria from USA (Pilarska et *al.*, 2000; 2006) and yet were observed some strong epyzootics on *L. dispar* by it at different places in the country (Georgiev et al., 2007).

Our opinion is that in suitable conditions (presence of host larvae) the pathogen could be also introduced in the forests around Razgrad as a classical element of biological control. Acclimatization of *E. maimaga* in a long-term perspective, with no debt, will lead to following results:

- Better stability of forest ecosystems as a result of increasing of the regulation role of biotic limitation complex of *L. dispar*;
- Rarely appearance and faster attenuation of pest calamities;
- Significant decrease of insecticides usage as well as *L. dispar* control expenses;
- Conservation of biological diversity and improvement of forests' recreational qualities.

As a conclusion it could be noted that the investigated forest ecosystems in the region of Razgrad are in a relatively good health status. They are influenced to a different degree by pathological, entomological and abiotic effects and need differentiated silvicultural and forest protection activities. Mitigation of the damages by pathogens could be realized by cuttings, sanitary and other types of cuttings and not allowing mechanical damages on tree stems.

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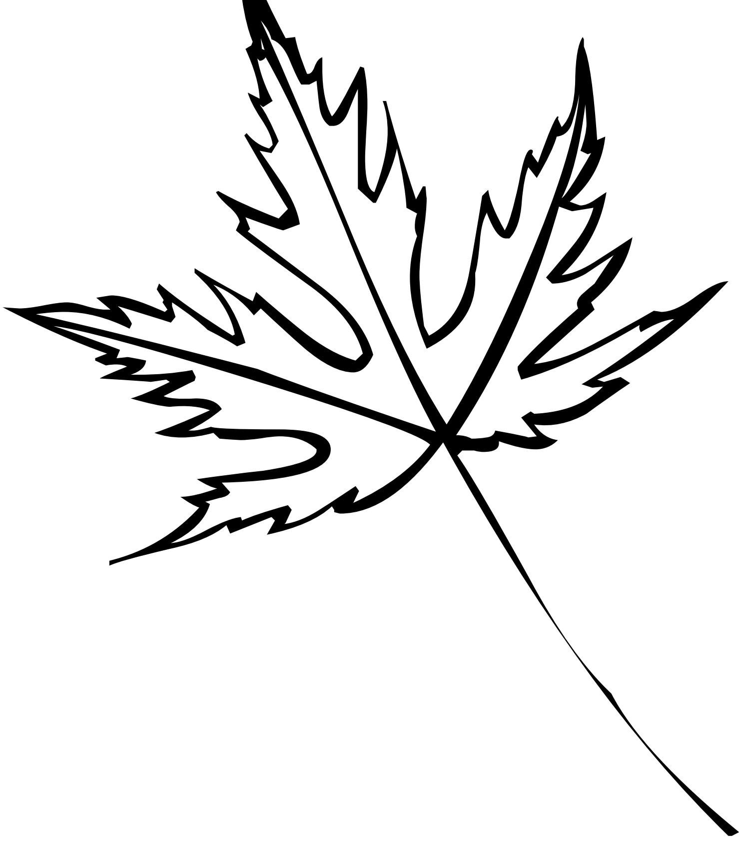

ALTERNATIVE LAND USE

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Summary: *Modern agricultural production is characterised by use of certified seeds, intensive soil cultivation, intensive use of pesticides for control of diseases, insect pests and weeds, monocultures on large areas, and low diversity. In the Vojvodina region, agricultural production is the most dominant form of plant production. To ensure a sustainable agricultural production, as well as protection and enhancement of environmental quality, it is necessary to increase the extent of the present forest area up to 140,000 ha, i.e. from the present 6.37% to 14%. However, increasing the forest cover percentage is difficult because of lack of forest land. It is therefore necessary to apply alternative systems of land use to increase yield, and this objective can be achieved by combining the agricultural and forestry technologies, making the ecosystem more stable. Agro-forestry systems are alternative land uses with the main purpose of creating diverse, productive, profitable, healthy and ecologically more stable ecosystems. Agro-forestry is considered an alternative land use, which includes: alley cropping, windbreaks, riparian buffer strips, silvo-pastoral and forest farming. Agro-forestry systems will have special significance by the full implementation of the Kyoto Protocol, as carbon sequestration by perennial plants (trees) will be more adequately economically valorised. This paper presents a survey of different types of agroforestry plantations, as well potential solutions, related to selection of species.*

Key Words: *Agro-forestry, Afforestation*

INTRODUCTION

Agro-forestry is an intensive system of land management which benefits from the biological interactions when trees or shrubs are grown with agricultural crops or livestock. It should be emphasised that agro-forestry is not a new technology, but a concept neglected in our region, which has been instead been practised in tropical and sub-tropical regions. Agroforestry systems help attaining sustainable agricultural land use systems in many ways. They provide protection for topsoil against erosion, protection of livestock and agricultural crops; increase productivity, reduce inputs of energy, increase water-use efficiency of plants and animals, improve water quality, enable diversification of revenues and enhance biodiversity and landscape amenity, and finally, the quality of life (Rietveld, 1996). The potential uses of agro-forestry are numerous, but they can be reduced to the following categories: alley cropping, windbreaks, riparian buffer strips, silvopastoral systems and forest farming.

ALLEY CROPPING

Alley cropping is established on agricultural lands to increase the diversity of returns, reduce wind and water erosion, increase productivity in agriculture, enhance the utilisation of nutrients, improve the habitats with the aim of diversity conservation. They are actually tree plantations in single or multiple tree rows, at a wide spacing between the plants (minimum 6m), in which agricultural or horticultural plant species are planted. The most widely used trees are high-value hardwoods or soft hardwoods, as well as the species which produce fruits that can be used as food for people or livestock. Also, it is possible to establish intensive plantations of soft broadleaves (poplars) for the production of wood for pulp and paper. Frequently used species in the establishment of such plantations are walnut (good-quality and high-value wood and fruits), oaks (good-quality and high-value wood, acorn for livestock), ash (good-quality and high-value wood), wild cherry (high-value wood). Alley cropping may enhance economic stability, increase plant and animal diversity, sustainable management of agricultural systems and landscape diversity. One of the main questions when designing an alley cropping system is the issue of selecting tree species and spacing of tree rows. Due to the interaction between trees and agricultural species and the optimisation of economy, alley cropping should be created so as to ensure the highest rates of return of the investments in the establishment. If Black Walnut (*Juglans nigra*) is planted as a woody species, it is best to start with 270 plants/ha and to reduce the number of plants to 75/ha by later thinning (Garrett, *et al*, 1994). In alley cropping, care must be taken about the characteristics of tree species, as well as the landowner objectives and priorities. Special attention must be focused in the selection of plant species that are compatible with the site. Selected tree species should have the following characteristics:

- To produce high-value products, such as wood, fruits, chemical compounds, etc., with a viable market;
- To ensure fast yield;
- To adapt to different site and soil conditions;
- To provide shade for the growth of some agricultural species;
- To have deep root systems, to avoid the competition of agricultural species;
- To have the foliage which have a minimal potential of soil acidification;
- Do not produce substances which inhibit the growth of agricultural species;
- To have the same growth season as the agricultural species grown between the rows,
- To enhance diversity.

In the selection of tree species, it is necessary to take into account the growth characteristics of the species for alley cropping, the number of rows - single or multiple tree rows, the application of single or multiple species, spacing, as well as the species demands for light. It is especially necessary to know the growth characteristics of tree species during the juvenile stage, because of the possible competition with the crops.

The spacing between rows and between plants is very important in the design of alley cropping. It depends on the special purpose of the plantations, i.e. if alley cropping is intended for the production of wood for fibres, spacing is narrower. If the plantations are intended for erosion control spacing is also narrower, but instead for production of fruits spacing is wider. If the species selected for alley cropping do not tolerate shade, the planting spaces should be wider. Also, the spacing depends on the demands for fertilisation, maintenance, or weed suppression during plant growth. Very often, an intensive multi-crop system (cultivation of several plant species) ensures a much higher profit than tree growing only for timber (Kurtz, *et al.,* 1984)

WINDBREAKS

Windbreaks are line plantations of trees and shrubs which are established to reduce wind velocity in a given area. Windbreak establishment and management are the main components of management and growing of agricultural crops or livestock. Special significance of windbreaks is environmental protection, i.e. reduction of wind erosion. In addition, the quality of water and air is enhanced, thanks to the filtering and remediation. The lower wind speed enables the higher efficiency of water utilisation, through the reduced evapo-transpiration. Windbreaks also increase the economic benefits because they reduce the energy costs for heating; they increase the yield, enhance the livestock health and vitality and enable the production of fruits or wood. The research shows a significant increase of yield in agricultural crops (10 - 20%) if plants are protected by windbreaks. The percentage varies depending on the soil conditions and the year (Baldwin, 1988; Kort, 1988). Windbreak establishment leads to the reduction of height of the plants within the shelterbelt, but this loss is compensated by the increased yields on the remaining area, thanks to the reduction of wind speed (Baldwin, 1988). Efficiency of pesticide application, as well pollination success, is also significantly higher (Wight & Stuhr, 2002).

Windbreaks have an important role in the protection of livestock from cold and severe winds during autumn, winter and spring, also providing protection from insolation in summer (Quam *et al.,* 1994). Due to reduction of wind speed, livestock is protected from colds, it is healthier, efficacy of food is higher and reproduction cycle is enhanced. Windbreaks also provide the increase of yield in bee keeping, because they provide favourable plants for bees, as well as the shelter to bees from wind (Gordon & Newman, 2006).

Windbreaks have a favourable effect on the increase of biodiversity due to cultivation of multiple species in windbreaks, which increases the potential of nourishment for animals, birds and insects; at the same time windbreaks provide corridors for movement and protection. Also, thanks to greater diversity, there are better opportunities for hunting, which is a significant activity for the enhancement of rural revenues. Landscape beauty is enhanced by colours, shapes and noises (Johnson & Brandle, 1991).

Windbreaks decrease energy consumption for heating and cooling. During winter months the decrease of velocity of cold winds, may determine saving ranging between 10 and 40%. If windbreaks are established along the roads, they reduce noise and protect roads from blowing snow (Wight and Stuhr, 2002).

In the Autonomous Province of Vojvodina, there is a significant demand for the establishment of about 90,000 ha of windbreaks (Orlovic, 2005). They are necessary to provide the ecosystem stability and to maintain the level of agricultural production.

RIPARIAN BUFFER STRIPS

The lack of permanent vegetation, i.e. trees and shrubs, grass and annual plants leads to the increase of water runoff containing different sediments and dissolved contaminants which are delivered to surface waters. The increased runoff causes stream-bank erosion which leads to degradation of aquatic habitats and increased deposition of sediments in rivers and lakes. In most cases water transports a number of contaminants, such as: fertilizers, pesticides, nutrients and bacteria from livestock operations, sediments from croplands and urban areas and eroding stream-banks, oils (Gordon & Kaushik, 1987). When these pollutants reach rivers or seas, they create hypoxic zones in which fish cannot survive. Hypoxic zones are created also when excess of nitrates reaches the rivers, especially in the spring. To prevent this negative trend, riparian buffer strips, or tree and shrub plantations, need to be established to decrease the harmful impacts and to enhance the conditions for fish and wildlife habitat. There are numerous data on the adverse effects of removal of forest plantations along the stream-banks, but there are few data on the cumulative rehabilitation effects of artificially established forests on degraded waterways (Borman & Likens, 1979). Data from a locality in Ontario (Canada, 1987) show that three years after tree planting along the Grand River, the number of plants was significantly higher compared to some non-rehabilitated areas. The same study also showed that after 5 years the planted trees provided a significant shade, lower air temperature, a 20% reduction in nitrates concentrations in water, and the production of significant biomass quantities (4-5 oven-dry Mg ha^{-1} $year^{-1}$) [5]. Recent research in this area showed a significant increase in the number of fish and the populations of mammals and birds, from which it can be concluded that riparian corridors are significant for the activities on restoration and rehabilitation of biological diversity (Spackman & Hughes, 1995), as well as that they have a significant buffering capacity to remedy the contamination. This practically means that the establishment of riparian buffer strips is one of the methods for the enhancement of diversity of agricultural lands.

Intensive production in animal husbandry in Vojvodina leads to production of high quantities of organic waste (slurry, manure), a great part of which is delivered to watercourses. By establishing and intensifying the existing riparian buffer strips, the deposition of nitrates, oil derivatives and heavy metals in the existing watercourses will be significantly reduced. Poplar plantations established for different purposes can be useful to achieve this objective. It is especially important to take into account the selection of poplar clones, because different poplar clones have specific reactions to the pollutants.

SILVOPASTORAL SYSTEMS

The traditional interaction between trees and livestock occurs at places where trees are used for shade (shade trees in pasture), where forests are used for grazing and where there are trees and shrubs on pastures. A silvo-pastoral system consists of livestock, forage - pasture and trees. The trees provide protection against cold winds as well as the sun and dry winds. In addition, farmers can provide significant returns by harvesting the fruits and wood. The land use for pasturage and tree growing can provide significant quantities of wood for mechanical and chemical wood processing. Numerous fruits, such as black walnut, hazelnut, almond and berries can be produced as secondary products. On such areas, the enhancement of biodiversity is significant. In the Autonomous Province of Vojvodina, there are large areas under pastures. Part of these lands is undergoing a significant process of salinization, and is now used only as pastures. Planting some tree species on such lands (common oak, blackthorn, wild pear, honey locust, etc.) would provide shade during summer days, and grass vegetation serving as pasturage, decreasing dryness due to insolation. In addition, this would create a significant pasture for honey bees, because the greatest numbers of tree and shrub species that thrive on such soils are also melliferous. Production of honey and bee products would be a significant contribution to the economic stability of farmers and to rural development.

CONCLUSIONS

Agro-forestry is a method of land management that enables integration of production and environmental management, which results in a healthier and sustainable agricultural production important for future generations. Agro-forestry systems, which are almost absent in Serbia, can provide significant economic effects, such as: diversifying income, reduction of inputs of energy, increase in agricultural production; positive effects on the environment: quality of air, water, soil and plants, as well as enhancing of biodiversity and could be appropriate ways for harmonisation of demands from agriculture, forestry and environment.

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ROLE OF GRAND FIR AS A SOIL IMPROVEMENT SPECIES

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Abstract: *The presentation aims to evaluate the role of the Grand fir (Abies grandis (Dougl. ex D.Don) Lindl.) as a soil improving species. The state of humus forms in the stand part with Grand fir was compared with the mature Norway spruce without regeneration, further with young beech, Norway spruce and oak stands (pole stage). The site is characterized as 4P1 – acid oak-fir site with Luzula nemorosa, the geological bedrock is formed by cretaceous sandstone with loess overlays, the soils are characterized as Luvisols, terrain is flat in the altitude 420 – 440 m a.s.l.. The humus form samples (L, F, H, Ah horizons) were sampled in 4 replications, quantitatively for the holorganic layers. The dry mass amount and total nutrient contents were analyzed for holorganic, the basic pedochemical characteristic (pH, soil adsorption complex characteristics, exchangeable acidity and exchangeable nutrients) for all horizons. The results confirmed considerably visible and positive effects of the Grand fir litter on the surface layers characteristics. This tree species supports the litter with good composition, transforming easily and forming humus forms with higher quality comparing with coniferous as well as studied broad-leaved species.*
Key words: *forest ecosystems, Grand fir, introduction species, humus forms, accumulation, soil chemistry, soil amelioration*

INTRODUCTION

Introduction of the Grand fir (*Abies grandis* /Dougl. ex D.Don/) was more in the center of the forestry research in the Czech Republic in the past (Hofman 1963, Šika 1983). This species is among those ones with the highest production potential in the conditions of the Central Europe. It is characteristic by rapid growth, production of high amount of technologically important wood and with remarkable landscaping and gardening value. Production of branches for ornamental use is also not negligible. The decline of domestic White fir (*Abies alba*) contributed partially to the interest in this species in the past century. This aspect is less topical at present because of partial revitalization of White fir in the last decade. Also the environmentalistic obstacles represent certain limit for introduced tree species in general. But, as the result of past activities, the stands of Grand fir occupy several hundreds of hectares in the Czech Republic (about 950 ha - Novotný, Beran 2008), this area being even more large in other European countries. Grand fir is studied as for its growth and production relatively well in the series of research plots and provenance experiments, documenting satisfactorily its production potential in the younger age (Beran 2006, Vančura 1990).

There are only few publications documenting the effects of this tree species on the environment (e.g. Podrázský 2003a, Podrázský & Remeš 2007, 2008). Aim of the presented study is to evaluate the Grant fir as the soil improving species on the School Training Forest in Kostelec nad Černými lesy territory. In this region, typical for larger areas of the Central Bohemia, the structure and development of humus form was studied in close-to-nature stands (Podrázský & Remeš 2007a) as well as the soil changes after conversion into spruce monocultures (Podrázský 2003b). Now, the re-conversion of the stands and effects of particular tree species on the humus forms is studied in this process and the results are presented.

MATERIAL AND METHODS

Research was provided on the territory of the School Training Forest Kostelec nad Černými lesy, in the stand 409 F. The bedrock is sand-stone with loess over-layers, site is characterized by the forest type 4P1 – *acid oak-fir forest on pseudogley soils.* The concrete stand part studie dis characterized by transition from Luvisol (dominant) to Pseudogley. The particular stand parts are:

- mature Norway spruce stand, in full density (age 120 years), SMold
- European beech (BK), Norway spruce (SM), oak (DB), and especially Grand fir (JD) areas in the pole stage (age 35-50 years).

The humus form samples were taken in all these stand parts, in number of replication 4, holorganic layers quantitatively by iron frame 25 x 25 cm. The analyses were performed in the Laboratory Tomáš by standard analytical methods, yearly tested.
It was analyzed:

- amount of surface humus (holorganic layers) at 105 °C,
- content of total nutrients after mineralization with sulphuric acid and selene, amount of nutrients within holorganic layers per 1 ha,
- pH in water and 1 N KCl solutions,
- soil adsorption complex characteristics by Kappen (S – bases content, H – hydrolytical acidity, T – cation exchange capacity, V – base saturation),
- total carbon (humus) content by Springer – Klee and total nitrogen content by Kjeldahl,
- exchangeable nutrient content in the Mehlich III solution.

The statistical evaluation was performed using the statistical software S-PLUS by the analysis of variance method. The results were evaluated by the Scheffe's method by multiple comparison on the 95% significance level. The ecologically corresponding horizons were compared. Important note: In the tables, different indexes determine statistically significant differences at the significance level 95%, in the same/corresponding horizons, the same indexes or their absence means no significant differences occur.

RESULTS AND DISCUSSION

The establishing of stand parts with different species composition lead together with the small-clearcut effects to lowering of the surface humus amount (Table 1). The young spruce stand showed less prominent decrease, all other species were very similar as for their effects. In the young stands, non-significant decrease of the nitrogen concentration was documented, the decrease indicated only non-significantly being higher in the broadleaved stand. This is connected with the higher demand of these species, documented also in other cases (Podrázský & Remeš 2008). Amount of nitrogen fixed within the holorganic layers did show the same trend as the surface humus amount. The total phosphorus content had showed the same level in all stand excluding significantly higher values in the Grand fir one – recycling this element very effectively. In all the young stands were documented significantly higher concentrations of the total potassium, the total sum was higher then in the old stand because of bigger accumulation of necromass. Potassium is so recycled very intensively by the fast growing tree stands.

In the young spruce stand decreased the amount and content of the total calcium, the increase was observed in the other species. The only significant increase was documented in the Grant fir ecosystem again. All young stands showed increase of the total magnesium content, the decrease of the amount was similar as the total surface humus weight.

In similar conditions, the decrease of the surface organic matter and increase of the basic macroelements content was documented after canopy lowering (Podrázský & Remeš 2007a), changes of the bases concentrations as well (Podrázský & Remeš 2005, Podrázský & Remeš 2007b, Podrázský & Viewegh 2005).

Table 1. Accumulation of the surface humus and amount of fixed macronutrients in particular stands

Stand	Horizon	Dry mass kg/ha	N		P		K kg/ha		Ca kg/ha		Mg kg/ha	
			%	kg/ha	%	kg/ha	%	kg/ha	%	Kg/ha	%	kg/ha
Smold	L+F1	11572 b	1.47 a	170	0.08 a	9	0.10 a	12	0.83 a	96	0.06 a	7
SM_{old}	F2	14364 bc	1.52 a	218	0.07 a	10	0.10 a	14	0.20 a	29	0.05 a	7
SM_{old}	H	39924 a	1.57 b	628	0.07 a	26	0.14 a	56	0.07 a	28	0.03 a	13
sum		65860		1016		45		82		90		27
SM	L+F1	8516 ab	1.53 a	130	0.07 a	6	0.12 a	10	0.56 a	48	0.07 ab	6
SM	F2	11260 abc	1.33 a	150	0.06 a	7	0.15 ab	17	0.21 a	24	0.07 a	6
SM	H	25868 a	1.28 ab	330	0.06 a	16	0.27 b	69	0.04 a	9	0.04 a	10
sum		45644		610		29		96		81		22
BK	L+F1	4844 a	1.48 a	72	0.07 a	3	0.14 a	7	1.04 ab	50	0.11 d	5
BK	F2	6284 a	1.15 a	72	0.06 a	4	0.18 ab	11	0.42 a	26	0.09 a	6
BK	H	27276 a	0.89 a	242	0.07 a	15	0.40 c	109	0.09 a	23	0.02 a	5
sum		38404		386		22		127		99		16
DB	L+F1	3590 a	1.40 a	50	0.07 a	2	0.14 a	5	0.81 a	29	0.11 cd	4
DB	F2	7084 bc	1.25 a	89	0.07 a	5	0.19 b	13	0.26 a	18	0.08 a	6
DB	H	29048 a	0.94 a	274	0.05 a	14	0.36 bc	105	0.10 a	28	0.02 a	5
sum		39722		413		21		123		75		15
JDo	L+F1	5568 ab	1.41 a	79	0.08 a	4	0.15 a	8	1.57 b	87	0.08 bc	5
JDo	F2	7620 bc	1.40 a	106	0.08 a	6	0.20 b	15	0.29 a	22	0.06 a	5
Jdo	H	22320 a	1.02 a	227	0.06 a	12	0.30 bc	67	0.04 a	8	0.02 a	5
Sum		35508		412		22		90		117		15

Grand fir showed the biggest, statistically significant, effects on the soil chemistry: increase of the soil reaction (both types), bases content, base saturation (Table 2). The hydrolytical acidity decreased less comparing to the broad-leaved species, but in other soil chemistry characteristics this species was superior to other tree species. The old and young Norway spruce stands differ in the same trend, the broad-leaved tree species showed the middle intense effects. These effects were documented in other case too – the effects of Grand fir consisted in higher accumulation of the surface humus with high quality. The broad-leaved species showed more rapid composition of the litter, but sometimes with less favorable soil chemistry characteristics (Podrázský 2003b, Podrázský et al. 2003, Podrázský & Remeš 2008). This can be very often ascribed to the specific demand of particular tree species and to more prominent demands of broad-leaved trees as for especially phosphosru, potassium and bivalent bases.

The total humus content (total carbon content x 1.724 coefficient – rough estimation only) was lowered in the young spruce stand due to more intense surface humus transformation – mineralization and humification – in the period of stand regeneration. This can occur both due to the process of natural regeneration (Podrázský & Remeš 2007a), thinning (e.g. Šarman 1982, 1985), as well as clear-cutting (Podrázský & Remeš 2005a). The broadleaved species with their litter of higher quality caused even more prominent lowering of the total humus contents comparing to coniferous monocultures. Grand fir was fully comparable with the broadleves in the studied case. The total nitrogen content by Kjeldahl showed similar trends, but the Grand fir exhibited even lower nitrogen losses. The regeneration phases together with high demands of fast growing young stages of the forest tree species is leading to extraction of nitrogen from the soil (Table 3).

Table 2. Soil reaction and soil adsorption complex characteristics in particular stands

Stand	Horizon	pH/H2O	pH/KCl	S (mval/ /100g)	H (mval/ /100g)	T (mval/ /100g)	V (%)
SMold	L+F1	4.13 a	3.66 ab	27.35 a	21.5 a	48.85a	56.36 a
SMold	F2	3.94 a	3.31 a	26.32 a	50.11 c	76.43 b	34.52 a
SMold	H	3.55 a	2.78 a	18.28 a	71.39 b	89.66 c	20.44 ab
SMold	Ah	3.40 a	2.76 a	3.04 a	22.07 a	25.11 a	11.45 ab
SM	L+F1	4.57 a,b	3.88 abc	27.02 a	24.81 a	51.83 a	51.82 a
SM	F2	4.17 ab	3.57 ab	27.78 a	44.94 bc	72.72 ab	38.28 ab
SM	H	3.76 ab	3.03 a, b	12.4 a	46.87 ab	59.27 ab	21.02 ab
SM	Ah	3.56 ab	2.98 a, b	2.68 a	15.48 a	18.16 a	14.27 abc
BK	L+F1	4.38 ab	3.96 abc	47.98 ab	21.9 a	69.88 a	68.66 ab
BK	F2	4.35 ab	3.86 ab	36.78 a	25.86 a	62.64 ab	58.74 b
BK	H	4.03 b	3.42 bc	12.89 a	28.2 a	41.09 a	31.45 b
BK	Ah	3.89 c	3.18 bc	4.56 a	14.52 a	19.08 a	23.24 c
DB	L+F1	5.03 b	4.46 bc	41.18 ab	21.9 a	63.08 a	65.21 ab
DB	F2	4.34 ab	3.74 ab	31.94 a	28.63 a	60.57 a	52.65 b
DB	H	4.19 b	3.43 bc	11.33 a	29.81 a	41.14 a	27.62 ab
DB	Ah	3.86 c	3.40 c	2.71 a	10.57 a	13.28 a	20.49 bc
JDo	L+F1	5.09 b	4.59 c	56.6 b	16.82 a	73.42 a	76.46 b
JDo	F2	4.70 b	4.11 b	39.45 a	31.08 ab	70.53 ab	55.85 b
JDo	H	4.22 b	3.52 c	12.49 a	32.73 a	45.22 a	29.25 ab
JDo	Ah	3.73 bc	3.25 bc	1.64 a	13.84 a	15.48 a	10.74 ab

Table 4 documents the contents of plant available (exchangeable) nutrients in individual horizons of particular stands. The contents of the plant available phosphorus increased especially in the oak and Grand for stands, showing growing tendencies in all young stand parts. Potassium concentrations grew especially in the broad-leaved stands, partly in correspondence with the total form of macronutrients. The bivalent cations showed decreasing tendencies in lower (H, Ah) horizons of both young spruce, Grand fir and broad-leaved stands, the increase in upper horizons (L, F) was documented for fir and broad.leaves. This indicates probably the increased uptake of these elements and transport in litter and slightly transformed humus matter. The humus forms underwent considerable quantity and quality changes and indicate visible changes after forestry treatments studied. The Grand fir effects are visible too and results represent important evidence of its environmental functions.

Table 3. Total humus and nitrogen contents in particular stands

Stand	Horizon	Humus Springel-Klee (%)	Nitrogen Kjeldahl (%)
SM_{old}	L+F1	71.45 a	1.39 a
SM_{old}	F2	81.38 c	1.39 a
SM_{old}	H	63.79 bc	1.46 b
SM_{old}	Ah	13.15 a	0.31 a
SM	L+F1	62.60 a	1.33 a
SM	F2	59.34 ab	1.42 a
SM	H	44.86 ab	1.15 ab
SM	Ah	10.31 a	0.30 a
BK	L+F1	60.72 a	1.42 a
BK	F2	47.26 a	1.14 a
BK	H	31.80 a	0.77 a
BK	Ah	10.52 a	0.36 a
DB	L+F1	64.84 a	1.44 a
DB	F2	46.55 a	1.20 a
DB	H	27.87 a	0.82 a
DB	Ah	7.49 a	0.24 a
JDo	L+F1	64.40 a	1.37 a
JDo	F2	51.87 a	1.40 a
JDo	H	36.63 a	1.03 ab
JDo	Ah	6.92 a	0.22 a

CONCLUSIONS

All the results, obtained in the presented study, document the increased humus mineralization and transformation after the forestry treatments and during the regeneration processes. The reduction or removal from the canopy results in lowering of the surface humus amount and changes of its qualitative characteristics.

In spite of the Grand fir, the quantitative effects were comparable to the studied the most important broad-leaved tree species: beech and oak. European beech is considered as one of the most important site improving and stabilizing tree species. The Grand fir is as for quantitative aspects even so effective.

Also the characteristics of the soil chemistry, i.e. the soil reaction, soil adsorption complex characteristics and humus as well as nutrient contents improved visibly, often significantly, after the regeneration and tree species change. The young Norway spruce did show less prominent changes.

From the qualitative point of view, the Grand fir was fully comparable with the studied broad-leaved tree species and its characteristics as site improving species is without doubt confirmed. The high production potential – in contrast to beech and oak in coniferous monocultures – supports its cultivation in broader extent. Introduction of the Grand fir as production increasing and site improving species has to be recommended.

Table 4. exchangeable nutrients content in the Mehlich III solition in particular stands

Stand	Horizon	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)
SM$_{old}$	L+F1	51.33 a	488.67 a	2329.33 a	228 a
SM$_{old}$	F2	35 a	341 a	2845 a, b	249 a
SM$_{old}$	H	22 ab	257.5 a	2047.5 b	232 b
SM$_{old}$	Ah	2.75 a	77.25 a	425.75 a	65 a
SM	L+F1	50.67 a	594.67 a	2414 ab	278.67 a
SM	F2	42 a	444 a, b	2656.67 a	272.67 a
SM	H	29 a, b	316.5 a	1438.5 a	179.5 ab
SM	Ah	9.00 a	91.75 a	347.75 a	54.5 a
BK	L+F1	87 abc	963 b	4224 bc	712 b
BK	F2	54 ab	595 b	3731 ab	532 b
BK	H	28 ab	256.5 a	1494.5 a	205.5 ab
BK	Ah	15.5 a	115.5 a	449.25 a	68.5 a
DB	L+F1	132 c	973 b	3345 a bc	624 b
DB	F2	74 b	549.5 b	2920.5 ab	496 b
DB	H	34 a, b	300.5 a	1253.5 a	224.5 b
DB	Ah	10.00 a	94 a	292.25 a	58.25 a
JDo	L+F1	100 b, c	681.33 ab	4823.33 c	316.67 a
JDo	F2	62.67 ab	512.67 ab	4130.67 b	286.67 a
JDo	H	42.5 b	352.5 a	1718 a, b	156.5 a
JDo	Ah	4.75 a	68.5 a	328.25 a	48.25 a

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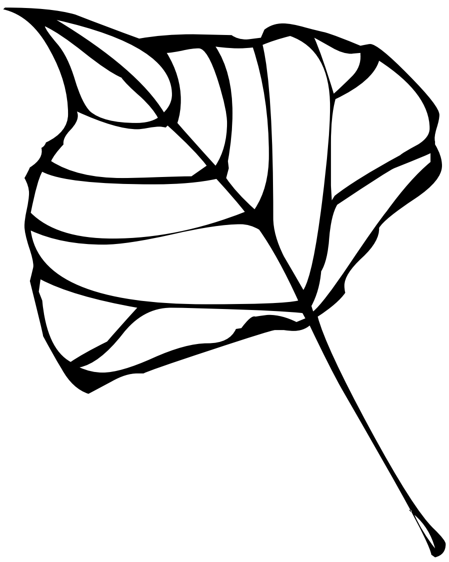

ESTIMATE OF ENERGY POTENTIAL OF POPLAR BIOMASS FROM SHORT ROTATION PLANTATIONS

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Abstract: *The paper presents the estimates of energy potential of poplar biomass based on the amount of energy obtained through combustion of total aboveground biomass. Plantations of the most common clone, P.x euramericana cl.-I 214 were considered, of 10 years of age with different number of rotations (2 to 9), and different plant densities (2,500 to 40,000 plants/ha). We estimate the potential amount of energy plantations of 6 new poplar clones (P.x euramericana cl. Ostia, P.nigra cl.53/86, P deltoides cl. PE 19/66, P. xeuramericana cl.I-214, P.x euramericana cl. S6-7, P.x euramericana cv. Robusta), whereby two different plant densities were tested (38,461 plants ha^{-1} and 83,333 plants ha^{-1}). Analysis also included experimental clones which are in the phase of selection at the Institute, with seven new experimental clones of eastern cottonwood, P. deltoides cl. B-229, P. deltoides cl. B-81, P. deltoides cl. 129/81, P. deltoides cl. 182/81, P. deltoides cl. 181/81, P. deltoides cl. PE 4/68, P. deltoides cl. 665 and euramerican poplar P. x euramericana cl. M-1, planting space of 16667 plants ha^{-1}. The biomass produced varies in a very wide range from about 3 t/ha to over 20 t/ha depending primarily on the plant's age, and plant density, and only secondarily on the poplar clone type. Differences in the higher heating values among the tested clones are relatively minor, except for the clone I-214, which also has a significantly lower value of wood density as compared to other clones; this markedly affects the yield of dry biomass (o.d. mass). Estimated amount of energy per hectare of plant varies between 200 GJ/ha and 400 GJ/ha, depending on the weight of biomass and higher heating values of wood (stem and bark) of each individual clone.*
Key words: *poplars, biomass, energy*

INTRODUCTION

Most projections of global energy use predict that biomass will be an important component of primary energy souces in the coming decades, and that SRWC will be a primary source of biomass (Berndes et al., 2003). In addition to combustion and gasification conversion pathways for power and heat production, SRWC represent a uniform, locally available feedstock for the production of bioproducts - liquid fuels, chemicals and advanced materials - curently made from petroleum products.

According to the European Commission's White Paper (1997), the overall aim is to double the share of renewable energy from 6 to 12% of the total energy consumption in the European Union by 2010. According to the White Paper, the major part of this renewable energy could come from woody biomass. This means that, additionally, over 160 million m^3 of woody biomass per year would be used for energy in Europe (Parikka, 2004).

Plantations help ease shortage of forestry wood. The establishment of new plantations is assumed to increase between 160 and 235 million ha in year 2050 (Whityeman and Brown, 1999). Around 2030 the industrial wood supply from plantations is estimated to be 45% of the total consumption of industrial wood compared to 22% in 1997. Thus, the above-identified regional and global shortages of wood supply would be much worse without the establishment of plantations.

Large-scale production of energy crops should not compete for land needed for food and fibre production. There have been careful calculations made that there is enough suitable land available to provide the world›s population with all its needs for food, fibre and energy throughout this century (FAO, 2000), althoough equitable distribution of these basic necessities is another issue yet to be resolved. In some regions the availability of water will be the constraining factor to growing energy crops rather than available land.

The objective of this study is to present the results of multi annual research of the creation and improvement of the selected poplar clones intended for the establishment of the so-called energy plantations, i.e. plantations with a very high number of plants per hectare and short rotation cycles.The aim is to present the results of examination of mean annual increment and estimated energy production of plantations with the high number of poplar plants per hectare. Comparison of the results of registered and experimental poplar clones is presented and discussed.

MATERIALS AND METHODS

The results of the research of poplar clones in the first part of paper refer to *Populus x euramericana* Guinier (Dode) cl. I-214, and the spacing in the plantaiton from 1.00 x 0.25 m (density 40,000 plants ha^{-1}), to 2.0 x 2.0 m (i.e. 2,500 plants ha^{-1}). All the experimental plantations were established on alluvial (fluvisol) and humofluvisol soils of the Middle Danube Basin. Five experimental plantations were selected for investigations consisted of the above poplar clone, on the soils of approximate productive capacity, with planting density and spacing which enable relatively short rotations (one to five years) and plantation renewal by coppice vigor, and with maximum wood and bark biomass productivity.

Second experimental field plantations were established in experimental estate "Kacka suma" (N 45°17' 36,7'' E 19°52' 56,4''). In the field trial 6 poplar clones (*P.x euramericana* cl. Ostia, *P.nigra* cl.53/86, *P deltoides* cl. PE 19/66, *P.x .euramericana* cl.I-214, *P.x euramericana* cl. S6-7, *P.x euramericana* cv. Robusta), with two different plant densities (38,461 plants ha^{-1}, and 83,333 plants ha^{-1}) are being tested. All clones were planted as 25-cm-long dormant, unrooted cuttings obtained from the Institute. The cuttings were stored at 4^0 and than soaked in water for 24 hours prior to planting.. Cuttings were planted manually to a depth of 22-23cm, leaving one or two buds above the soil surface. Above ground biomass was harvested at the and of the first growing season.

Third experimental field plantations were established by sprouting one shoot per stool after harvesting in the experimental estate "Kacka Suma" with seven experimental clones of eastern cottonwood *P. deltoides* cl. B-229, *P. deltoides* cl. B-81, *P. deltoides* cl. 129/81, *P. deltoides* cl. 182/81, *P. deltoides* cl. 181/81, *P. deltoides* cl. PE 4/68, *P. deltoides* cl. *665* and euramerican poplar *P. x euramericana* cl. M-1, planting space with 16667 plants ha^{-1} (1.5 m between rows, and 0.4 m within rows). Experimental plantations were established on two different forms of fluvisol – sandy and loamy (Plantation 1 and Plantation 2). The main physical and chemical characteristics of the soil were determined by standard methods, based on which the soil was characterised as very favourable for poplar growing.

After the selection of characteristic sample trees, measured parameters of growth elements were determined. Sample trees were chosen as average plants based on average diameter and height on the experimental plot. Biomass volume per unit area was calculated, as well as volume increment, and biomass weight (aboveground biomass weight) was determined based on bulk density of the analysed clones. The heat which could be produced by full combustion of the aboveground biomass per hectare was calculated based on the calorific value of wood (and bark) of individual clones.

For the determination of moisture content, wood samples were oven dried at 104^0C to a constant weight. All analyses were done in duplicate and the results were expressed on a dry weight basis.

Wood density was determined on the basis of oven-dry weight per green volume of an individual wood specimen.

The calorific value was determined for ground air-dried samples. Pellets were combusted in C200 IKA Werke calorimeter (three replications for each sample).

RESULTS AND DISCUSSION

***Populus x euramericana* Guinier (Dode) cl. I-214**

This part of paper presents the results of the research of biomass production of *Populus x euramericana* Guinier (Dode) cl. I-214, in one-year, two-year, three-year, four-year and five-year rotations. In this way, the production process during 8-10 years and from two to nine rotations lasting from one to five years, produces annually on the average between 40.9 and 53.9 m^3, i.e. 14.8 to 19.8 tons (of oven-dry mass) of wood and bark per ha, which can provide (combustion of whole tree chips) energy from 216 to 285 GJ (Markovic et al., 1996).

In the plantations with one-year rotations (Table 1), average diameter amounts to 2.0cm to 2.8 cm, height 3.0 m to 3.5 m. Volume percentage of bark in the total biomass in the plantations with one-year rotations is about 47%. The quantity of bark per hectare of plantation was calculated based on the bark percentage.

Table 1. One-year rotation in nine-year production process (clone I-214)

Plant spacing, m	Rotation number	Plant number per ha, N	Dimensions		Volume			Biomass (o.d.)		
			ds	Hs	Total	Wood	Bark	Wood	Bark	Total
			cm	m	m³/ha			DMt/ha		
1.00 x 0.25	I	40,000	2.0	3.0	15	7	8	2.1	3.6	5.7
	II	80,000	2.5	3.5	50	25	25	7.5	11.25	18.75
	III	100,000	2.8	3.5	80	45	35	13.5	15.75	29.25
	IV	100,000	2.8	3.5	80	45	35	13.5	15.75	29.25
	V	100,000	2.8	3.5	80	45	35	13.5	15.75	29.25
	VI	80,000	2.8	3.5	60	35	25	10.5	11.25	21.75
	VII	60,000	2.8	3.5	50	25	25	7.5	11.25	18.75
	VIII	50,000	2.8	3.5	40	20	20	6.0	9.00	15.00
	IX	40,000	2.8	3.5	30	15	15	4.5	6.75	11.25
Total for 9 years					485	262	223	78.6	100.3	178.9
Annual average					53.9	29.1	24.8	8.7	11.2	19.95
%					100.0	54.0	46.0	43.9	56.1	100.0
Energy (GJ/ha)					for 9 years			1354	1216	2570
					Annual average			150	135	285
					%			52.7	47.3	100.0

Due to the fact that this study deals with the biomass of very young trees, bark weight per unit area is presented separately, disregarding the fact that the bark is not removed from so young plants, i.e. the trees are not barked before chipping. However, as the bark has a relatively high calorific value, it is significant to present the percentage of bark in the total energy released by biomass combustion.

Under planting density of 40,000 plants per hectare (Table 1) production cycle is one year, attaining small dimensions: diameter 2-3 cm and height 3.0-3.5 m, i.e. volume 15-80 m^3/ha (average 55 m^3/ha) depending on production cycle. Such plantations produce high percentage of bark, almost 50% of the volume and above 50% of the mass, so that it is possible to produce averagely 285 GJ per hectare annually.

Registered poplar clones

Biomass yield per unit area and energy were estimated of 6 poplar clones (*P.x euramericana* cl. Ostia, *P.nigra* cl.53/86, *P deltoides* cl. PE 19/66, *P.x .euramericana* cl.I-214, *P.x deltoides* cl. S6-7, *P.x euramericana* cv. Robusta), with two different plant densities (38461 plant/ha and 83333 plant/ha) aged one year rooted cuttings.

The study results of biomass yield after the first year show (Table 2) that the increase of planting density has not the same effect on all the study clones. Namely, cl. I-214 shows the rise of biomass yield for about 8%. Biomass yield of the clone PE 19/66 has a downward tendency for about 9%. The clones Ostia and Robusta are significantly behind, because their yield is lower for 60%. Maximal values of biomass yield in the plantations with 38.461 plants/ha were attained by the clones S6-7 (28.769 t/ha year), Robusta (24.038 t/ha year), and PE 19/66 (23.846 t/ha year). It should be noted that PE 19/66 had the maximal yield also in a denser plantation (21.667 t/ha year). Clone S6-7, with 13.083 t/ha year, is the second by the yield in a denser plantation, although this is only cca 55% of its yield attained in the thinner plantation.

The minimal amount of energy is produced by cl. I-214 (188.728 GJ/ha). Clones Robusta and PE 19/66 have the advantage over the other study clones in the plantations with a lower number of trees. It is interesting that the thermal energy obtained by the combustion of PE 19/66 trees is similar also in a denser plantation (396.354 GJ/kg) and that the drop is only 3%, which is minor compared to the drop of almost 70% for Ostia or 61% for Robusta. Clone I-214 showed a slight increase (cca 8%) in the denser planting, which is explained by insignificant changes of biomass yield.

Experimental poplar clones

Experimental field plantations were established by sprouting one shoot per stool after harvesting in the experimental estate “Kacka Suma” with seven experimental clones of eastern cottonwood *P. deltoides* cl. B-229, *P. deltoides* cl. B-81, *P. deltoides* cl. 129/81, *P. deltoides* cl. 182/81, *P. deltoides* cl. 181/81, *P. deltoides* cl. PE 4/68, *P. deltoides* cl. *665* and Euramerican black poplar *P. x euramericana* cl. M-1, planting space with 16667 plants per hectare.

Table 2. Biomass and energy production after first growing season

Clone	Biomass yield, DM t/ha	Energy, GJ/ha
Plant density 38461 trees/ha		
Ostia	22.692	398.993
53/86	16.654	315.144
PE 19/66	23.846	407.051
I-214	11.923	188.728
S6-7	28.769	589.908
Robusta	24.038	470.159
Plant density 83333 trees/ha		
Ostia	6.917	121.622
53/86	8.750	160.064
PE 19/66	21.667	396.354
I-214	12.999	205.215
S6-7	13.083	268.267
Robusta	9.417	184.187

The minimal values of average biomass weight after the first year were measured for clone 181/81 (1.458 t ha^{-1} in plantation 1 and 1.781 t ha^{-1} in plantation 2), while the maximal yield was shown by clone B81 in plantation 2 (6.617 t ha^{-1}). As for the average value in both plantations, the best clone was B229, because it attained a high biomass yield on both soil types - average 5.101 t ha^{-1}. Biomass yield after the second year was much more uniform in the study plantations, which means that the effect of soil type was much lower (Fig.1). However, the differences among individual clones were great – e.g., the maximal yield (cl.B229 - 22.183t ha^{-1}) was more than three times higher than the minimal yield (cl.181/81 - 7.076t ha^{-1}).

During only for the second year, the increase in yield was from 5.601t ha^{-1} for clone 181/81 (min), to 15.002t ha^{-1} for clone B229 (max). This agrees with the literature data, because the yields given in the literature for poplars in SRC differ considerably. Maximum yields lie between 20 and 35 o.d.tha^{-1} yr^{-1} mean annual increment (Ciria et al., 1995; Scarascia et al., 1997), but other publications report mean annual increment in the range of 2 - 3 o.d.t ha^{-1} yr^{-1} (Schneider, 1995). Average harvestable yields of poplars from SRC in temperate regions of Central Europe and North America range between 10 and 12 o.d.t ha^{-1} yr^{-1} (Kauter et al., 2003). The yield after the first year (18,000 plants ha^{-1}) ranges from 2.2 to 3.6 o.d.t ha^{-1} for poplar clones and 2 to 2.5 o.d.t ha^{-1} $year^{-1}$ for willow clones (Hanson, 1991). Riddel-Black et al., (1996) report that the yield of six poplar clones (16,500 plants ha^{-1}) after the first growing season was 4.88 to 9.54 o.d.t ha^{-1}.

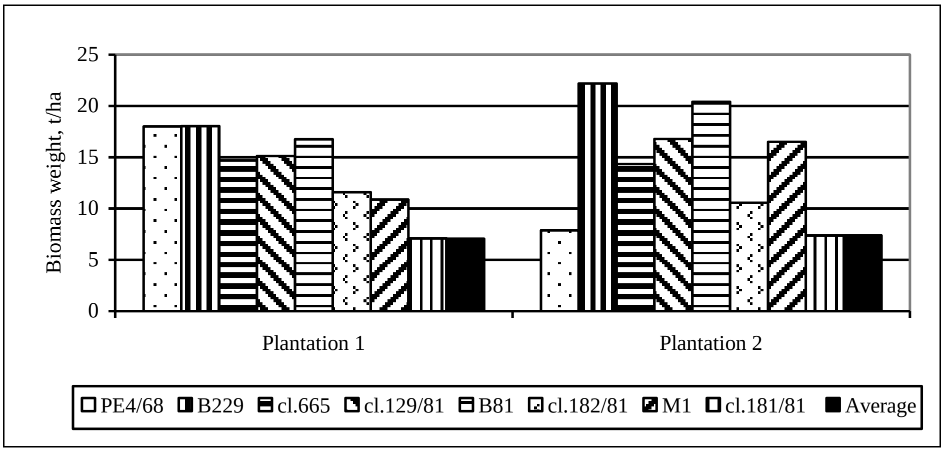

Figure 1: Estimated biomass yield after the second year

Average heating value of the analysed poplar clones ranged in a very narrow interval from 17,340 MJ/kg (clone M1) to 18,743 MJ/kg (clone B229). This agrees fully with the values of our previous research (Klasnja et al., 2002, 2006, 2008), and the values reported by Ciria et al., (1995) for heating values of 3 – 5-year old SRIC poplar wood (stem and branches) 18.1 – 18.3 MJ/kg. Benetka et al. (2002) for 1-3 year old poplar clones (wood at breast height and basal part, and branches) reported heating value from 18.60 MJ/kg to 19.27 MJ/kg.

The values of estimated energy yield per plantation unit area, depending on the soil type (locality), after the second year is presented in Fig.2.

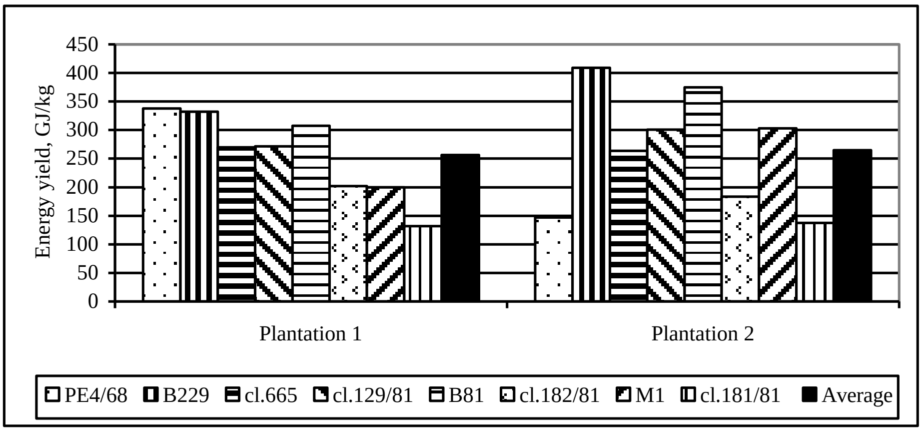

Figure 2: Estimated energy yield after the second year

Estimated energy in average of 64.883 GJ ha^{-1} (for all clones) after the first year, is significantly higher in second year and amounts to 260.741 GJ ha^{-1}, which is the increase by about four times. The ratios between the clones were similar to biomass yield, so the ratio of the maximal energy yield (408.84 GJ ha^{-1}max. clone B229), and minimal energy yield (137.53 GJ ha^{-1} min. clone 181/81) was about 3:1.

CONCLUSIONS

This paper presents the comparative results of the study of harvested biomass in the plantations with a high number of plants per hectare. The experiments were established by different methods: classical method by cuttings of experimental clones, and by stump sprouting of one shoot per stool of the new, experimental clones.

Under planting density of 40,000 plants per hectare (cl. I-214), production cycle is one year, average weight of biomass was 11.25 DM tha^{-1}. Such plantations produce high percentage of bark, almost 50% of the volume and above 50% of the mass, so that it is possible to produce averagely 285 $GJha^{-1}$annually.

The minimal value of average biomass weight for the experimental poplar clones after the first year was measured for clone 181/81 (1.458DM tha^{-1}), while the maximal yield was shown by clone B81 (6.617 DM tha^{-1}). During the second year, the increase in biomass weight was from 5.601DM tha^{-1} for clone 181/81 (min), to 16.644DM tha^{-1} for clone B229 (max).

Average estimated energy which can be produced by the combustion of the total (aboveground) biomass of experimental clones after the first year was 73.339 $GJha^{-1}$ (for all clones), is significantly higher after second year and amounts to 264.943 $GJha^{-1}$, which is the increase more than three times. The ratios between the clones were similar to biomass yield, so the ratio of the maximal energy yield (408.843 $GJha^{-1}$ max. for clone B229), and minimal energy yield (137.531 $GJha^{-1}$ min. for clone 181/81) was about 3:1.

Maximal values of biomass yields for registered poplar clones (plant density 38.461 plants ha^{-1}) were attained by the clones S6-7 (28.769DM tha^{-1} $year^{-1}$) and minimal by the clone I-214 (11.923DM tha^{-1} $year^{-1}$). The calculated amounts of energy show that max value is recorded for the clone S6-7 (589.908 $GJha^{-1}$), and the minimal amount of energy is produced by cl. I-214 (188.728 $GJha^{-1}$).

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UN/ECE ICP FORESTS IN CROATIA – A REVIEW OF CROWN CONDITION IN THE PERIOD 1998 - 2007

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Summary: *The assessment of crown condition in Croatia has been conducted since 1987, according to the methods and in cooperation with the International Cooperative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests). In this paper the data on crown condition of forests in Croatia on the 16 x 16 km network of bioindication plots is presented for the 10-year period 1998-2007, separately for all species, broadleaves, and conifers. The crown condition of three most prominent tree species in Croatia, Pedunculate oak (Quercus robur L.), Common beech (Fagus sylvatica L.) and Silver fir (Abies alba Mill.) are presented in comparison with the results of European assessments.*
The percentage of moderately to severely damaged trees in Croatia does not fluctuate significantly in the research period. While the damage of oak and beech is higher in Europe, silver fir is more damaged in Croatia.
Keywords: *ICP Forests, crown condition, Pedunculate oak, Common beech, Silver fir, Moderate to severe damage*

INTRODUCTION

Given the common view on widespread forest dieback being caused primarily by atmospheric pollution, the International Cooperative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests) were founded in 1985 under the umbrella of United Nations Convention on long-range transboundary air pollution (CLRTAP).

Croatia takes part in the ICP Forests program since 1987, when first large-scale and permanent monitoring of forest ecosystems started on 16x16 km grid network of plots (European network – Level 1), 4 x 4 km network of plots (national network of plots) and intensive monitoring plots (plots in characteristic forest associations of Croatia – Level 2) according to common ICP Forests methods. The major aim of transnational Level I survey is to provide a periodic overview on the spatial and temporal variation in forest condition in relation to anthropogenic and natural stress factors in a European and national large-scale systematic networks (Eichhorn et al. 2006). Forest Research Institute is the National Focal Center of ICP-Forests. Since 2007, programme in Croatia is funded by the Ministry of Rural development, Forestry and Water Management.

With the gradual development of knowledge on functioning of forest ecosystems it has become clear that a multitude of ecological factors play a role in the dieback of forests. Therefore the main task of the program has become to collect and interpret the data on vitality of forests ecosystems and their reaction to various stress factors on regional, national and international level. While in the past the degradation of forest ecosystems was attributed almost exclusively to atmospheric pollution, in recent years there has been a growing tendency to attribute the deterioration of crown condition of forest trees to various factors responsible for health condition of forests. With recent lowering of the amount of sulphur and nitrogen compounds being deposited into forest ecosystems, the attention of the scientific community operating as a part of ICP Forests network has shifted towards the climate change and the damaging influence of ground-level ozone.

In this paper the data on crown condition of forests in Croatia for the period 1998 – 2007 are presented as totals and separately for broadleaves and conifers, as well as for the three dominating tree species – Pedunculate oak (*Quercus robur* L.), Common beech (*Fagus sylvatica* L.) and Silver fir (*Abies alba* Mill.). The share of moderately to severely damaged trees is compared to Europe-wide data for these three species.

MATERIALS AND METHODS

Within the transnational survey (Level I – 16 x 16 km grid network) sample plots and trees Kraft classes 1-3 (1 = dominant; 2 = codominant, 3 = subdominant) should be selected according to a statistically sound procedure which includes the random principle. An example is the 4-point cross cluster, with 4 subplots oriented along the main compass directions at a distance of 25 m from the grid point. On each subplot the 6 trees nearest to the subplot centre are selected as sample trees, resulting into 24 sample trees per plot.

Figure 1. ICP Forests Level I plots in Croatia

The crown present at the moment of the assessment is to be considered, regardless of the potential or theoretical crown which may have existed in previous years. The influence of any present or absent (removed) trees on the crown of the sample tree must be taken into account when determining its condition. In cases where the sample tree crown is influenced by competition, the assessable crown includes only those parts that are not influenced by other crowns i.e. shading. Parts of the crown directly influenced by interactions between crowns or competition are excluded.

Table 1. Defoliation classes

Class	Defoliation	Defoliation (%)
0	none	0-10 %
1	slight	>10-25 %
2	moderate	>25-60 %
3	severe	>60-99 %
4	dead	100%

The assessable crown of a freely developed tree is defined as the whole living crown from the lowest substantial living branch upwards. In Croatian practice the part of crown that is assessed is defined as the «light crown», i.e. the part of the crown that is in contact with direct sunlight. Defoliation is generally estimated in 5% classes relative to a tree with full foliage. The results are then grouped into classes as shown in Table 1. All trees with defoliation over 25 % belong to the class of moderately to severely damaged trees that is often used as an indicator of forest damage. The reference tree can be either a healthy tree in the vicinity (of the same crown type), a photograph locally applicable, representing a tree with full foliage or a conceptual (imaginary) tree. Observers should have a satisfactory view of the tree from several observation points. On level ground, the optimal view is given at a distance of one tree length. On slopes, trees should be observed at a distance of about one tree length above the tree or at least on the same level. It is recommended that assessments

should be done by two trained observers. When the estimates produced of the two observers differ, both should change their observation position.
The number of Level I plots varies from year to year of assessment due to various reasons (fire, storm, end of rotation period etc.). In Croatia there are 101 plots (Figure 1.) of which 83 were assessed in 2007.

RESULTS AND DISCUSSION

In 2007, the percentage of trees of all species within classes 2-4 in 2007 (25,1%) is somewhat higher than in 2006 (24,8 %) and comparable to the year 2004 (25,2%). The highest percentage of moderately to severely damaged trees was recorded in 2005 (27,1%), and lowest in 2002 (20,6 %). The share of moderately to severely damaged trees before 2002 never reached values above 25 % (Fig. 2).

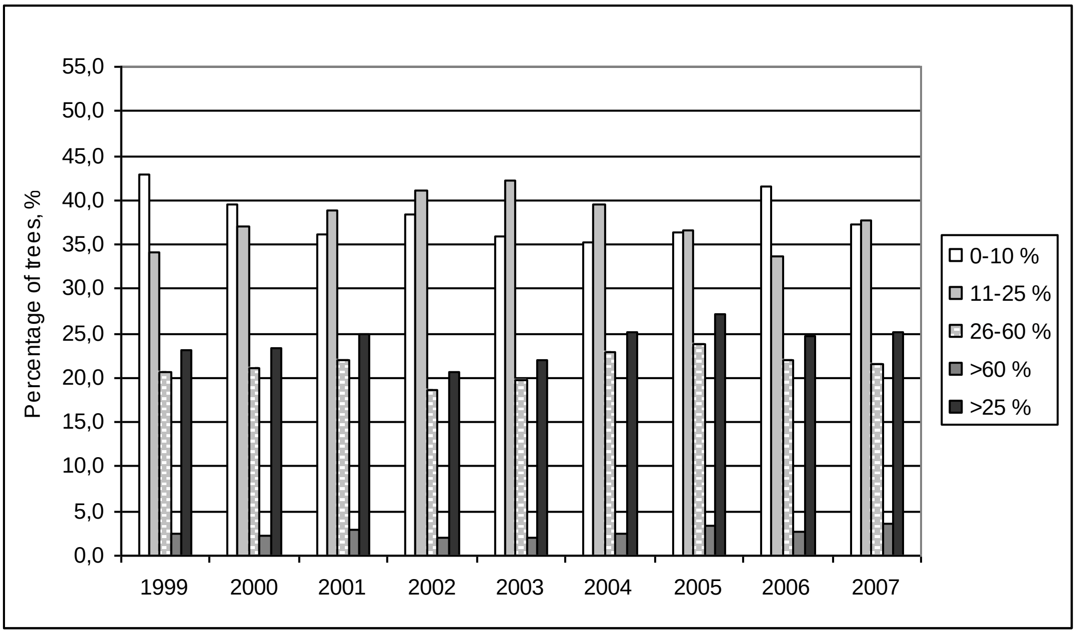

Figure 2. Crown defoliation on Level I plots in Croatia 1999 – 2007 (all species)

The trend of defoliation of broadleaved species follows closely the pattern of all species. The share of trees in classes 2-4 is highest in 2007 (20,0%), somewhat higher than in 2006 (18,2 %). The lowest value was recorded in 2003 (14,3 %).

For conifers, the percentage of damaged trees of classes 2-4 in 2007 (61,1%) is significantly lower than in 2006 (71,7 %) and the value reported in 2005 (79,5%). The share of moderately to severely damaged trees was lowest in 1999 (53,2 %) and highest in 2005.

Although the percentage of moderately to severely damaged conifers is high, it does not have a stronger impact on the overall percentage of trees of all species for the same damage class, due to the low representation of conifer trees in the sample (252 conifer trees vs. 1760 broadleaves in survey 2007).

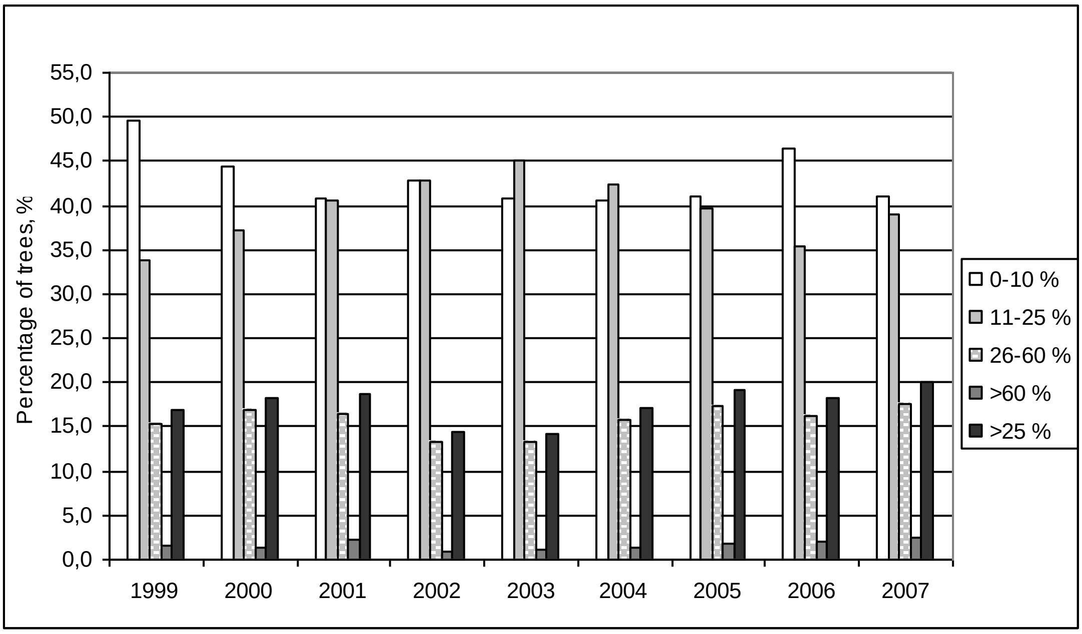

Figure 3. Crown defoliation on Level I plots in Croatia 1999 – 2007 (broadleaves)

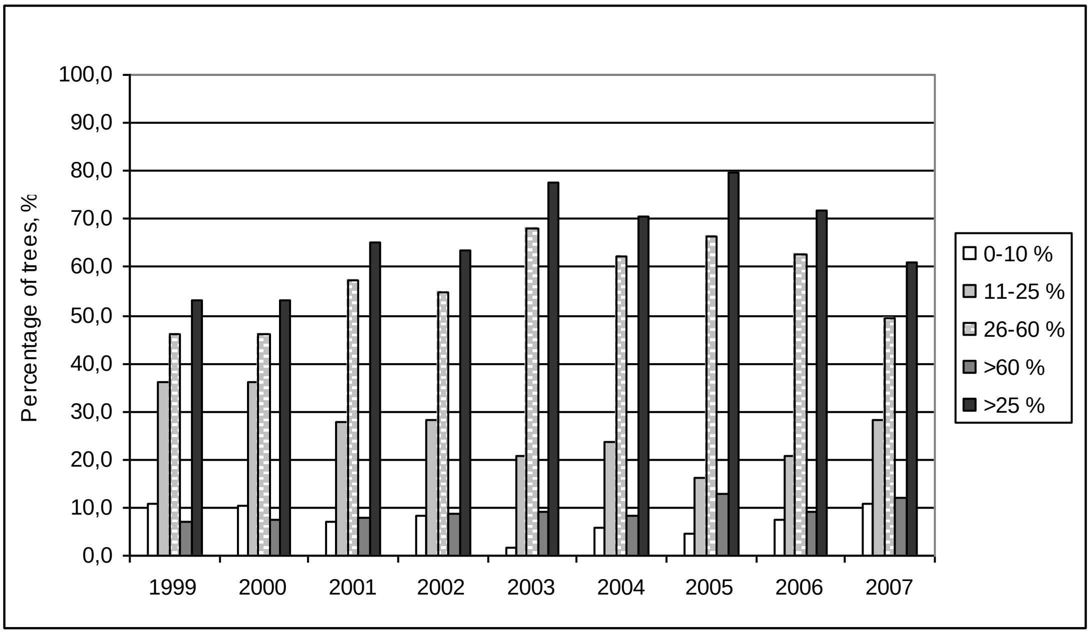

Figure 4. Crown defoliation on Level I plots in Croatia 1999 – 2007 (conifers)

Silver fir and Common oak (along with Common beech) are the most widely distributed and the most ecologically and economically important tree species in Croatia, therefore their high defoliation is a matter of great concern.

The share of moderately to severely damaged Silver fir was rising from 1999 to 2005. The lowest value of moderately to severely damaged fir trees was recorded in 2007 (67,9 %), compared with 69,7 % in 2006 and the highest value reached in 2005 (88,5). In Europe the values were significantly lower, but the trends are similar. It is interesting that the negative effects of 2003 drought could still be seen in Croatian data in 2005, one year longer than in Europe-wide data (Fig. 5).

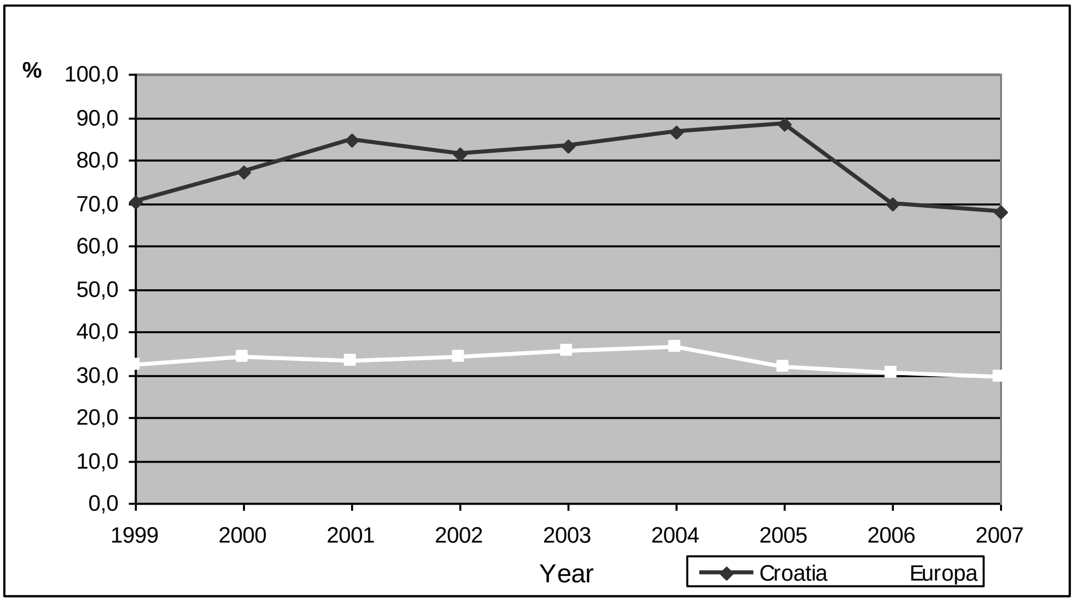

Figure 5. Moderate to severe damage of Silver fir – comparison between Croatia and Europe (All regions) (European data from Lorenz et al. 2008)

The lowest damage of Pedunculate oak was recorded in 2007 (19,8%), the highest in 2004 (30,6%), and in 2001 (30,2%). The Europe-wide values are higher in the same period, but following a similar trend (Fig. 6).

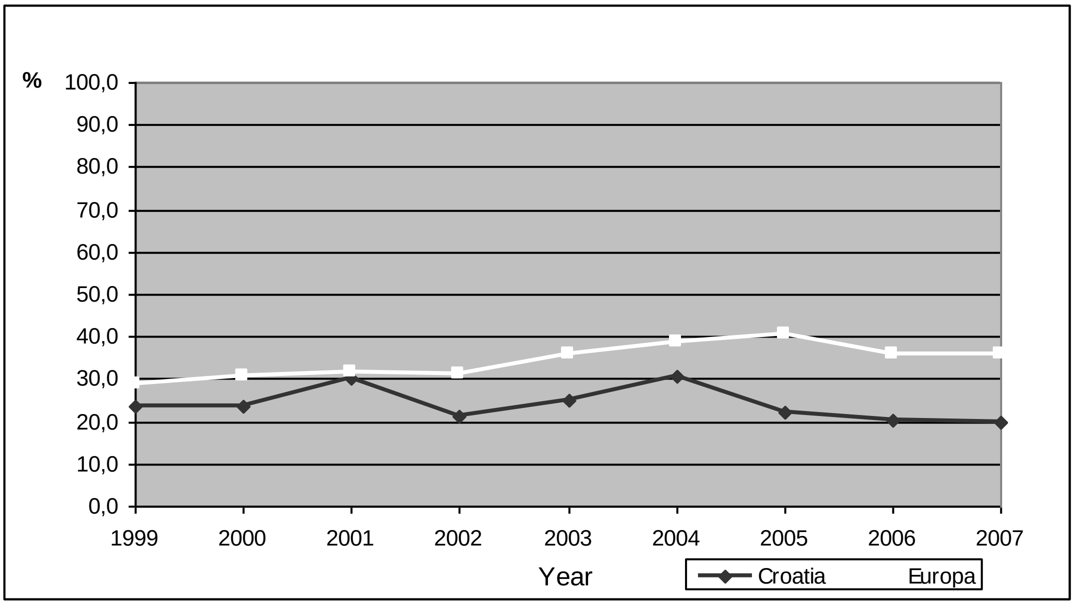

Figure 6. Moderate to severe damage of Pedunculate oak – comparison between Croatia and Europe (All regions – European oaks) (European data from Lorenz et al. 2008)

Common beach remains the least damaged tree species in Croatia. The percentage of moderately to severely damaged beech trees is rarely higher than 10 % (the highest value was recorded in the year 2001 - 12,5 %), and it was i the range 5-8 % ever since. The lowest value was assessed in 1999 (3,8%). These values are far lower than the European share of moderately to severely damaged beech trees (Fig. 7).

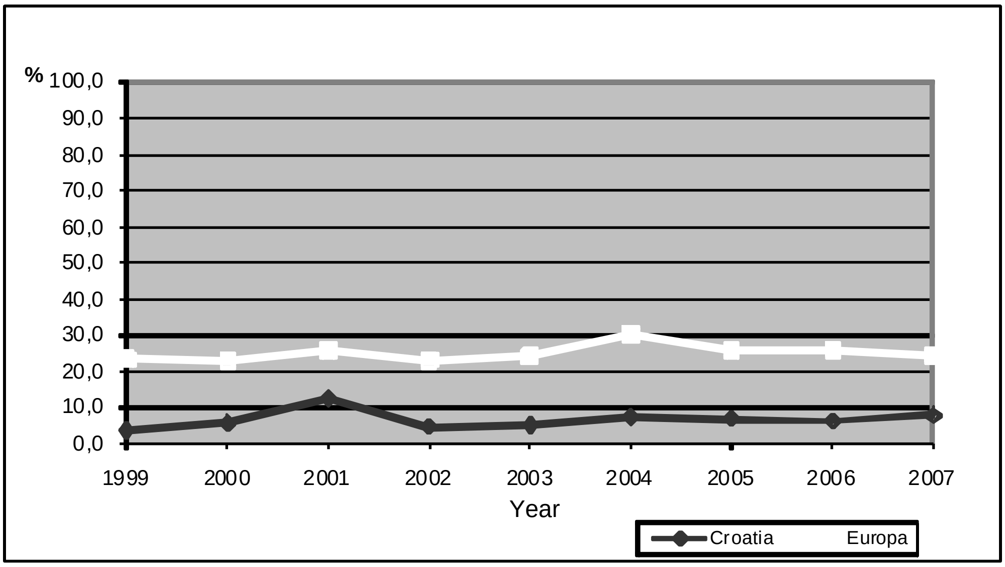

Figure 7. Moderate to severe damage of Common beech – comparison between Croatia and Europe (All regions) (European data from Lorenz et al. 2008)

Overall, the state of crown defoliation in Croatia did not change significantly in the period 1999 – 2007. A cyclic pattern can be discerned, with starting points one year after drought years (2000, 2003).

Although the crown condition of some important tree species is poor, the practice of sustainable, close-to-nature forest management, as well as the enforcement of environmental laws, help protect the stability of the forest ecosystems in Croatia. The new challenges for the condition of Croatian forests are opening of new motorways and the expected increase in traffic-related pollution.

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VARIABILITY OF ROOTING CHARACTERS IN ONE-YEAR OLD BLACK POPLAR (*Aigeiros* Duby) ROOTED CUTTINGS

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Abstract: *The successful rooting of planting material is one of the key moments of the establishment of black poplar (section Aigeiros Duby) plantations. In that sense it is important to evaluate the significance of factors that affect rooting of planting material and characters that are strongly dependent on these factors. In this work the rooting of one-year old rooted cuttings of five selected genotypes of section Aigeiros Duby were examined. The experiments were established on two sites in three repetitions. Number of first-order roots and sum of cross-section areas of roots near the root base were examined for whole plant and for three parts of plant: stem, cutting and remains of last year formed roots. Their contribution to the total amount for whole plant was also examined. According to analysis of variance the differences among sites significantly affected examined sum of cross-section areas of roots on almost all examined parts of plant except on cutting. Characters for stem of rooted cutting were rather responsive on differences among sites as well as among examined clones. Better rooting and root development were gained on site with more silt+clay fraction in surface horizon. Best results achieved P. x euramericana cl. "Pannonia". The most significant influence of examined factors was achieved on the contribution of number of roots from remains (mainly edge of cross-cut) of formerly formed roots and particularly stem to the total number of roots. The roots of shoot had mostly perpendicular orientation against plant axes. Roots that emerged from old the cuts of last year formed roots emerged in different angles against plants axes (some of them had the orientation of tap-roots). These results could be implemented in breeding and design of cultivar-adjusted plantation establishment technology.*
Key words: *Black poplars, plantation establishment, breeding*

INTRODUCTION

Rooted cuttings are often used in the establishment of Short and Middle Rotation Coppice as well as long-term poplar plantations. High rooting potential of black poplar plants allows successful establishment of plantations. Beside high rooting potential of the remaining of roots, rooting is also based on preformed primordia in primary bark of the shoot that were formed during previous growing period. However, activation of primordia is complex process, influenced by many factors: genetic factors (within and between species), factors of variation within genotype (among and within plants) and numerous factors of environment and technological procedures (Ying & Baglay, 1976; Fege, 1983; Zalesny *et al.*, 2005; Kovacevic *et al.*, 2007; Kovacevic *et al.*, 2008).

The age of plants that are used in the establishment of black poplar plantation defers, but the results of Smit & Wareing (1972) suggest that the best rooting capacity could be expected in one-year old plants. Because different genotypes are used in poplar plantation production, differences among them in their reaction on factors environment and technological procedures could be expected. That applies for the design of technology adjusted to specific characteristics of cultivar in course of optimal utilization of genotype potentials, as it was emphasized by Heilman *et al.* in 1991 (according to De Bell & Harington, 1997), Zalesny *et al.* (2005) and Kovacevic *et al.* (2008).

The aim of this work was to examine and evaluate the effect of clone and soil types, as well as their interactions on variation of rooting characters of one-year old rooting cutting.

MATERIAL AND METHODS

The experiment was established on Experimental Estate of "Institute for Lowland Forestry and Environment" near Novi Sad, Serbia. Five genotypes were examined: *Populus deltoides cl.* PD100, *Populus deltoides cl.* S6-7, *Populus deltoides cl.* B-17, *Populus x euramericana cl.* Pannonia and *Populus deltoides x ? cl.* 182/81. Genotype Pannonia is registered in Serbia and the others are in experimental phase. Experiment was established on two sites with sandy fluvisol soil type, in spacing: 1,00 x 1,00 m, in the middle April

1997. Two sites deferred in the depth of ground water level (200 cm and 250 cm for site "Vrbak" and site "Fister", respectively). Also, soil in Fister had considerable loamy sand layer from the surface horizon to ground water level that interrupted upward capillary flow from ground water. Used plants had 200-250 cm high stem, with the old cutting and the 5-10 cm long remainings of roots left on. There were three plants per clone randomly distributed, as a repetition. Plants were planted at a depth of 80 cm. The experiment was mechanically cultivated on regular base. At the end of growing period vital plants were carefully dug out and following rooting characters were measured: total number of roots (TRN), number of roots on shoot (SRN), number of roots on cutting (CRN), number of roots on last-year roots remainings (PRN), total root cross-section area (TRA), root cross-section area for shoot (SRA), root cross-section area for cutting (CRA), root cross-section area for roots on last-year roots remainings (PRA), percentage of number of roots on shoot (SRNP), percentage of number of roots on cutting (CRNP), percentage of number of roots on last-year roots remainings (PRNP), ratio SRA/TRA (SRAP), ratio CRA/TRA (CRAP), PRA/TRA (PRAP).

The variation of examined characters was analyzed by two-way analysis of variance, fixed model:

$$X_{ijm} = \mu + a_i + b_j + ab_{ij} + \varepsilon_{m(ij)} ,$$

where a_i stands for effect of i^{th} genotype, b_j stands for effect soil characteristics of j^{th} site, while ab_{ij} is the effects of interaction of examined factors. Label $\varepsilon_{m(ij)}$ stands for the residual effect of repetition. Results of two-way ANOVA were used for calculation of expected variances for examined sources of variation (Kirk, 1968).

Relative contribution of expected variance for a source of variation X to the total variance of a variable ($\frac{\sigma_X^2}{\sigma_T^2} * 100\%$) and coefficient of variation ($Cv = \frac{\sigma_A}{\overline{X}} * 100\%$) were used as a measure of influence of that source on variation of variable in concern.

Data for number of roots was transformed by square-transformation ($\sqrt{X_{ijm} + 1}$), while the percentage of roots data was transformed by arcsin – transformation ($\arcsin \sqrt{X_{ijm}}$).where X stands for average value for cuttings of the plot with i^{th} genotype, j^{th} cuttings' length, and k^{th} bud position and m^{th} repetition.

Group means were compared by Fisher's LSD-test.

The program package STATISTICA 7.1 (StatSoft, 2006) was used in the statistical analysis.

RESULTS AND DISCUSSION

Results presented in this work show that examined characters differed in the dependence of their variability of examined sources of variability. For the characters describing rooting properties in total and on old cutting, only total (TRA) and root cross-section area for new roots on old cutting (CRA) showed significant dependence on the differences between sites. Worse performance was in the soil with deeper ground water level. The number of roots on preformed roots (PRN) and their cross-section area (PRA) did not depended significantly on examined sources of variation. The effect of error was dominant. However, their contribution to the TRN and TRA (PRNP and PRAP, respectively) were under significant effect of genotype. The character PRNP was also significantly influenced by the main effect of site and interaction genotype × site.

Examined sources of variation achieved the most considerable effect on variation of characters concerning the roots localized on the part of shoot in the ground. Only the effect of interaction genotype × site on SRA and main effect of site conditions on SRAP were not significant. The best rooting on the shoot part achieved clone Pannonia, while the poorest rooting on the shoot achieved clones 182/81 and S6-7.

The root formation mostly occurred on the shoot and on the cut of the remaining of the old roots. There were no significant differences among genotypes in number and cross-section area of roots at the previous year root remaining, but were significant for roots on shoot. The rooting from the stem is based on preformed primoria in primary bark. This premordia were initiated and formed during the previous growing period. According to the previous results on cuttings (Kovacevic *et al.*, 2005; Kovacevic *et al.*, 2008), there is positive correlation between early root formation and survival of cuttings. As we did not follow the early rooting we can not say which roots emerged the first. However, according to Smith & Wareing (1972) and Kovacevic *et al.* (2008), the emergence of roots from preformed primordia of cutting precedes the formation of wound roots. Wound roots are initiated de novo, at the basal cut of the cutting. It takes time for them to initiate and start the formation of root, while pre-formed primordia has just to be activated to start root formation. In that sense, we suggest that roots from preformed primordia on shoot of plant also started to emerge before roots on the remainings of previous year roots.

Table 1. Analysis of variance for examined characters of black poplar genotypes

1) / 2)	MS_G	MS_S	$MS_{G×S}$	MS_{Err}	F_G	F_S	$F_{G×S}$	CV_G	CV_S	$CV_{G×S}$	CV_{Err}	$LSD_{0.05}$
TRN	2,240	5,018	0,287	1,343	1,668	3,736	0,214	5,604	7,174	0,000	15,334	2,178
TRA	1286423	9107675	531248.7	516246.8	2.492	17.642**	1.029	14.705	31.063	2.902	26.921	1350.155
CRN	1,556	0,978	0,534	0,654	2,377	1,494	0,816	17,188	6,513	0,000	32,754	1,520
CRA	139140.3	620969.5	135884	135679.7	1.026	4.577*	1.002	9.265	69.392	3.184	129.724	692.169
PRN.	0,162	0,003	0,791	1,598	0,101	0,002	0,495	0,000	0,000	0,000	22,550	2,375
PRA	484622.9	1077788	415541.6	280403.5	1.728	3.844	1.482	14.132	17.661	16.258	37.028	995.054
SRN	6,026	12,050	1,583	0,531	11,355**	22,706**	2,983*	23,978	21,957	14,840	16,662	1,369
SRA	682746.5	1420175	205391.2	113905.9	5.994**	12.468**	1.803	35.323	33.854	20.033	35.344	634.203
CRNP	102.117	57.383	62.322	73.994	1.380	0.776	0.842	13.853	0.000	0.000	50.245	16.164
CRAP	0,011	0,025	0,008	0,011	1,045	2,282	0,770	10,677	36,039	0,000	112,529	0,197
PRNP	354.575	688.694	220.273	60.421	5.868**	11.398**	3.646*	15.496	14.323	16.155	15.704	14.607
PRAP	0,084	0,043	0,032	0,013	6,635**	3,404	2,497	21,757	8,987	15,859	20,494	0,212
SRNP	350.054	606.115	183.819	36.003	9.723**	16.835**	5.106**	23.345	19.894	22.651	17.675	11.275
SRAP	0,044	0,002	0,038	0,010	4,397*	0,248	3,874*	23,010	0,000	29,934	27,918	0,187

1) Labels of examined characters: TRN – total number of roots, SRN – number of roots on shoot, CRN – number of roots on cutting, PRN – number of roots on last-year roots remainings, TRA – total root cross-section area, SRA – root cross-section area for shoot, CRA – root cross-section area for cutting, PRA – root cross-section area for roots on last-year roots remainings, SRNP – percentage of number of roots on shoot, CRNP – percentage of number of roots on cutting, PRNP – percentage of number of roots on last-year roots remainings, SRAP – SRA/TRA, CRAP – CRA/TRA, PRAP – PRA/TRA

2) MS – Mean squares, F – F-value from F-test, Cv – coefficient of variation (%) $LSD_{0.05}$ – the least significant difference at α=0.05

The evaluation of the significance of the examined rooting traits for the breeding purposes and design of cultivar technology depends on the dependence of variability of particular trait on the differences among genotypes and its relationship with plants' survival.

The roots that emerged on the cut of the remainings of last-year roots in our trail could also be considered as wound roots. These roots were found on the cut of last-year roots' remainings and their primordia were initiated after the plant preparation. The number (PRN) and cross-section area (PRA) did not depend significantly on genotype. Also, it could be said that the contribution of genotype to the total variation of their percentage in total (PRNP and PRAP, respectively), is primarily due to the close relationship to the percentage of number (SRNP) and cross-section area (SRAP) for roots on shoot. According to Kovacevic *et al.* (2005) and Kovacevic *et al.* (2008), the emergence of wound roots was not related to the survival of cutting, but only the emergence of roots from preformed primordial in the primary bark. Together with the fact that only the emergence of roots on shoot was under significant effect of genotype, this emphasize the rooting on shoot as an important trait for utilization in breeding and the design of cultivar technology.

The poor rooting was expected for S6-7 that in previous studies showed poor rooting potential of its cuttings (Kovacevic *et al.*, 2007; Kovacevic *et al.*, 2008). These results suggest that the information of rooting of cutting could be utilized in the evaluation of rooting potential in plants.

The rooting on shoot in 182/81 was poor. It was especially poor in site that could be less favorable for rooting (deeper ground water level), while there was no clear difference among sites in rooting on previous root remainings. Some results from previous studies (Kovacevic, 2003) suggest severe lost of newly formed roots in 182/81 during the developmentally caused water stress at the beginning of growing period (Kovacevic et al., 2008). However, additional studies are needed in order to reveal the cause of such reaction.

Table 3. Average values of examined characters

Genotype	Site	TRN[1)]	TRA		
182/81	Fister	5,89	1993,86		
182/81	Vrbak	7,39	2042,04		
B17	Fister	7,56	1974,75		
B17	Vrbak	7,98	3365,17		
Pannonia	Fister	8,22	1804,32		
Pannonia	Vrbak	8,76	3332,44		
PD100	Fister	6,86	2646,01		
PD100	Vrbak	8,16	4234,34		
S6-7	Fister	7,03	1907,73		
S6-7	Vrbak	7,74	3388,47		
		CRN	CRA	CRNP	CRAP
182/81	Fister	1,33	140,85	6,04	0,07
182/81	Vrbak	2,50	62,05	19,42	0,03
B17	Fister	1,85	133,26	12,05	0,06
B17	Vrbak	2,48	497,16	17,05	0,16
Pannonia	Fister	3,37	146,08	23,13	0,07
Pannonia	Vrbak	2,76	189,02	17,36	0,06
PD100	Fister	2,00	27,49	14,80	0,01
PD100	Vrbak	2,24	572,82	14,49	0,13
S6-7	Fister	2,80	184,04	22,01	0,10
S6-7	Vrbak	3,35	886,71	24,86	0,24
		PRN	PRA	PRNP	PRAP
182/81	Fister	5,64	1685,73	76,71	0,84
182/81	Vrbak	5,63	1485,97	48,40	0,67
B17	Fister	6,52	1328,89	59,45	0,66
B17	Vrbak	5,24	1173,65	40,37	0,34
Pannonia	Fister	5,03	916,04	36,95	0,51
Pannonia	Vrbak	5,91	1969,78	41,90	0,59
PD100	Fister	5,29	1405,08	50,01	0,53
PD100	Vrbak	5,55	2279,23	42,18	0,56
S6-7	Fister	5,49	776,50	50,61	0,41
S6-7	Vrbak	5,75	1279,94	48,39	0,39
		SRN	SRA	SRNP	SRAP
182/81	Fister	1,62	167,29	9,73	0,09
182/81	Vrbak	4,26	494,02	34,72	0,29
B17	Fister	3,32	512,60	24,64	0,28
B17	Vrbak	5,62	1694,37	44,31	0,50
Pannonia	Fister	5,67	742,20	43,91	0,41
Pannonia	Vrbak	5,97	1173,65	42,86	0,35
PD100	Fister	4,12	1213,44	36,14	0,46
PD100	Vrbak	5,65	1382,30	43,68	0,32
S6-7	Fister	3,65	947,19	30,70	0,49
S6-7	Vrbak	3,83	1221,82	28,79	0,37

Genotype		CRN	CRA	CRNP	CRAP
182/81		1,92	101,45	12,73	0,05
B17		2,17	315,21	14,55	0,11
Pannonia		3,07	167,55	20,24	0,07
PD100		2,12	300,15	14,64	0,07
S6-7		3,08	535,38	23,44	0,17
		PRN	PRA	PRNP	PRAP
182/81		5,63	1585,85	62,55	0,76
B17		5,88	1251,27	49,91	0,50
Pannonia		5,47	1442,91	39,42	0,55
PD100		5,42	1842,15	46,09	0,54
S6-7		5,62	1028,22	49,50	0,40
		SRN	SRA	SRNP	SRAP
182/81		2,94	330,65	22,22	0,19
B17		4,47	1103,48	34,48	0,39
Pannonia		5,82	957,92	43,39	0,38
PD100		4,89	1297,87	39,91	0,39
S6-7		3,74	1084,50	29,74	0,43
		TRN	TRA		
182/81		6,64	2017,95		
B17		7,77	2669,96		
Pannonia		8,49	2568,38		
PD100		7,51	3440,17		
S6-7		7,39	2648,10		
	Site				
		CRN	CRA	CRNP	CRAP
	Fister	2,27	126,34	15,61	0,06
	Vrbak	2,67	441,55	18,64	0,12
		PRN	PRA	PRNP	PRAP
	Fister	5,59	1222,45	54,75	0,59
	Vrbak	5,62	1637,71	44,25	0,51
		SRN	SRA	SRNP	SRAP
	Fister	3,68	716,54	29,02	0,35
	Vrbak	5,07	1193,23	38,87	0,37
		TRN	TRA		
	Fister	7,11	2065,34		
	Vrbak	8,01	3272,49		
LSD0.05	- values				
TRN	2,178	SRN	1,369	SRNP	11.275
TRA	1350.155	SRA	634.203	SRAP	0,187
CRN	1,520	CRNP	16.164		
CRA	692.169	CRAP	0,197		
PRN.	2,375	PRNP	14.607		
PRA	995.054	PRAP	0,212		

[1)] Labels of examined characters: TRN – total number of roots, SRN – number of roots on shoot, CRN – number of roots on cutting, PRN – number of roots on last-year roots remainings, TRA – total root cross-section area, SRA – root cross-section area for shoot, CRA – root cross-section area for cutting, PRA – root cross-section area for roots on last-year roots remainings, SRNP – percentage of number of roots on shoot, CRNP – percentage of number of roots on cutting, PRNP – percentage of number of roots on last-year roots remainings, SRAP – SRA/TRA, CRAP – CRA/TRA, PRAP – PRA/TRA

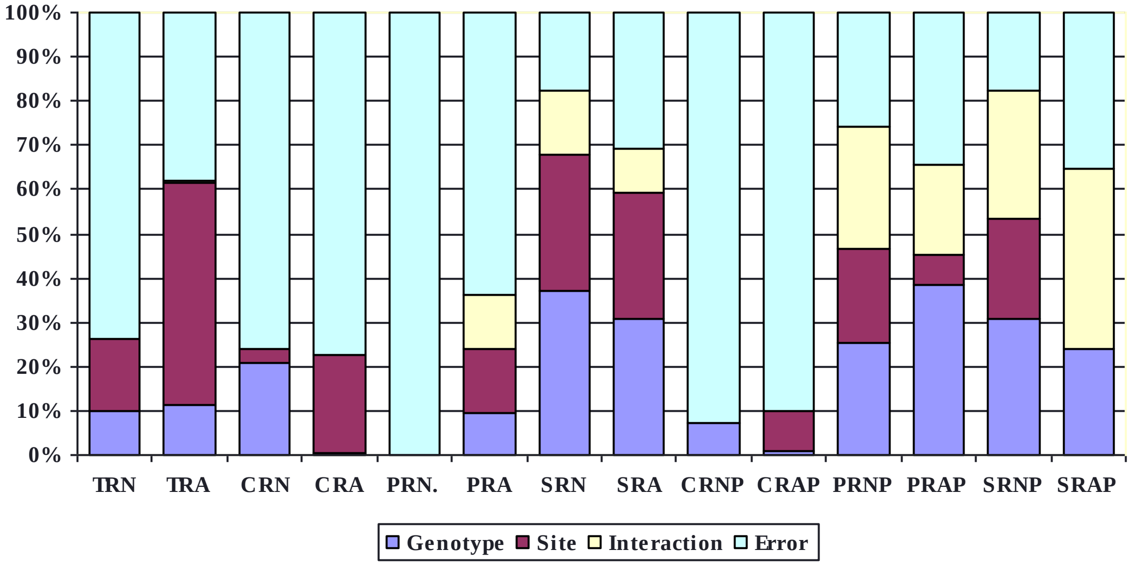

Figure 1. Contribution of expected variances to the total variance for examined characters in black poplar genotypes

The roots of shoot had mostly perpendicular orientation against plant axes. However, roots that emerged from last year formed roots emerged in different angles against plants axes. Some 50 – 70% of roots of total were oriented perpendicular against plant axes, 20 – 45% were oriented downwards, and less then 10% upwards (data not shown). Some downward oriented roots are rather thick, suggesting that they took the role of tap-roots.

CONCLUSION

According to presented data it could be concluded that significant differences for controlled sources of variation were found for traits concerning roots that emerged on stem, but not for the rooting on old cutting and remainings of last year found roots. Only among roots that emerged on the remainings of last year roots were those that were oriented downwards, performing the role of tap-roots. Best results achieved *P. x euramericana cl.* Pannonia. The clone *P. deltoides cl.* S6-7 achieved the poorest rooting on stem and because of that the poorest rooting in total. The information of rooting on stem could be utilized in breeding process and design of cultivar technology.

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ESTIMATION OF VOLUME AND METHOD OF FELLING IN HIGH BEECH STANDS BY STUMP INVENTORY

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Abstract: *The volume, type and spatial distribution of fellings were researched in high beech stands based on stump inventory on sample plots. Five high beech stands were investigated in East and West Serbia, at the altitude of 500-1000 metres. They belong to submontane beech forest (Fagetum moesiacae submontanum) and montane beech forest (Fagetum moesiacae montanum). By structural form, they are group selection, all-aged stands. The stand inventory was performed by the systematic sample, intensity 5% of the stand area. The size of sample plots in circular form was 500 m*^{2}*; the plots were established by square distribution 100x100 m. The diameters of all stumps that could be measured were recorded. Altogether 100 sample plots were established, i.e. 5 ha were measured in detail. It was concluded that the fellings could be followed up for the past 20-25 years. Their volume, type and spatial distribution can be estimated rather reliably. The data were processed by the method of regression analysis, and they were presented by GIS.*
Keywords: *beech, felling, inventory, stand, stump*

INTRODUCTION

In the previous forest inventories in Serbia, the principal objective was to determine the current state of the forests. During the collection of data in the field, attention was primarily paid to get the precise information whose processing produces the size and structure of the basic taxation elements: number of trees, basal area, volume and volume increment. These data are used for the construction of forest management plans. In recent times, in the national inventory and in some special forms of forest inventory, in addition to the above information, the information on the health state and tree quality, state of the regeneration, dead wood, and the fellings performed in the past are also collected.

As in the forestry practice in Serbia frequently there were no recordings on the past fellings, stump inventory of removed trees can be carried out in order to determine (check) the volume and type of fellings in the recent past. In this way, (in combination with other researches), some dynamic changes in the stands can also be traced down, such as e.g. the change of the structure of individual taxation elements.

Also, by stump measurement, we can estimate the productivity, i.e. stand increment. Stand increment should be distinguished from productivity of an area. Stamenković & Vučković (1988) report that stand increment refers only to the case when the number of trees in the beginning and in the end of the study period remain the same and when it is the case of the same trees, otherwise it is the productivity of an area. When the volume of the removed trees is also taken into account, and the volume of the recruited trees is excluded, stand increment and productivity are the same (Klepac, 1963).

STUDY AREA AND METHOD

The study was performed in five high beech stands which are characterised by different site and stand properties. They belong to submontane beech forest (*Fagetum moesiacae submontanum*) and montane beech forest (*Fagetum moesiacae montanum*) at the altitude of 500-1000 metres and on different parent rocks and soil types. The slope is on the average 10-25°. By structural form, they are group selection, all-aged stands.

In the study stands tree felling was mostly operated by the tree selection or group selection systems.

Stand area varies from 12 to 29 ha, site quality class from I/II to III/IV, canopy from 84 to 90%, mean diameter from 30 to 37 cm, mean height from 18 to 28 m, and these are pure beech stands.

In the estimation of the size and structure of the basic taxation elements, as well in the estimation of volume and method of felling[1] in the analysed high beech stands, the me-

[1] In this context, the term felling method includes the type of cutting and the spatial distribution of cutting per area.

thod of a simple (systematic) sample was applied, with sample plots in circular form and sized 500 m^2 (radius 12.62 m), which were distributed in the square grid network in the stand, spacing 100 metres (one circle represents one hectare). The size of a sample was 10-29 sample plots.

General characteristics of the study beech stands are presented in Table 1.

Table 1. General characteristics of beech stands

Stand	Area (ha)	Site class	Canopy closure (%)	Mixture (%)	Mean diameter (cm)	Mean height (m)
33a	22.70	II	90	100	34.9	27.0
42a	17.52	III	87	100	32.8	21.7
42b	11.97	III/IV	84	100	32.1	18.0
122a	29.05	I/II	86	100	37.1	28.3
27a	18.17	II	85	100	29.8	23.7

The diameters and the height and diameter increments of all trees above the taxation limit (10 cm) were measured in all sample plots in the stand.

The data were processed by the special method developed to this purpose. The specificity of the method of data processing is the fact that a tariff series was determined separately for each sample plot, based on the height of the trees with diameter at breast height 30-70 cm. The standard quality-class set of heights for beech in Serbia was applied, after Mirković (Nikolić & Banković, 1992).

We also measured the diameters (two intersecting diameters) of the stumps which were in different phases of wood decomposition (decay). The diameters at breast height of the cut trees were calculated by using a regression equation based on stump diameters.

The regression equation for the calculation of the diameter at breast height of beech trees based on stump diameters is obtained based on Panić's data (Nikolić & Banković, 1992), and it has the following form:

$$d_{1.30} = 0.651965 \cdot d_p + 0.000766 \cdot d_p^2 + 0.000013 \cdot d_p^3$$

where:

$d_{1.30}$ = diameter at breast height

d_p = stump diameter.

Stump age, i.e. the time elapsed from the felling to the present time, is calculated based on the records of felling and tree ages of the spontaneous pioneer vegetation (*Sambucus nigra* and *Salix caprea*) on the felling sites in the stands.

Tree volume and volume increment were calculated by the regression equations (Koprivica & Matović, 2005) obtained based on the tariffs for beech in Serbia (Mirković, 1959 after Nikolić & Banković, 1992).

RESULTS

Taxation elements of the study beech stands are presented in Table 2.

Table 2. Taxation elements of the study beech stands

Stand	Taxation elements per hectare			
	Number of trees (pcs.)	Basal area (m^2)	Volume (m^3)	Volume increment (m^3)
33a	274	33.4	522.5	8.60
42a	321	31.7	379.6	6.61
42b	308	31.5	333.2	4.96
122a	214	29.0	503.7	10.50
27a	259	23.1	350.4	7.92

Number of trees per hectare (above 10 cm diameter at breast height) varies from 214 to 321, basal area from 23 to 33 m^2/ha, volume from 333 to 522 m^3/ha and volume increment from 5.0 to 10.5 m^3/ha.

The spontaneous pioneer vegetation (*Sambucus nigra* and *Salix caprea*) invades the area around the larger-size stumps after the establishment of the openings formed by tree felling. After the re-establishment of the canopy, the trees of pioneer species decline and die gradually. Elder and sallow have a great regeneration capacity, i.e., they have abundant yield every year, their seed is light so that on the study plots in the year after felling they occupy large openings in the stand, especially at places where the canopy closure is less than 0.5. The above conclusions regarding the stump age based on pioneer vegetation are confirmed by the felling data recorded in the files.

The process of beech stump wood decomposition to the phase when they cannot any more be used for the estimation of felling volume or method, most often lasts for up to 20 years, and in some cases up to 25 years after felling. After that period, the stumps are in the condition that the diameters cannot be measured objectively. Healthy stumps can be found only at places where felling was performed within the past three years.

The estimated volume of fellings performed in beech high stands in East and West Serbia, based on stump inventory, is presented in Table 3.

Table 3. Estimated volume of fellings

Stand	Volume per hectare (m^3)		
	1*	2	Total
33a	23.31	9.33	32.64
42a	-	69.11	69.11
42b	-	68.45	68.45
122a	1.15	181.00	182.14
27a	1.12	133.50	134.62

1*- based on healthy stumps
2 - based on decayed stumps

Stand 33a is, according to its current state, close to a virgin forest. In the past 20-25 years there were almost no fellings in it. In the past years, only one (tree) selection felling of moderate intensity was performed in one part of the stand.

In the stands 42a and 42b in the past 20-25 years there was only one selection (predominantly tree-selection) felling in almost the entire stand area, 10 years ago, intensity about 20 % of the stand volume before felling.

In the stands 122a and 27a in the past 20-25 years there were two selection (predominantly tree-selection) fellings over the entire stand area, felling intensity in both felling cycles accounted for 18-20 % of the stand volume before felling.

Estimated structure of the volume of the removed trees (yield) per diameter classes in the beech high stands is presented in Table 4.

Table 4. Volume structure of the removed trees per diameter classes

Stand	Volume of the removed trees per diameter classes (m^3/ha)										Total
	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	100-110	
33a	4.5	2.1	8.9	14.0	3.2	-	-	-	-	-	32.6
%	13.8	6.3	27.2	43.0	9.8	-	-	-	-	-	100.0
42a	1.2	1.4	2.9	7.4	25.2	24.4	6.6	-	-	-	69.1
%	1.8	2.0	4.3	10.7	36.4	35.3	9.5	-	-	-	100.0
42b	0.3	-	2.7	18.6	20.1	26.8	-	-	-	-	68.5
%	0.4	-	3.9	27.1	29.4	39.2	-	-	-	-	100.0
122a	3.6	8.0	10.5	36.9	38.4	42.2	15.4	14.7	-	12.5	182.1
%	2.0	4.4	5.8	20.2	21.1	23.2	8.4	8.1	-	6.8	100.0
27a	1.6	6.7	14.6	22.7	25.7	11.8	8.0	19.2	24.4	-	134.6
%	1.2	5.0	10.8	16.9	19.1	8.8	5.9	14.2	18.1	-	100.0
Average	2.6	4.3	8.6	21.7	23.5	22.0	7.1	7.8	4.5	3.6	105.7
%	2.5	4.0	8.1	20.5	22.2	20.8	6.7	7.4	4.2	3.4	100.0

Table 4 shows that, in almost all stands, the most represented trees are with diameter at breast height ranging between 40 and 70 cm (for all five stands, averagely about 64% of the yield). The trees with diameter at breast height below 40 cm account for about 15 %, and the trees with diameter at breast height above 70 cm account for about 21 % of the felling volume.

It is interesting to analyse also the structure of the present standing volume of timber in the study stands per diameter classes (Table 5).

Table 5. Present structure of the standing volume per diameter classes

Stand	Structure of standing volume per diameter classes (m^3/ha)										Total
	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	100-110	
33a	12.2	31.5	62.0	68.4	134.2	112.7	53.7	37.0	10.7	-	522.5
%	2.3	6.0	11.9	13.1	25.7	21.6	10.3	7.1	2.1	-	100.0
42a	6.2	35.0	89.7	103.9	94.3	43.8	6.6	-	-	-	379.6
%	1.6	9.2	23.6	27.4	24.8	11.5	1.7	-	-	-	100.0
42b	6.4	26.6	34.6	94.7	91.3	55.9	23.7	-	-	-	333.2
%	1.9	8.0	10.4	28.4	27.4	16.8	7.1	-	-	-	100.0
122a	8.0	20.4	48.5	96.4	107.0	149.6	59.2	14.5	-	-	503.7
%	1.6	4.1	9.6	19.1	21.2	29.7	11.8	2.9	-	-	100.0
27a	12.5	30.7	65.2	69.1	95.1	62.9	14.9	-	-	-	350.4
%	3.6	8.8	18.6	19.7	27.1	18.0	4.2	-	-	-	100.0
Average	9.3	28.1	60.2	86.2	106.9	95.4	36.3	12.7	2.5	0.0	437.6
%	2.1	6.4	13.8	19.7	24.4	21.8	8.3	2.9	0.6	0.0	100.0

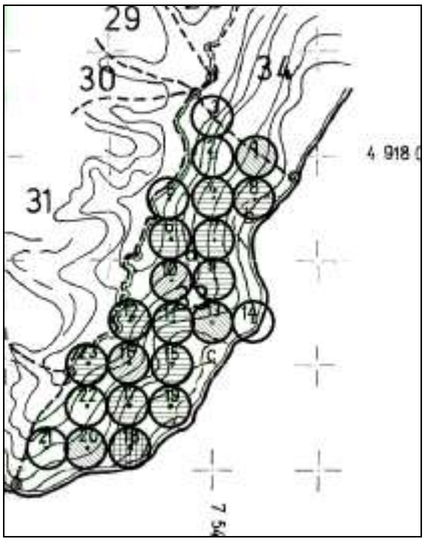
The highest percentage in the standing volume of the stand is that of the trees with diameter at breast height 40-70 cm (for all five stands averagely about 66 %), which corresponds to the estimated volume of felled trees in the past 20-25 years. Tree diameter at breast height below 40 cm accounts for about 22 % in the standing volume and tree diameter at breast height above 70 cm accounts for about 12 %.

As this is the case of uneven-aged stands and as tree selection was the predominant method in the past 20-25 years, it can be concluded that the percentage of trees with the largest and medium diameters was the highest, and the percentage of thin trees was lower. This is in harmony with the forest increment science, i.e. that the percentage structure of the yield is moved "to the right" compared to the percentage structure of the volume (Matić, 1980). It is important that the felling included all diameter classes and that the character of felling was primarily silviculture, and not exploitation. The principle of sustainable production was implemented. Thanks to these facts, the present quality of the study beech stands is better compared to the state 20-25 years ago, except in the stand 33a.

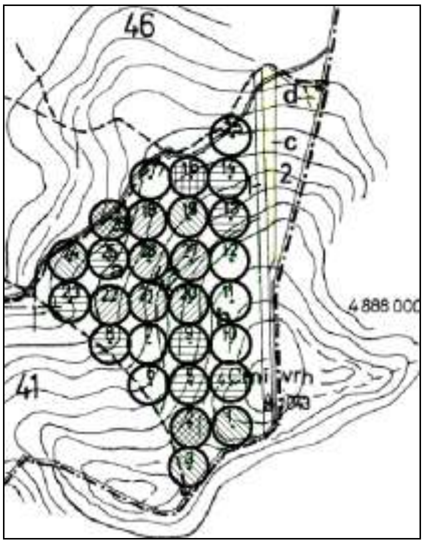
Spatial distribution of felling in the study stands was analysed by GIS (Figure 1).

It was concluded that the fellings in the stands 122a and 27a were rather homogeneous throughout the stand area, i.e. there was not a greater concentration of felling along the roads, skidding roads and landings. In the stands 42a and 42b there are some parts of the area in which there was no felling (stand 42a - 20% and stand 42b - 30%). In the stand 33a, the felling weight was light and it was carried out only in the upper part of the stand which was the most accessible for harvesting.

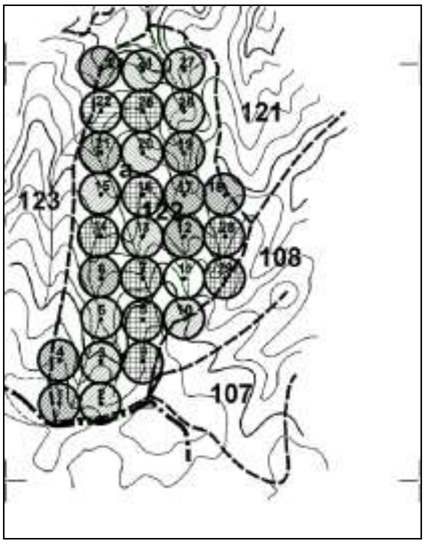
Similar studies of the spatial distribution of structural elements and felling volume were performed also in Croatia (Čavlović et *al.*, 2001).



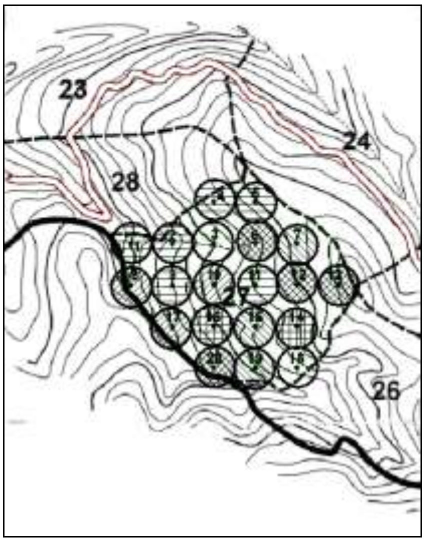
Stand 33a



Stands 42a, 42b



Stand 122a



Stand 27a

Legend: Total volume of cut trees on the sample area (m^3/ha)

no felling 1-50 50-100 100-150 150-200 >200

Figure 1. Spatial distribution of felling per stand area

CONCLUSION

The study results show that, during the inventory of beech high forests in Serbia, stump inventory can be implemented for the successful analysis (reconstruction) of the felling volume and the structure of felled volume per diameter classes. Also, the method of past fellings can be successfully deduced, i.e. the type of felling and its spatial distribution.

The applied method is simple and it consists of the systematic distribution of sample plots in the stand, the measurement of stump diameters and the calculation of the tariff series, separately for each sample plot. By using the regression equation for the calculation of diameter at breast height of the felled trees and the corresponding volume tariffs, the data can be computerised rather simply and the results can be displayed by GIS. Also, GIS can find out the correlation of fellings with stand and site characteristics.

The volume of fellings carried out in the past 20-25 years corresponds to the felling plans in almost all study stands, i.e. there were no fellings of exceptionally heavy weight. Also, spatial distribution shows that felling was most often performed throughout the stand area, except in the stand 33a and on smaller parts of the stands 42a and 42b.

The method of stump inventory for the estimation of the felling volume and method can be implemented in the control of the felling plans, i.e. in the control of the realisation of the forest management plans. This leads to the additional knowledge on the effects of the fellings, significant for future management.

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PHOTOSYNTHESIS AND WATER USE EFFICIENCY (WUE) OF POPLAR AND WILLOW CLONES DEPENDING ON HEAVY METAL AND DIESEL FUEL SOIL CONTAMINATION

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Summary: *To evaluate the phytoremediation capacity of some poplar and willow clones, a screening for Pb, Cd, Ni and diesel fuel tolerance was investigated. Heavy metals, especially in excessive concentrations are well known as inhibitors of the development and function of the photosynthetic apparatus. Also, disturbance of water balance in plants is considered as an early effect of heavy metal stress. Physiological base of plant tolerance to excessive pollutants is combination of high rates of photosynthesis and high water-use efficiency (WUE), calculated as rate of photosynthesis per unit water loss (transpiration). Those characteristics of genotype suggest its unique suitability for remediation. It is of great interest to concern weather and to which extant, photosynthetic features and water regimen of woody plants benefit their survival and remediation potential at unfavorable ecological conditions, primary at chemical contamination of soil. According to these facts, here we present measurements of gas exchange parameters and WUE of four poplar and two willow clones grown in green house on soil culture. There were control group of plants and six treatments of pollution: Pb, Cd, Ni, combination Pb+Cd+Ni, Diesel fuel and combination Diesel fuel+ Pb+Cd+Ni.*
The rate of photosynthetic CO_2 fixation (µmol m^{-2} s^{-1}) of the youngest leaves significantly relied upon the pollution treatment and genotypic variations in control poplar clones were from 10.77 to 8.22 µmolCO_2 m^{-2} s^{-1}. Two control willow clones showed similar photosynthesis (about 6.5 µmolCO_2 m^{-2} s^{-1} in average). Photosynthesis and transpiration of plants grown on soil with individually applied heavy metals decreased significantly, but it was less obvious in Cd treatment. Heavy metals mixture in soil induced significant reduction in photosynthesis (more than 50%). Inhibition of transpiration (mmol $H_2O$$m^{-2}$ s^{-1}) caused by heavy metal stress was less expressed than inhibition of photosynthesis. Diesel fuel in soil caused very strong and significant inhibition of photosynthesis and transpiration of willow clones, but the same treatment did not provoke drastic decrease of photosynthetic rate in poplar clones. The treatment of combined pollutants - excessive Pb, Cd, Ni and diesel fuel has approved to be very strong stress factor for photosynthetic productivity. Relatively high photosynthesis combined with good water economy in polluted environment indicated high WUE and good remediation potential of plants. According to this fact, our results indicated three poplar genotypes and one willow genotype as potentially good tools in remediation.
Key words: *photosynthesis, WUE, poplar, willow, phytoremediation*

INTRODUCTION

Phytoremediation utilizes plants and rhizosphere microorganisms to remove and transform toxic chemicals from soils, sediments, ground water, surface water, and the atmosphere (Kumar et *al.*, 1995; Susarla et *al.*, 2002; Ghosh & Singh, 2005; Pajevic et *al.*, 2008). Heavy metals accumulation ability of plants is useful as phytoextraction technique in phytoremediation which refers to the use of plants that can extract and translocate contaminants to the harvestable parts (Marchiol et *al.*, 2004).

Over the last few years, metal-accumulating woody species have been considered for phytoextraction of metal-contaminated sites (Greger & Landberg, 1999; Rosselli et *al.*, 2003; Dickinson & Pulford, 2005).

Except of cleaning environment, another advantage of using forest plants in this technology is high biomass production, which can eventually be used in producing energy (Laureysens et *al.*, 2004).

Great attention has been drawn to the study of water use efficiency (WUE), in order to define interdependence of water use and biomass production and detect genotypes that consume less water and are photosynthetically more efficient (Orlovic et *al.*, 2002; Martin & Stephens, 2006). Genotype adaptive values should be expressed in high photosynthetic potential, relatively high and stabile WUE, and high biomass production with high accumulation of pollutants (heavy metals in particular) in tissue.

The aim of this investigation is to quantify the photosynthetic capacity of 4 poplar clones and 2 willow clones depending on growing conditions according to heavy metals and diesel fuel soil treatment. Relatively high ratio of photosynthesis / transpiration in contaminated soil would distinguish genotypes with high adaptive potential in contaminated environment, high biomass production and high phytoextraction capacity.

MATERIAL AND METHODS

The experimental material consisted of four poplar and two willow genotypes (Table 1). These genotypes were obtained from the Institute for Lowland Forestry and Environmental Protection, Novi Sad, Serbia. Genotypes chosen for screening are result of long period selection of genes that cause high biomass production.

Table 1. Poplars and willows used in the experiment.

No.	Species	Working name
1	*Populus* x *euramericana*	Panonia
2	*Populus deltoides*	PD 3
3	*Populus deltoides*	B 81
4	*Populus deltoides*	B 229
5	*Salix alba*	V 1
6	*Salix alba*	V 2

Experiment was set in May of 2008. Plants were grown in a semi-controled environment (greenhouse), in soil culture method. Temperature was kept under 30°C. Illumination was natural deepending on the outside light conditions. Six woody cuttings, 20 cm long, with one shoot per cutting, were grown in each of the Mitscherlich pots. Each treatment consisted of 24 poplar and 36 willow cuttings. Pot distribution was randomized. Soil was watered permanently to maintain optimal soil humidity. Every four weeks 0.2 l of full strength Hoagland solution was added to pots. There were control group of plants and six treatments of pollution: Pb, Cd, Ni, combination Pb+Cd+Ni, Diesel fuel and combination Diesel fuel+ Pb+Cd+Ni. Concentration of metals in soil in individual metal treatments was: Pb - 360.35 mg/kg, Ni - 205.37 mg/kg and Cd – 8.14 mg/kg. In joined metal treatments concentrations were: Pb – 382.29 mg/kg, Ni – 185.25 mg/kg and Cd – 1.34 mg/kg. Total soil hydrocarbons in diesel treatment were 9.825 g/kg of dry soil. Metal concentration in soil was achieved using metal water solutions. The metals which were used are Cd (10^{-4} M and 10^{-5} M, supplied as $CdCl_2 \cdot H_2O$), Ni (10^{-4} M and 10^{-5} M, supplied as $NiSO_4 \cdot 6\ H_2O$) and Pb (10^{-4} M and 10^{-5} M, supplied as Pb-EDTA). Photosynthesis and transpiration were measured in June of 2008, after 42 days of treatment.

Photosynthesis and transpiration were measured using LC pro+ Portable Photosynthesis System, manufactured by ADC BioScientific Ltd. Light conditions were set using LCpro+ light unit, which emitted Photosynthetically Active Radiation (PAR) at 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Air supply unit provided flow of ambient air to leaf chamber at constant rate of 100 $\mu mol \cdot s^{-1}$. Temperature, CO_2 concentration and humidity were at ambient levels.

Parameter WUE (water use efficiency) was calculated as ratio of photosynthesis to transpiration and expressed in unit $\mu molCO_2 \cdot m^{-2} \cdot s^{-1}$ / $mmolH_2O \cdot m^{-2} \cdot s^{-1}$.

Stomatal conductance of water vapor has been measured automatically *in vitro* and expressed in unit $molH_2O \cdot m^{-2} \cdot s^{-1}$.

Statistical analyses were conducted using Duncan's Multiple Range Test, at the level of significance $p<0.05$, using 1-way factor analyses. The average values shown in tables followed by the same letter, did not differ significantly. Values decreased following the alphabetical order. The least significant difference (LSD), between the average values of treatments is shown at the end of each table.

RESULTS

Photosynthetic capacity of the youngest group of poplar and willow leaves was under significant influence of heavy metals and diesel fuel soil contamination. Investigated genotypes showed specific photosynthetic answer under different pollution treatments which was evident comparing photosynthesis of controlled plants: there were no significant differences in photosynthesis in poplar group and also in willow group of genotypes. The obtained photosynthetic CO_2 assimilations of control poplar plants were from 10.77 μmolCO_2 m^{-2} s^{-1} (genotype 1 - Panonia) to 8.22 μmolCO_2 m^{-2} s^{-1} (genotype 3 - B 81) (Table 2).

The highest inhibitory effect on photosynthetic assimilation of all investigated clones was detected for combined pollution treatment, diesel fuel+ Pb+Cd+Ni, and for the most genotypes it was impossible to detect any photosynthetic activity in these conditions (except for poplar genotype 1). Also, very strong inhibitory effect on photosynthesis we detected for plants grown on soil contaminated with heavy metals mixture (Cd+ Ni+Pb).

Analyzing single genotype reaction on pollution treatment, it can be concluded that poplar genotype Panonia had the lowest photosynthesis in soil with heavy metals mixture (3.32 μmolCO_2 m^{-2} s^{-1}), which is photosynthetic decreasing of about 70% comparing to control plant photosynthesis (10.77 μmolCO_2 m^{-2} s^{-1}). Statistically similarly decreasing of photosynthesis for the same genotype we registered for plants grown on Ni and diesel fuel+Pb+Cd+Ni. This genotype *Populus* x *euramericana* (Panonia) could be distinguished according to photosynthetic capacity as suitable for phytoextraction of Cd and diesel fuel in single contamination treatments, because in these conditions it had stabile and relatively high photosynthesis (Tab. 1). Genotype 4 (*Populus deltoides* - B 229) showed the lowest photosynthesis and it has potentially lowest remediation potential because all applied treatments induced decreasing in photosynthesis for more than 60%.

Table 2. The rate of photosynthetic CO_2 assimilation poplar and willow leaves (μmolCO_2 m^{-2} s^{-1})

	Poplar genotypes				Willow genotypes	
Species/ clones / **Treatment**	*Populus* x *euramericana* - Panonia	*Populus deltoides* - PD 3	*Populus deltoides* - B 81	*Populus deltoides* - B 229	*Salix alba* - V 2	*Salix alba* - V 1
Pb	5.04 c	4.41 d	3.83 d	4.63 b	3.25 c	2.68 b
Cd	8.05 b	8.63 a	8.72 a	4.56 b	3.12 c	2.24 b
Ni	3.76 d	7.47 b	5.44 c	3.49 c	4.88 b	1.44 c
Pb+Cd+Ni	3.32 d	2.65 e	7.13 b	0.69 d	1.33 d	1.06 c
Diesel fuel	7.32 d	6.53 c	6.68 b	0.00 e	0.00 e	0.00 d
D.f.+Metals	4.25 cd	0.00 f	0.09 e	0.00 e	0.00 e	0.00 d
Control	10.77 a	9.16 a	8.22 a	8.87 a	6.88 a	5.92 a
$LSD_{0.05}$	0.92	0.68	0.73	0.67	0.65	0.44

Registered photosynthetic activities of willow clones were lower than that of poplar clones, and the average values for control plants were 6.88 μmolCO_2 m^{-2} s^{-1} (*Salix alba* - V1) and 5.92 μmolCO_2 m^{-2} s^{-1} (*Salix alba* - V2). Similarly to poplar clones, the most obvious inhibitory effect regarding photosynthesis of willow clones was detested for diesel fuel + Cd+Ni+Pb and heavy metal mixture treatments. Willow genotypes have been defined as intolerant for phytoextraction of diesel, because their photosynthetic rates were very low, under level of registration (Table 2). According to obtained results of photosynthetic activity, willow clone V1 could be used for remediation of Ni from soil polluted with this heavy metal. Excessive concentrations of Cd in soil had the lowest effect on photosynthesis of almost all investigated poplar and willow clones (Table 2).

Transpiration rates measured at the youngest leaves were similar according poplar clones and ranged from 2.37 mmol H_2O m^{-2} s^{-1} (clone B 229) to 1.78 mmol H_2O m^{-2} s^{-1} (clone Panonia). Statistical data analysis showed very similar pollution effect on transpiration of different clones (Table 3). Similar to photosynthetic rate, clone B 229 exhibited the lowest transpiration rate in polluted conditions, diesel fuel + metals especially, which distinguished it as the genotype of the lowest remediation potential.

Investigated willow genotypes showed significant metabolic disturbance in pollution conditions: their photosynthetic and transpiration rates were very low (under registration level) in soil conditions with diesel and heavy metals (Table 3).

Stomatal conductance of water vapor in leaves of poplar and willow clones were in correlation with transpiration rates. Inhibition of stomatal conductance of plants grown under soil contamination indicated that photosynthesis and transpiration depression happened to a great extant at the stomatal level.

Water use efficiency (WUE) is a good economical indicator of biproduction per unit of water volume consumed, and we expressed it as photosynthesis / transpiration ratio (Table 4).

Obtained results for WUE of poplar clones indicated that diesel fuel and diesel fuel + metals in soil significantly disturbed water consumption in plants and consequently photosynthetic organic production (Table 4).

Table 3. The rate of transpiration of poplar and willow leaves (mmolH_2O m^{-2} s^{-1})

	Poplar genotypes				Willow genotypes	
Species/ clones / **Treatment**	*Populus x euramericana* - Panonia	*Populus deltoides* - PD 3	*Populus deltoides* - B 81	*Populus deltoides* - B 229	*Salix alba* - V 2	*Salix alba* - V 1
Pb	1.02 bc	0.74 b	0.73 bc	0.97 b	0.72 c	0.75 b
Cd	1.53 ab	1.62 a	1.62 a	1.15 b	0.83 bc	0.61 bc
Ni	0.84 c	1.56 a	1.39 ab	0.96 b	1.11 ab	0.50 c
Pb+Cd+Ni	0.85 c	0.77 b	1.69 a	0.74 b	0.69 c	0.63 bc
Diesel fuel	1.91 a	1.63 a	1.75 a	0.00 c	0.00 d	0.00 d
D.f.+Metals	1.07 bc	0.00 c	0.25 c	0.00 c	0.00 d	0.00 d
Control	1.78 a	2.21 a	1.95 a	8.87 a	1.41 a	1.11 a
LSD 0.05	0.64	0.62	0.68	0.65	0.32	0.18

Genotypes Panonia and B 81 showed very stabile WUE during growing in polluted soil and only treatment of diesel fuel and metal mixture put in soil together provoked severe WUE depression in genotype B 81. Stabile WUE in unfavorable environmental conditions is an indicator of high organic production and good remediation potential of those genotypes.

Table 4. WUE of poplar and willow leaves (µmolCO_2 ·m^{-2} · s^{-1} / mmolH_2O ·m^{-2} · s^{-1})

	Poplar genotypes				Willow genotypes	
Species / clones / **Treatment**	*Populus x euramericana* - Panonia	*Populus deltoides* - PD 3	*Populus deltoides* - B 81	*Populus deltoides* - B 229	*Salix alba* - V 2	*Salix alba* - V 1
Pb	5.09 ab	6.16 a	5.36 a	5.32 a	4.69 a	3.59 b
Cd	5.16 ab	5.50 ab	5.25 a	4.42 ab	4.04 a	3.88 b
Ni	4.60 ab	4.97 ab	4.25 a	3.91 b	4.47 a	2.92 b
Pb+Cd+Ni	4.28 ab	3.85 b	4.44 a	1.24 c	1.53 b	1.71 c
Diesel fuel	3.88 b	4.08 b	3.84 a	0.00 c	0.00 c	0.00 d
D.f.+Metals	4.08 ab	0.00 c	0.45 b	0.00 c	0.00 c	0.00 d
Control	6.15 a	4.17 b	4.31 a	3.78 b	4.90 a	5.48 a
LSD 0.05	1.96	1.68	1.52	1.30	1.19	1.06

Willow clone V_2 grown in contaminated soils showed significantly lower WUE in soil contaminated conditions comparing to V_1 and other poplar genotypes, which distinguished it as the least suitable for fitoextraction and remediation.

DISCUSSION

Heavy metal accumulator species *Populus* sp. and *Salix* sp. have been suggested for phytoremediation by many authors (Robinson et *al*., 2000; Unterbrunner et *al*., 2007). Poplar trees may be used for the *in situ* decontamination of polluted soils. Average metal concentrations in plant tissues followed the order: leaves>bark>twigs>wood for Zn and Cd, and bark>wood>twigs ≈leaves for Pb (Pierzynski et *al*., 2002). Rosselli et *al*. (2003) found that *Salix* and *Betula* transferred zinc and cadmium to leaves and twigs, but *Alnus*, *Fraxinus* and *Sorbus* excluded them from their above-ground tissues. None of the species considered transferred copper to the shoots.

Defining CO_2 photosynthetic assimilation of different woody species is important in order to choose genotypes suitable for phytoremediation breeding programs. Heavy metal uptake and translocation from the root zone to stems and leaves of plants are driven by transpiration, and because of that the water status of the plant tissues and soil moisture are of crucial importance for photosynthesis and organic assimilation (Marchiol et *al.*, 2004).

Our results suggest that morphological and ecological characteristics in clones and species were affected by heavy metal and diesel fuel treatment in different ways. The most depressive effect on photosynthetic CO_2 assimilation rate according to all investigated poplar and willow genotypes was evident in plants grown on diesel fuel and metal mixture in soil. Also, diesel fuel as the only pollutant in soil, caused very strong and significant inhibition of photosynthesis and transpiration of willow clones, but the same treatment did not provoke drastic decrease of photosynthetic rate in poplar clones, except in poplar clone B 229. The treatment of combined excessive Pb, Cd, and Ni has also approved to be very strong stress factor for photosynthetic productivity, especially for willow clones and poplar clone B 229. The negative effects of a mix of contaminating metals on the physiology (assimilation and growth) of some other plant species (*Brasica napus* and *Raphanus sativus* in the presence of metals has been verified by Marchiol et *al.*, 2004. The substitution of heavy metals in chlorophyll leads to a breakdown in photosynthesis. Küpper et *al.* (2007) observed that during excessive Cd - induced stress, a few mesophyll cells became more inhibited and accumulated more Cd than the majority and this heterogeneity disappeared during acclimation in good remediator - plants.

Our results indicated that combination of metals (Pb+Cd+Ni) in soil had more inhibitory effect on photosynthesis in comparing with single metal effect.

Among the poplar genotypes, clone B 229 expressed the lowest photosynthetic potential in contaminated environment which distinguished it as the least suitable for good biomass production in polluted soils. Also, comparing results referring for photosynthetic potential, *Salix* species could be distinguished with significantly lower biomass production and remediation ability in chemically loaded environment.

The good photosynthetic gas exchange parameters and better capability of nitrogen nutrition is a consequence of the optimal water supply.

In our study WUE of poplar clones did not decrease in the pollution treatments as photosynthesis did. This is because the transpiration rate was also affected by heavy metal stress, and plants that are more adapted to polluted environment perform lower water costs for nutrient absorption. Plant populations which suffer strong selection pressure of the contaminated conditions, perform lower WUE and higher N, suggesting that plants may be "wasting water" to increase N delivery for photosynthetic apparatuses via the transpiration stream (Donovan et *al.*, 2007).

Good adaptation potential is in plant capability to keep high WUE and good distribution of nitrogen in photosynthesis during the heavy metal induced-stress, in other words good resource investment in bioproduction. Our results are in accordance with that, confirming that all genotypes are partially tolerant to heavy metals and indicating poplar clones Panonia, PD 3 and B 81 and willow clone V1 as genotypes with high bioproduction potentials in contaminated environmental conditions.

Our results pointed out genotypic specificity of all investigated physiological parameters, and marked poplar clones as very useful in phytoextraction technology of bio-cleaning of chemically polluted soils.

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SESSILE AND TURKEY OAK FORESTS IN PEŠTER PLATEAU

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Summary: *Research was conducted in the Sessile and Turkey oak forest (Quercetum petraeae-cerris Jovanović (1960) 1979) in Pešter Plateau. Sessile and Turkey oak forests are transitions from the monodominant Sessile oak forests of the hilly region to the zonal vegetation, mostly Hungarian and Turkey oak forests in Serbia. One hundred and fifty-seven taxons were reported: 14 tree species, 7 bush species and 136 species of ground flora. The stands are degraded to a great extent, at some localities with the completely interrupted canopy, which caused the plant presence from the forest and meadow communities in the vicinity. In the spectre of life forms the plants adapted to the more extreme life conditions are dominant. In the spectre of the floristic elements most dominant are the Mid-European (35.06%) and Euro-Asian floristic elements (24.68%). The Submediterranean floristic elements account for 12.34%, as much as Pont-Central Asian. The circumpolars and cosmopolitans account for 7.14%, Arctic and Boreal for 3.25%. Ecological indexes of the Sessile and Turkey oak forests have: average value 2.45 (from 2.26 to 2.63) of the humidity; average value 3.21 (from 3.05 to 3.32) of the soil chemical reaction; average value 2.36 (from 2.21 to 2.53) of the nutrients; average value 3.21 (from 2.94 to 3.61) of the light; average value 3.34 (from 3.18 to 3.45) of the temperature. Forty-seven medicinal and 53 honey plant species have been reported. Forests cover 428.41 ha in Pešter Plateau. The high forests account for only 3.9%, and the rest are sprout stands of the different level of degradation*
Key words: *Sessile oak forests, natural resources, unfavorable resources, measures for the improvement of the condition*

INTRODUCTION

The contemporary developments in the use of the renewable nature resources cause a well-founded fear that the existent nature ecosystems in the regard to the biodiversity and ability to adapt to the drastic climate and other changes might not be preserved. The disappearance speed of forests 10 million ha a year points to the gravity of the problem and requires urgent measures with the aim of the stopping of the existent forest ecosystems degradation and devastation. The alternative to this approach is ecocentric (or biocentric) use of the resources, based on the principles of the management which is close to nature. Ecosystem presents the complexity of living organism and has ITS OWN SIGNIFICANCE. These ecological paradigms consider human needs and their attitude towards nature in the other way. The way in which nature creates and preserves ecosystems is respected. There are no undesirable species in natural ecosystems, except for the species induced by man. For instance, in forest, during removal, each tree or product is estimated from the point of view of functioning of the ecosystem. Nature on its own continues the development and steadiness of the ecosystems, and humans on the bases on their perceptions of it. Ecosystem belongs to only the species which live in it, including the humans. Thus, man does not have the right to destroy it because of his interests. Ecocentric concepts protects, maintains and renews the functioning of the nature ecosystems with the simultaneous use of all the goods and services aimed at providing human needs on the steady and permanent bases. The priority is given to the ecological processes in the ecosystems, so that they provide the economic needs of the society, but not in the way of the industrial use. Ecocentric concept insists on the protection of the remaining old nature forests as the last haven of the bird species, as well as of other types of organisms. The integral part is the care of the soil, water, biodiversity and biomass. Policy in the use of the ecosystems in Pešter Plateau should provide the extension of the forest areal to the areas which are not used in other purposes (demarcation between the agricultural and forest land), improvement of the condition of the degraded and devastated forests and shrubberies, increase and improvement of the production of the wood volume, and other products and services, as well as the protection of the remaining nature forests from degradation and devastation. The achievement of these goals is based on the ecological, socio-demographic and economic criteria. Its activities are based on the Concept of the Sustainable development and it is focused on the smaller territorial units (areas).

MATERIALS AND METHODS

Research was conducted in the Sessile and Turkey oak forests in Pešter Plateau. Pedological characteristics were studied on the basis of the pedological profiles, and the soil types were determined by the soil classification Škorić *et al.* (1985), as well as by FAO classification. Vegetation characteristics were determined by Braun-Blanquet. Study on vegetation was conducted through phytocenological records, which encompass the basic properties of the stands, floristical content, compostion and habitat conditions. Flora of SR Serbia" (1970–1986), "Ikonographie der flora des südöstlichen Mitteleuropa" Jávorka & Csapody (1979), "Flora and Vegetation of Golija and Javor Gajić (1990), Flora of National Park Tara were used for the determination of species. The spectres of the floristic elements were processed in accordance with the systematization of the plant-geographical elements of Gajić (1970, 1984). The bilogical spectres of the plants were processed by Kojić *et al.* (1994). The area covered by forests and forest stands classification of the area according to the types of the stands, wood volume and volume increment were analyzed. The percentage of the trees in the total volume thinner than 30cm was specially analyzed.

RESULTS

Sessile and Turkey oak forests (**Quercetum petraeae-cerris** Jovanović (1960) 1979; Palaeartic classification **Illyrian termophyle turkey oak - sessile oak woods CODE 41.734)** are transitions from the monodominant Sessile oak forests of the hilly region to the zonal vegetation, mostly Hungarian and Turkey oak forests in Serbia. They cover the lower belt of the Sessile oak forests, lower expositions, and have also been reported on brown and loess soils on different geological bases. The forests are somewhat more mezophilic than monodominant Turkey oak forests. The community is more spread and frequent than the monodominant Turkey oak forests. The great areas have been reported in Norhwestern and Western Serbia (in Gučevo, Maljen, Cer, branches of Tara and Zlatibor) Vukićević (1966), as well as in Eastern and Northeastern Bosnia. The community is also present in Fruška gora, at altitudes to 400 meters above sea level. Jović *et al*, (1989). In Eastern Serbia it is presented only in fragments (Tomić & Cv j etičanin (1993/1994). The community of Sessile and Turkey oak in Voloder is geographically close to the Sessile and Turkey oak forests in Pešter Plateau Tomić (1988.).

In Pešter Plateau the Sessile and Turkey oak forests are present to the ultimate limits of its altitudinal spread. There are two deposits. The first one is in Babinjača, at altitudes up to 1,200 m, below Gradac karst. It covers a wide reef of crest Rašće and Plandište, to Tubić Houses in Donje Lopiže on northern exposition to Krmske doline, from the west of Uvac Village. From Babinjača the community goes down to Uvac Valley, then it spreads upstream to Mašović Village. In Uvac gorge, from Čedovo to Lupoglav there are several preserved Sessile and Turkey oak reserves. In this area Sessile and Turkey oak forests are often accompanied with the hazel groves, in which poplars and birches are present. The community of Sessile and Turkey oak was reported in Vrhovi (in Javor piedmont area), in Ljutska Reka river basin, on south slopes of Golija to Novi Pazar, where besides degraded bushes under Šarski karst, there are several private estates of a better quality. Good renewal of Sessile and Turkey oak forest has been observed in Strmac, below Duga Poljana.

The community cover long crests, gentle plateaus, and long levelled reefs at altitutudes from 900-1,200 m, on eastern, southestern, southwestern, northwestern and other expositions. The terrains range from gentle plateaus to inclination greater than 20°. Geological base consists of neogenic sediments; sandstones, limestone and clays, and philittes on smaller area. Soil area is devoid of huge stones and rocks since parent rock is subject to the process of physical decomposition. Skeleton is not expressed to the level of the geological base, where the transition is gradual. The most frequent soils in this community are district cambisols, pseudogley and luvisol. The soils are deeper than 70cm.

In Sessile and Turkey oak forest 157 taxons have been reported: 14 tree species, 7 bush species and 136 types of ground flora. Sessile and Turkey oak stands in Pešter Plateau are degraded to a great extent, in some localities with the completely interrupted canopy. It conditioned the presence of the plants from the neighbouring forest and meadow communities which found favourable life conditions in the phytoclimate of this phytocenosis.

Floristic content of Sessile and Turkey oak forests

Crataegus monogyna, Potentilla australis, Crepis viscidula, Potentilla erecta, Cynosurus cristatus, Potentilla heptaphylla, Cytisus scoparius, Primula acaulis, Dactylis glomerata, Primula veris, Dactylorhiza incarnata, Prunela vulgaris, Danaa cornubiensis, Prunus avium, Danthonia provincialis, Prunus spinosa, Deschampsia flexuosa, Pteridium aquilinum, Dianthus armeria, Pulmonaria officinalis, Digitalis ambigua, Quercus cerris, Dorycnium herbaceum, Quercus petraea, Euphorbia amygdaloides, Ranunculus steveni, Evonymus latifolius, Rosa agrestis, Fagus silvatica, Rubus canescens, Festuca amethystina, Rumex acetosa, Festuca heterophylla, Salix capreae, Festuca valesiaca, Scabiosa triniaefolia, Filipendula hexapetala, Selinum carvifolia, Fragaria vesca, Serratula tinctoria, Galium aparine, Silene italica, Galium cruciata, Silene nutans, Galium kitaibelianum, Silene roemeri subsp. sendtneri, Galium rubioides, Silene vulgaris, Galium silvaticum, Solidago virga-aurea, Galium vernum, Stachys officinalis, Galium verum, Stellaria graminea, Genista ovata, Stellaria holostea, Geranium sanguineum, Tanacetum corymbosum, Geum rivale, Telekia speciosa, Helianthemum nummularium, Teucrium chamaedrys, Helleborus odorus, Thalictrum minus, Hieracium bauhini, Thymus longicaulis, Hieracium cymosum, Thymus serpyllum, Hieracium murorum, Tilia cordata, Hieracium pilosella, Torilis anthriscus, Hieracium transsilvanicum, Trifolium alpestre, Hypericum maculatum, Trifolium hybridum, Hypericum montanum, Trifolium medium, Hypericum perforatum, Trifolium rubens, Inula hirta, Verbascum lanatum, Inula salicina, Veronica chamaedrys, Juniperus communis, Veronica officinalis, Knautia arvensis, Veronica praecox, Koeleria gracilis, Veronica teucrium, Koeleria pyramidata, Viburnum lantana, Lamium galeobdolon, Vicia cassubica, Lathyrus hallersteinii, Vicia cracca, Lathyrus niger, Viola silvestris

In the **spectre of life forms** (Table 1) there is a great presence of chemicryptophytes (56.69%), as a result of the aggravated life conditions for the plants in this altitude zone. The plants adapted to the more extreme life conditions are dominant in the community. Percentage of geophytes (15.29%) points to the favourable edaphic conditions (moisture, structure and soil depth). The number of terophytes (3.18%) points to the climate transition to the mountain.

Table 1. Spectre of life forms of the plants in Sessile and Turkey oak forests

Life forms (%)							
Phanerophytes	Nano-phanerophytes	Woody chamaephytes	Zeljaste Herbaceous chamaephytes	Chemicryptophytes	Geophytes	Terophytes	Terophytes/ chamaephytes
p	np	dc	zc	h	g	t	th
8.92	4.46	3.18	5.10	56.69	15.29	3.18	3.18
13.38		8.28					

In the **spectre of the floristic elements** (Table 2) the most dominant are Mid-European (35.06%) and Euro-Asian floristic elements (24.68%), which cover the most part of the spectre. It points to the Mid-European and Euro-Asian influences. Submediterranean floristic elements account for 12.34%, as much as Pont-Central Asian. The circumpolars and cosmopolitans account for 7.14%, Arctic and Boreal for 3.25%. The plants of xero-mezophilic character are dominant, as a result of the transitory area between Illyrian and Mezian province. This community was incorporated into the vegetation maps as the community of East and Souteastern Bosnia Stefanović *et al.* (1983).

Table 2. Spectre of the floristic elements in Sessile and Turkey oak Forests

THE NAME OF THE GROUP OF THE FLORISTIC ELEMENTS	FLORISTIC ELEMENT	PERCENTAGE %	
1 FLORISTIC ELEMENTS OF NORTH AREAS			
Arctic floristic elements			
Boreal floristic elements	Subboreal-European- West Siberian	0.65	
	Subboreal-circumpolar	1.95	
	Boreal-Euro-Asian	0.65	3.25
2 MID-EUROPEAN FLORISTIC ELEMENTS			
Mid-European and	Mid-European	10.39	
European	Sub-Mid-European and	24.03	
	Sub-Mid-Russian	0.65	35.06
3 SUBATLANTIC FLORISTIC ELEMENTS			
Sub-Atlantic and Antlantic	Sub-Atlantic	0.65	
	Subatlantsko-submediteranski Sub-Atlantic-Sub- Mediterranean	3.90	4.55
4 Sub-Mediterranean floristic elements			
Sub-Mediterranean	Sub-Mediterranean	3.90	
East-Sub-Mediterranean	East-Sub-Mediterranean	3.25	
Balkan and Balkan-Apenian	Mezian	0.65	
	Subilirski Sub-Illyrian	1.30	
	Mezijsko-karpatski Mezian- Carpathian	0.65	
	Mid-Balkan	0.65	
	Sub-Mid-Balkan	0.65	
	Balkan-Central-South-Apenian	0.65	
	Sub-Dacian	0.65	12.34
5 PONTIC-CENTRAL ASIAN FLORISTICAL ELEMENTS			
	Sub-Pontic-Central Asian	1.95	
	Sub-Pontic-Sub-Central Asian-Sub-Mediterranean	0.65	
Pontic	Pontic	1.30	
	Sub-Pontic	3.90	
	Pontic-Sub-Mediterranean	2.60	
	Pontic-Eastern-Sub-Mediterranean	0.65	
	Subpontsko-submediteranski Sub-Pontic-Sub-Mediterranean	0.65	
	Pontic-Pannonian	0.65	12.34
6 FLORISTIC ELEMENTS OF DESERT AREAS			
	Iranian- Euxinian	0.65	0.65
7 EURO-ASIAN FLORISTIC ELEMENTS			
	Sub-South Siberian	3.25	
	Euro-Asian	15.58	
	Sub-Euro-Asian	5.84	24.65
8 CIRCUMPOLAR AND COSMOPOLITAN FLORISTIC ELEMENTS			
	Circumpolar	4.55	
	Sub-Circumpolar	0.65	
	Cosmopolitans	1.95	7.14

The Sessile oak and Turkey oak forest is a bi-dominant community, because the both of the said species dominate the layer of trees and bushes. The canopy degree in the first layer is ranging from 0. 3 – 0. 8. The tree height goes from 9 to 25 m; and the average trunk diameter of the trees in the first layer ranges from 8cm (on Strmac slopes) to 30cm (in Babinjaca). The least preserved stands are found near village meadows and pastures due to constant anthropogenic influence. This community stands on the river and brook banks are almost destroyed, because the forest has not succeeded to regenerate itself owing to the increased erosion. There is no Sessile oak on these localities, only old and stunted Turkey oak trees have remained. The Sessile oak dominates in stands, forest enclosures and in places distanced from the country roads and in spite of the habitat conditions it has a notable size. *Corylus avellana, Pyrus pyraster, Crataegus monogyna, Betula pendula, Carpinus betulus, Evonymus latifolius, Populus tremula, Prunus avium, Prunus spinosa, Salix capreae* participate in forming the layer of bushes, beside Sessile oak and Turkey oak. *Crataegus calycina, Evonymus europaeus, Rosa agrestis, Viburnum lantana* are also found but separately. The layer of the lower flora is notably lush and the soil coverage ranges from 40 to 90 %. The third layer coverage degree is the highest in stands with pronounced devastation on the steep slopes toward Uvac, where the coverage is 0. 9. The lowest coverage (mostly 0. 4) is in the densely formed stands on the north slopes and mild plateaus less exposed to sun-light.

The ecologic indices of the Sessile and Turkey oak forests

The ecologic indices of the Sessile and Turkey oak forests have the following averages for:

- Humidity 2. 45 (from 2. 26 to 2. 63)
- Soil chemical reaction 3. 21 (from 3. 05 to 3. 32)
- Nutrients 2. 36 (from 2. 21 to 2. 53)
- Sun-light 3. 21 (from 2. 94 to 3. 61)
- Temperature 3. 34 (from 3. 18 to 3. 45).

Medicinal herbs in Sessile and Turkey oak forests

The existence of 157 plant species has been recorded in the Sessile and Turkey oak forests. The 47 of the said species are medicinal herbs, i.e. 29. 9%. Nine species belong to the first category of herbs with medicinal properties, i.e. 5. 7% **(**Betula pendula Roth., Crataegus monogyna Jacq., Hypericum perforatum L., Juniperus communis L., Ononis spinosa L., Primula veris Huds., Quercus petraea (Matt.) Lieb., Thymus serpyllum L., Tilia cordata Mill.). All these species are on the market and under certain regulations concerning collecting and selling. Quercus petraea is the only specie, from this plant group, not subjected to limitations in collecting and selling. The second category of herbs with medicinal properties contains 3 species or 1. 9% (Pulmonaria officinalis L., Rumex acetosa L., Solidago virga-aurea L.). These recorded species are on the market, Rumex acetosa is not under the legal protection. The third category numbers 9 species or 5. 7% (Veronica officinalis L., Ajuga reptans L., Asarum europaeum L., Fagus silvatica L., Helleborus odorus W.et K., Melittis melissophyllum L., Populus tremula L., Potentilla erecta (L.) Raucsh., Prunus spinosa L., Telekia speciosa (Schr.) Baumg.). Species *Ajuga reptans, Melittis melissophyllum, Populus tremula* and *Telekia speciosa* are not on the market and they have importance in the folk medicine. The species *Fagus silvatica, Helleborus odorus, Prunus spinosa* are on the market. The collection of species *Asarum europaeum* and *Potentila erecta* is under control. The forth category of herbs with medicinal properties includes 13 plant species or 8. 3% (Corylus avelanna L., Cytisus scoparius L., Digitalis ambigua Murr., Euphorbia amygdaloides L., Filipendula hexapetala L., Fragaria vesca L., Galium verum L., Plantago major L., Prunus avium, Quercus cerris L., Stachys officinalis (L.) Trev., Teucrium chamaedrys L., Veronica chamaedrys L.). There are no limits set to the amounts collected in the natural habitat concerning the specie *Plantago major.* Species *Corylus avelanna, Digitalis ambigua, Fragaria vesca, Galium verum, Teucrium chamaedrys* and *Veronica chamaedrys* are subjected to controlled collecting and selling, while species *Cytisus scoparius, Euphorbia amygdaloides, Prunus avium, Quercus cerris* and *Stachys officinalis* are significant in folk medicine. The fifth category has 12 species or 7. 6% **(**Aegopodium podagraria L, Alchemila vulgaris L., Anemone nemorosa L., Galium vernum Scop., Hieracium pilosella L., Lilium martagon L., Primula acaulis (L.) Gr., Prunela vulgaris L., Pteridium aquilinum (L.) Kuhn., Salix capreae L., Thalictrum minus L., Trifolium rubens L.). Specie *Thalictrum minus* is on the market without any restrictions imposed, while *Alhemila vulgaris* is subjected to limited collection and selling; other species do not have a larger economic significance.

Fruit trees in Sessile and Turkey oak forests

The following species of fruit trees are found in the Sessile and Turkey oak forests: Corylus avelanna, Crataegus monogyna, Fragaria vesca, Juniperus communis, Pirus piraster, Prunus avium, Prunus spinosa, Rosa agrestis and Rubus canescens.

Honey plant species in Sessile and Turkey oak forests

Based on the analysis performed 53 honey plant species are found in Sessile and Turkey oak forests out of which 8 are woody plants: (Prunus avium, Tilia cordata, Populus tremula, Quercus petraea, Betula pendula, Carpinus betulus, Fagus silvatica, Quercus cerris), 12 are bushy (Corylus avelanna, Crataegus calycina, Salix capreae, Viburnum lantana, Crataegus monogyna, Prunus spinosa, Cytisus scoparius, Evonymus latifolius, Genista ovata, Teucrium chamaedrys, Chamaecytisus supinus i Ononis spinosa L.) and 33 are herbaceous (Anemone nemorosa, Campanula persicifolia, Geranium sanguineum, Lamium galeobdolon, Pulmonaria officinalis, Stachys officinalis, Trifolium alpestre, Trifolium hybridum, Veronica chamaedrys, Vicia cracca, Ajuga reptans, Astrantia major, Coronilla varia, Digitalis ambigua, Filipendula hexapetala, Geum rivale, Inula salicina, Lysimachia vulgaris, Phyteuma spicatum, Plantago major, Primula acaulis, Prunela vulgaris, Solidago virga-aurea, Thalictrum minus, Thymus serpyllum, Fragaria vesca, Aegopodium podagraria, Helleborus odorus, Hypericum perforatum, Knautia arvensis, Lilium martagon, Polygala comosa, Primula veris i Veronica teucrium). The community average honey yield is 3. 07. The number of honey plants in bloom is the highest during May, June, and July (chart 1).

The wood resources of Sessile and Turkey oak forests

The Sessile and Turkey oak forests are found on 428. 41 ha on Pester plateau (table 3). The tall forest occupies only 3. 9% of the area; others are shoot stands of different degradation degree. The total volume is 50,022m^3, the tall forest volume is 7,914m^3; and shoot stand volume is 42,108m^3.

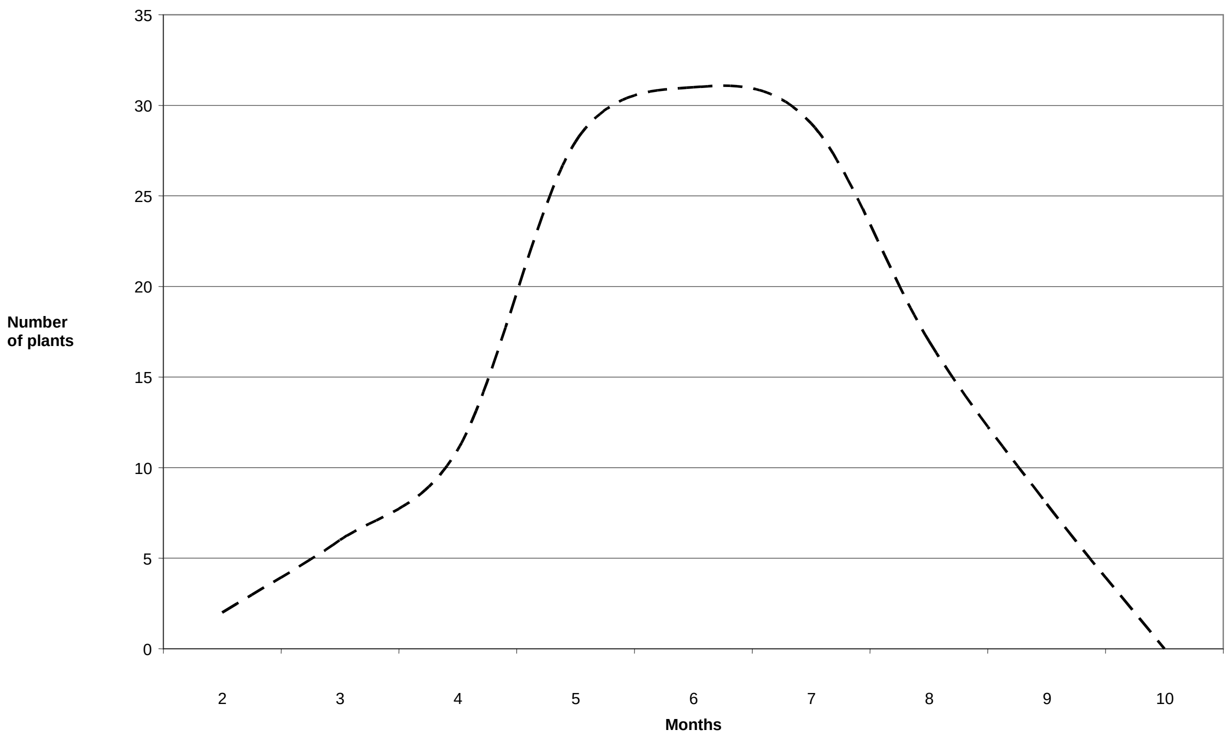

Chart 1. The honey plants in bloom in Sessile and Turkey oak forests over the year

Table 3. Wood resources of Sessile and Turkey oak forests

Area (ha)	**Total volume (m^3)**	**Volume in thickness degrees**								**Volume accretion (m^3)**
		< 10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	
Tall Sessile and Turkey oak forest										
16.22	7914		700	2173	2326	1576	720	188	230	138
Shoot forest of Sessile and Turkey oak										
412.19	42108	7219	21114	13724	5760	5055	1159	858		1018

DISCUSSION

Bearing in mind the fact that destruction of plant resources over the centuries has brought them into jeopardy; thus having the direct consequence on the socio – demographic status of the people inhabiting the Pester plateau area. The aim of these researches is to define the condition of the natural resources, give priority to the activities for stopping the negative influences and set measures for improving the state of things. Carrying into effect the research aims on the sustainable use of plant resources in Pester plateau sets the foundation for:

- Implementing of European standards, models, creating methodology for registering restorable plant resources;
- creating strategic span for sustainable management of restorable resources according to the sustainable development principles and the current exploration degree of the existing natural resources;
- maintaining and raising ecological, biological, climate, socio – cultural and economic contribution to plant resources use;
- protecting the environment, the social and spiritual function, natural ecosystem values achieved through establishment, expansion and adequate management of protected areas and communities, forest preservation in characteristic ecological systems and regions, wild life preservation and management, genofond preservation, setting up support and security measures for sustainable use of biological resources and biodiversity preservation;
- supporting and improving national afforestation programs and re – cultivation of degraded habitats, setting up new and improving the existing forests for various uses, in order to relieve the pressure on current forest ecosystems;
- founding the planning concept for permanent management of restorable plant resources on the criterion preserving the quality of environment, meaning that the economical use of restorable plant resources must not reduce numerous ecological functions, accompanied by sustaining and enhancing habitat biodiversity;
- creating conditions for ascertaining sustainable agricultural production;
- Preserving and improving biodiversity.

The data assembled enable the multilayer comparative analysis of the area by GIS technology, as well as the establishment of the natural ecosystems endangerment degree and spatial suitability for certain activities. It enables forecasting direst and indirect consequences of aimless use of the area, permitting prevention of higher level mistakes before reaching the final decision. The array of subprogram activities interacting can be formulated as a few general program tasks: environment protection, optimum natural resource use, strengthening of the secondary activities, market analyses and analyses of commercialization offers on this area, strictly in the function of preserving natural resources and environment protection (by applying the principle on sustainable development of restorable plant resources).

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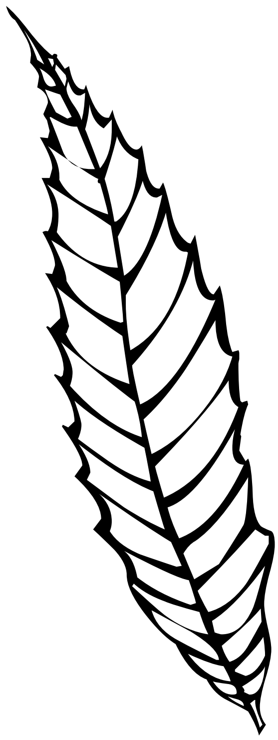

SOME PATHOLOGICAL CAUSES FOR WORSENING OF *Quercus rubra* L. STATUS IN BULGARIA

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Summary: *During more than 20 years investigations were realized on the health status of Quercus rubra L. in Bulgaria. It is established that a cancer disease on the stem bark caused by the fungus Pezicula cinnamomea (D.C.) Sacc. and its anamorph Cryptosporiopsis grisea (Pers.) Petr. The symptoms of the disease are formation of wet spots on the bark at the end of winter and/or the beginning of spring, discolouration, cracking and necroting of the stem bark and formation of perennial callus under the damaged places. The damages are more frequently seemed on dry sites as well as at and places that detain superficial water. Trees from all Kraft degrees are affected.*
Silvicultural activities to mitigate the damages are suggested, including selection of sites for afforestation, thinnings, decrease of mechanical damages during cuttings and elimination of damaged trees.
Key words: *Quercus rubra L., health status, pathogens*

INTRODUCTION

Quercus rubra L. is an introduced tree species in Bulgaria. First afforestations were mainly in parks and gardens, the oldest plantations being about 100 years old (Borisova gradina - Sofia, etc.). The last decades *Q. rubra* has been with wide application in afforestation practice but most frequently mixed plantations were established with participation of *Tilia argentea* Desf., *Quercus petraea* (Matt.) Liebl., *Fraxinus* sp., etc. These plantations were established in too different site conditions, especially about soils, exposures, humidity, etc.

It is known that *Q. rubra* is a fast growing species and its timber is with wide application in furniture production, constructions, etc. the interest by customers is high, especially about large timber but during the last decade some negative phenomena related to its health status were observed in many places were established totally dry trees.

The aim of this short communication is to present information about pathological causes for worsening of the *Q. rubra* status in forest plantations.

MATERIALS AND METHODICS

During more than 20 years observations were realized in pure *Q. rubra* plantation in the region of Shumen (Northeast Bulgaria): age – 48, soils – degraded Haplic Chernozem, medium deep, fresh, at a plain ridge. The changes in trees defoliation and leaf colour were evaluated, as well as presence of withering in different crown parts and pathological changes on bark, root neck and open roots, e.g. according to the Methodics for monitoring realization on forest ecosystems in Europe (ICP 'Forests', 1986).

The second sample plot is in Borisova gradina Park – Sofia. The plantation is aged 103, soils - Haplic Luvisol, exposure N, NW. it is observed only in relation to stems, bark, root neck and open thick roots.

Information about the status of *Q. rubra* is gathered from other plantations as well in Northeast Bulgaria.

RESULTS

Monitoring observations (SP Shumen) have shown gradually increasing of the defoliation level, in the middle and lower part of the crown it is significant, with appearance of some dry branches usually about 2 cm in diameter. Defoliation during the years is shown on Fig. 1 - in 2008 predominate trees with degree 1 (up to 25% defoliation of the crown), and with degrees 2-4 (e.g. 26-100%) are 32.5% from the trees, incl. 10% dead. In some trees it was observed leaf under sizing and in the base of the stems – wet spots with different forms and dimensions reaching several cm width. The spots are better visible at the end of the winter or beginning of spring. Later the necrotic part of the bark cracks and under it callus tissues grown permanently. The necrosis reaches cambium and in the conductive tissues tylosis was observed. Most frequently are damaged physiologically weak trees. In vital trees necrosis is possible but it does not reach cambium but only outer bark. This is the reason for them to survive despite of the damages, pathological processes in them flow very slowly and the damages are local.

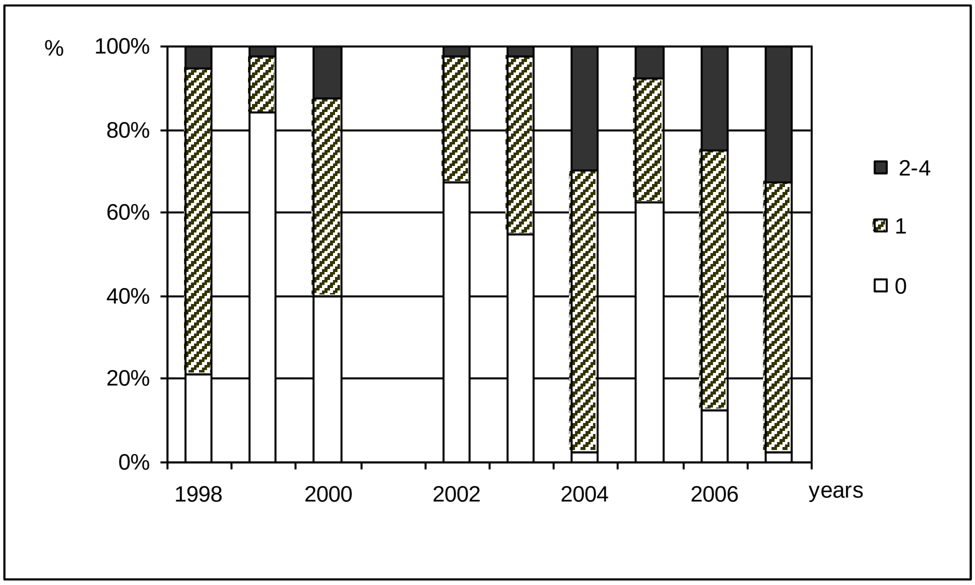

Figure 1. Evaluation of the defoliation of *Q. rubra* trees observed

Laboratory investigation show that the phenomenon described is with pathological character. The disease is caused by teleomeorphic fungus *Pezicula cinnamomea* (D.C.) Sacc. and its anamorph *Cryptosporiopsis grisea* (Pers.) Petr.

The fruit bodies (apothecia) were observed on the necrotic stem and branch barks. There were formed large quantities of stroma which at the beginning (in autumn) were yellowish but later, in next years, were dark brown to black.

The development of pathological process depends directly on some factors included in the complex of the site conditions. The observations showed that in dry to fresh sites and in sites with tendency to retain surface water, the size of the trees damaged is higher. Alternating dry with wet years leads to fluctuation of the underground water level and causes perturbances of physiological character in trees. Similar conclusions also have Butin et *al.* (1981) and Kehr (1991, 1992) in analogical investigations on *Q. rubra* status in Germany.

We established that the first symptoms of the disease were observed in forest plantations when the rough rhytidom starts to be formed in the stem base, e.g. at the age of about 25-30 years. The investigation showed that the distribution of the disease in the region of Northeast Bulgaria was significant and it was not observed in the plantations from Sofia, expert presence of mechanical damages caused during corn collection for planting material.

Climatic conditions in Bulgaria are suitable for *P. cinnamomea.* The fungus is with wide temperature range for development. During autumn-winter period, when the resistance of the host is decreased, it develops successfully even at temperature 2-4 ^{o}C (Kehr, 1991).

In order to mitigate the damages, the activities that should be realized in case of danger for Peziculian canker on *Q. rubra* are as follows:

1. Careful and competent choice of sites for creation of forest plantations from *Q. rubra*. On dry site, as well as sites with tendency for retaining of surface water, to use other tree species for afforestation.

2. During realization of cuttings first to remove trees showing Peziculian canker symptoms (wet spots, necroses, cancer in the field of root neck, dying and died trees, etc.). Do not tolerate damages on the stems, especially in the ground part during realization of silvicultural activity.

Q. rubra is now widely distributed tree species in the oak forest vegetation zone in Bulgaria. This is the reason to pay more attention on it, including its health status. More detailed further investigations on the agents causing diseases will lead to clarifying of their species' composition, pathogenesis, as well as to in due time silvicultural activities in order to mitigate the damages.

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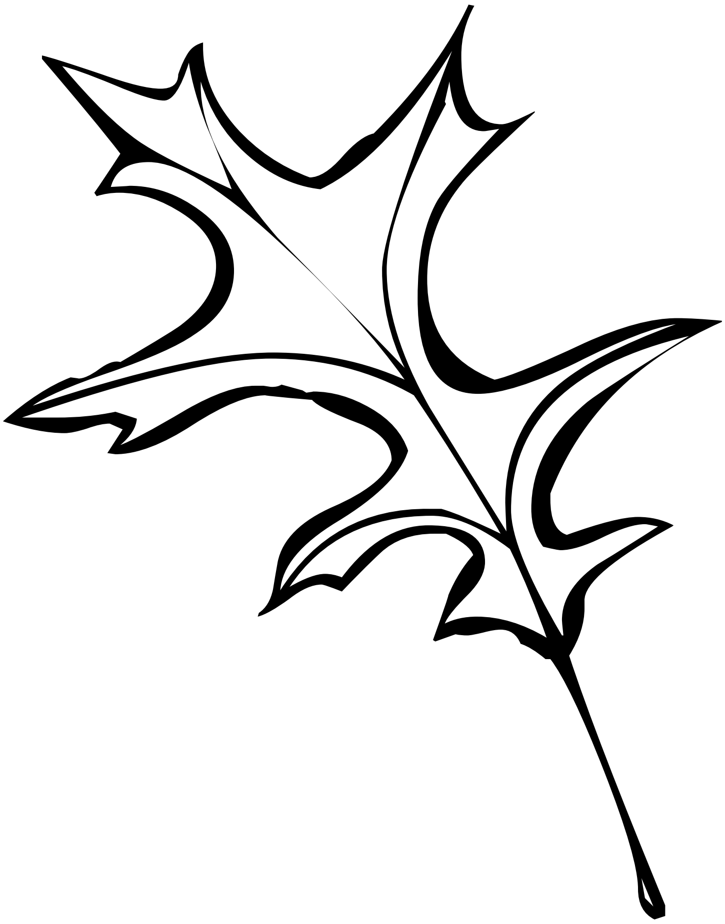

INFLUENCE OF KINETIN, 6-BENZYLAMINOPURINE, SILVER ION AND MYOINOSITOL ON GROWTH AND DEVELOPMENT OF WHITE POPLAR MICROSHOOTS IN VITRO

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Abstract: *Micropropagation is an efficient way of vegetative propagation of interesting genotypes of white poplar (Populus alba L.). The metabolism of auxins plays a crucial role in the regulation of plants growth and development. In this study we examined the effect of active substances that are known to affect the metabolism of auxins. Following substances were examined: kinetin (0 and 2 μM), 6-benzylaminopurine (0 and 2 μM), silver ion (Ag^+) (0, 1, 2 and 4 μM) and myoinositol (0, 30 and 100 mg l^{-1}, or 0, 166.5 and 555.06 μM, respectively). The microshoots were placed on ACM medium (Ahuja, 1983), the modified version of WPM. After four weeks of cultivation three characters were examined: percentage of rooting, percentage microshoots with new shoots and relative height increment. Results of analysis of variance suggest significant main effect on examined characters for cytokinines, but not for silver ion and myoinositol. Media with BAP strongly inhibited rooting, while the presence of kinetin lowered it. Good growth usually followed the good rooting percentage. Positive effects of silver ion presence on multiplication in some treatments suggest the presence of ethylene at level that inhibits flushing of new shoots. Better results for moderate then for high concentrations of myoinositol in treatments with BAP, suggest that higher attention should be paid on this important substance in 3-indolacetic acid (IAA) transport in plants. The best effect on multiplication was achieved by media with 2 μM kinetin, 2 μM BAP, moderate (30 mg l^{-1}) concentration of myoinositol and moderate or high (2 or 4 μM, respectively) concentration of silver ion.*
Key words: *Populus alba, micropropagation, axillary buds, rooting*

INTRODUCTION

The micropropagation "in vitro" is efficient way for rapid vegetative reproduction of interesting white poplar (*Populus alba* L.) genotypes. General interest in the utilization of white poplar in horticulture is piramidal or fastigiata tree shape and male sex, as well as tolerance to the environmental stress and prevalent pathogens and pests. Major goals in micropropagation are genotype preservation, high multiplication rate, high rooting rate and rapid growth. The growth and development of microshoots considerably depends on auxin metabolism. The substances that alter the effect of auxin are also important. Cytokinins kinetin and BAP are important in cell proliferation, but BUP has the impact on the accumulation of auxins in growing regions (Wilkins, 1984). Accumulation of auxins stimulates biosynthesis of ethylene (Nakagawa et al., 1991; Hansen and Grossmann, 2000), the hormone that promotes rooting but in higher concentrations suppresses growth and promotes necroses. Its activity could be suppressed by silver tiosulfate (STS) that is produced in the presence of silver ions (Ag^+). Myoinositol is involved in IAA transport, and its presence considerably speed the transport of IAA (more than 1000 times, after Gaspar and Hofinger, 1989).

The aim of this work was to examine the effect of kinetin, BAP, silver ions (Ag^+) and myoinositol on growth and development of microshoots of *Populus alba* L. cl. LBM in vitro.

MATERIAL AND METHODS

The genotype *Populus alba* L. cl. LBM is selected in Institute for lowland forestry and environment, Novi Sad, Republic of Serbia. It has "fastigiata" tree shape and male sex. It has strong vigor and tolerance against prevalent pathogens and pests.

The microshoots were grown on ACM medium (Ahuja, 1983) with 20 mg l^{-1} adenine sulphate, 100 mg l^{-1} lysine and 20 g l^{-1} sucrose. Forty eight treatments were formed by combination of kinetin (in concentrations 0 and 2 μM), BAP (in concentrations 0, 0.5 and 2 μM), Ag^+ (in concentrations 0, 1, 2 and 4 μM obtained by dissolving $AgNO_3$) and myoinositol (in concentrations (0, 30 and 100 mg l^{-1}, or 0, 166.5 and 555.06 µM, respectively). The agar was added in concentration 8 gr l^{-1}, while pH was adjusted on 5.5-5.6.

The experiments were established with 10 – 25 mm high microshoots (five per jar) set in 190 ml jars with 20 ml of medium. Three traits were examined: percentage of rooting (RP), percentage of microshoots with new shoots (MP) and ratio of length increment from the first till fourth week and length after the first week (RPH). The shoot length was measured after first and fourth week after the experiment establishment. The percentage of rooting and percentage of explants with new shoots were obtained after four weeks. The statistical analysis included four-way ANOVA, fixed model and Fisher's LSD-test. It was based on average value per jar for shoots length and value per jar for root and new shoot formation. The percentages of rooting and microshoots with new shoots were transformed by arcsine transformation (arcsine $\sqrt{X}$, where X stands for value in %). The magnitude of effect of sources of variation was evaluated by partial eta square: $\eta_p^2 = \frac{SS_X}{SS_X + SS_{ERR}}$, where SS_X stands for sum of squares for source of variation X.

The program package STATISTICA 7.0 (StatSoft Inc., 2006) was used.

RESULTS AND DISCUSSION

The results of four-way ANOVA indicate significant effect of examined cytokinin concentrations (kinetin and BAP) for all three examined traits, except for kinetin in multiplication percentage (Tab. 1). The presence of BAP considerably inhibited rooting and improved multiplication, while the effect of kinetin was the same but not so intensive. Thus, the presence of kinetin allowed rooting in considerable percentage. In the treatment with 2 µM kinetin, 2 µM silver ion and 100 mg l^{-1} myoinositol even more than 90% of microshoots achieved rooting (Tab. 2). This suggests considerable differences in action of BAP and kinetin, which could be utilized.

The main effects of silver ion and myoinositol concentrations were not significant on variation of examined characters, except negative effect of high concentration of myoinositol (100 mg l^{-1}) on multiplication. This negative effect could be related to ethylene accumulation caused by increased accumulation of IAA in growing tips regarding increased IAA transportation. The interactions of the concentration of myoinositol with kinetin, with BAP and with concentration of bought cytokinines for multiplication percentage were also significant. This was mostly due to negative effect of high concentration of myoinositol in presence of some of examined cytokinines. The effects of interaction of concentrations of myoinositol and silver ions on rooting and relative growth were also significant.

The effect of silver ions on examined characters was not significant. Only interactions with bought cytokinines were significant for relative growth. Silver ions altered negative effect of BAP alone on relative growth, suggesting the presence of ethylene in quantities that inhibited shoot growth.

The interaction conc. (Kinetin) × conc. (BAP) × conc. (Ag^+) achieved significant effect on microshoots growth. Microshoots usually improved growth by presence and by concentration increment, but on media without silver ion where there was no cytokinines or bought cytokinines were present good growth was also achieved.

Also, there was significant effect of interaction silver ion concentration x myoinositol concentration, where on in absence of myoinositol silver ion had negative, while in presence of myoinositol it had positive effect on rooting. This also suggests the relationship of IAA accumulation and accumulation of ethylene. This effect was expected for silver ions that act as an inhibitor of ethylene activity. It is known that ethylene in low concentration could improve rooting (Fuchs and Liberman, 1968; Mudge, 1989) by increment of tissue sensitivity to indole-3-acetic acid (IAA) (Visser et al. 1996). In high concentration ethylene favors secession and necrosis (Mudge, 1989). Positive effect of silver ions on multiplication and rooting suggest high presence of ethylene.

Table 1. Effect of kinetin, BAP, myoinositol and silver ion concentration in medium on variation of examined traits in *Populus alba cl.* LBM *in vitro*

Sources of variation	Degrees of freedom	RP[1)]			MP[2)]			RGH[3)]		
		MS	F-value	η_p^2 [4)]	MS	F-value	η_p^2 [4)]	MS	F-value	η_p^2 [4)]
c(Kinetin) (A)	1	10989.83	85.67**	0.309	1077.29	3.023	0.016	70906.89	32.13**	0.143
c(BAP) (B)	1	315213.53	2457.16**	0.928	154434.71	433.350**	0.693	498265.71	225.80**	0.540
c(Ag^+) (C)	3	111.60	0.87	0.013	676.20	1.897	0.029	2968.97	1.35	0.021
c(Myoinos.) (D)	2	17.68	0.14	0.001	2609.78	7.323**	0.071	1413.18	0.64	0.007
Int. A×B	1	12474.98	97.25**	0.336	981.06	2.753	0.014	61366.22	27.81**	0.127
Int. A×C	3	105.02	0.82	0.013	180.84	0.507	0.008	2528.72	1.15	0.018
Int. B×C	3	58.98	0.46	0.007	410.43	1.152	0.018	2086.68	0.95	0.015
Int. A×D	2	115.45	0.90	0.009	1887.20	5.296**	0.052	3160.21	1.43	0.015
Int. B×D	2	18.56	0.15	0.002	1393.83	3.911*	0.039	4913.18	2.23	0.023
Int. C×D	6	321.38	2.51*	0.073	520.32	1.460	0.044	8042.32	3.65**	0.102
Int. A×B×C	3	68.08	0.53	0.008	632.65	1.775	0.027	12354.25	5.60**	0.080
Int. A×B×D	2	280.99	2.19	0.022	2319.80	6.509**	0.064	10336.13	4.68**	0.047
Int. A×C×D	6	286.93	2.24*	0.065	149.60	0.420	0.013	2532.26	1.15	0.035
Int. B×C×D	6	278.51	2.17*	0.064	151.99	0.426	0.013	3608.72	1.64	0.049
Int. A×B×C×D	6	236.22	1.84	0.054	430.64	1.208	0.036	9181.86	4.16**	0.115
Error	192	128.28			356.37			2206.66		

[1)] RP - percentage of rooting (%); [2)] MP - percentage of microshoots with new shoots (%); [3)] RHG - ratio between increment between the first and fourth week and height after the first week (*100%); [4)] Partial eta square

The positive effect of silver on multiplication, i.e. activation of axillary buds, suggests that high presence of ethylene suppressed activation of buds. It is well known that IAA repress while cytokinines stimulates activation of sylleptic buds, as a part of regulation of apical dominance (Woolley and Wareing, 1972; Wilkins, 1984; Cline and Dong-Il, 2002). The connection of IAA accumulation with absence of bud activation was not completely understood yet. Eliason (1969) found that distribution of inhibitors along the shoot of *Populus tremula* L. decreases basipetaly. Those inhibitors (so called β-inhibitors), are generally related to abscisic acid (ABA) activity.

However, Gaspar in 1973 (after Gaspar and Hofinger, 1989) found among β-inhibitors, also the product of IAA oxidation - 3-methyleneoxindole, the substance with more than 10 times higher auxin activity. Later results (Nakajima et al., 2002) confirmed the influence of the products of IAA oxidation on the persistence of apical dominance. However, our results indicated that inhibition of the action of ethylene could promote bud activation. We suppose the ethylene could be included in apical dominance considering that accumulation of IAA promote ethylene biosynthesis (Nakagawa et al., 1991; Hansen and Grossmann, 2000).

The best results in rooting were obtained in media without cytokinines with the best growth on medium without any examined active substance but 100 mg l^{-1} of myoinositol (RHG > 250%). An intensive growth of these media could be partially related to simulative effect of roots, too. Considerable rooting was obtained in media with kinetin, but the values for RHG were smaller. This could be related also to lower rooting percentage.

Best results in multiplication was achieved on media with 2 µM kinetin, 2 µM BAP, 2 or 4 µM silver ion and 30 mg l^{-1} myoinositol (MP > 90%). It is interesting that the concentration of myoinositol is 30 mg l^{-1} and not 100 mg l^{-1} that is usual in the micropropagation praxis in white poplar (Ahuja, 1983) (Table 2). There is also clear positive effect of silver ion in media with bought cytokinines. Regarding the RHG, the best relative growth, in absence of rooting, was also achieved on these two treatments. Thus, these treatments could be suggested for further micropropagation of genotype LBM, and also should be tested on other white poplar genotypes. On usual media, proposed by Ahuja (1983) for aspen micropropagation: 2 µM BAP and 0.1 µM NAA Kovacevic et al (2008) achieved around 80% of micropropagation rate.

Table 2. Average values examined traits in *Populus alba cl.* LBM in vitro for combinations of examined concentrations of kinetin, BAP, myoinositol and silver ion concentration in medium

c(Kinetin) (μM)	c(BAP) (μM)	c(Ag^+) (μM)	c(Myoinositol) (μM)	RP[1)]	RP_t[2)]	MP[3)]	MP_t[4)]	RHG[5)]
0	0	0	0	100.00	90.00	12.00	15.94	206.15
0	0	0	30	92.00	79.37	4.00	5.31	161.43
0	0	0	100	100.00	90.00	0.00	0.00	253.95
0	0	1	0	92.00	79.37	0.00	0.00	130.54
0	0	1	30	100.00	90.00	0.00	0.00	124.33
0	0	1	100	100.00	90.00	0.00	0.00	162.67
0	0	2	0	92.00	79.37	0.00	0.00	167.62
0	0	2	30	100.00	90.00	8.00	10.63	182.88
0	0	2	100	100.00	90.00	20.00	18.00	181.67
0	0	4	0	96.00	84.69	0.00	0.00	243.49
0	0	4	30	100.00	90.00	4.00	5.31	146.97
0	0	4	100	100.00	90.00	8.00	10.63	173.13
0	2	0	0	0.00	0.00	64.00	56.53	39.18
0	2	0	30	0.00	0.00	48.00	46.85	27.44
0	2	0	100	0.00	0.00	68.00	56.06	49.79
0	2	1	0	0.00	0.00	84.00	71.53	62.71
0	2	1	30	0.00	0.00	76.00	63.68	83.18
0	2	1	100	0.00	0.00	73.00	67.85	36.81
0	2	2	0	0.00	0.00	76.00	63.68	29.60
0	2	2	30	0.00	0.00	72.00	61.37	56.82
0	2	2	100	0.00	0.00	72.00	61.37	81.37
0	2	4	0	0.00	0.00	88.00	74.06	50.65
0	2	4	30	0.00	0.00	68.00	61.62	86.67
0	2	4	100	0.00	0.00	44.00	38.53	53.32
2	0	0	0	76.00	63.68	20.00	21.01	134.84
2	0	0	30	60.00	54.00	8.00	10.63	101.21
2	0	0	100	44.00	41.31	0.00	0.00	26.52
2	0	1	0	72.00	61.15	22.67	28.30	117.52
2	0	1	30	76.00	63.68	8.00	7.85	111.48
2	0	1	100	61.00	54.69	5.00	6.00	124.25
2	0	2	0	76.00	66.69	12.00	15.94	115.41
2	0	2	30	52.00	46.38	8.00	7.85	77.74
2	0	2	100	92.00	79.37	17.00	16.15	147.45
2	0	4	0	60.00	54.00	36.00	33.47	145.96
2	0	4	30	84.00	74.53	20.00	18.00	176.78
2	0	4	100	55.00	47.88	0.00	0.00	59.39
2	2	0	0	0.00	0.00	56.00	51.69	40.49
2	2	0	30	0.00	0.00	88.00	79.85	30.34
2	2	0	100	0.00	0.00	29.00	26.53	103.64
2	2	1	0	0.00	0.00	64.00	53.53	45.82
2	2	1	30	0.00	0.00	80.00	68.99	50.48
2	2	1	100	0.00	0.00	50.00	45.00	29.58
2	2	2	0	0.00	0.00	80.00	66.21	38.93
2	2	2	30	0.00	0.00	96.00	84.69	66.73
2	2	2	100	4.00	5.31	68.00	61.85	83.46
2	2	4	0	0.00	0.00	72.00	61.37	80.56
2	2	4	30	0.00	0.00	92.00	79.37	31.21
2	2	4	100	4.00	5.31	52.00	46.38	27.54
			$LSD_{0.05}$		14.13		23.55	58.60

[1)]RP - percentage of rooting (%), [2)] RP_t – transformed RP, [3)] MP - percentage of microshoots with new shoots (%), [4)] MP_t – transformed MP, [5)] RHG - ratio between increment between the first and fourth week and height after the first week (*100%).

Table 2. Continue

c(kinetin) (µM)	c(BAP) (µM)	c(Ag^+) (µM)	c(Myoinositol) (µM)	RP[1)]	RP_t[2)]	MP[3)]	MP_t[4)]	RHG[5)]
0				48.83	43.45	37.04	32.87	116.35
2				34.00	29.92	40.99	37.11	81.97
	0			82.50	72.92	8.86	9.63	144.73
	2			0.33	0.44	69.17	60.36	53.60
		0		39.33	34.86	33.08	30.87	97.92
		1		41.75	36.57	38.56	34.39	89.95
		2		43.00	38.09	44.08	38.98	102.47
		4		41.58	37.20	40.33	35.73	106.31
			0	41.50	36.18	42.92	38.33	103.09
			30	41.50	36.75	42.50	38.25	94.73
			100	41.25	37.12	31.63	28.40	99.66
			LSD0.05		14.13		23.55	58.60

[1)]RP - percentage of rooting (%), [2)] RPt – transformed RP, [3)] MP - percentage of microshoots with new shoots (%), [4)] MPt – transformed MP, [5)] RHG - ratio between increment between the first and fourth week and height after the first week (*100%).

Our results suggest that relatively high concentration of BAP promoted activation of axillary buds which is in accordance with findings of Cline and Dong-Il (2002). However, silver ions stimulated growth suggesting inhibition by ethylene. We suppose that ethylene accumulation is mainly enforced by BAP-stimulated accumulation of IAA.

Our results enforce implementation of silver ion in micropropagation of white poplars and further work on the control of negative effect of ethylene in vitro culture.

CONCLUSION

According to the presented results following could be concluded:

- Significant main effect was on examined characters for cytokinines, but not for silver ion and myoinositol
- BAP strongly inhibited rooting, presence of kinetin just lowered it
- Good growth usually followed the good rooting percentage
- Positive effects of silver ion presence on multiplication in some treatments – high presence of ethylene?
- Better results for moderate then for high concentrations of myoinositol in treatments with BAP
- The best effect on multiplication was achieved by media with 2 µM kinetin, 2 µM BAP, moderate (30 mg l-1) concentration of myoinositol and moderate or high (2 or 4 µM, respectively) concentration of silver ion.

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ANTIMICROBIAL INFLUENCE OF FOREST SPECIES OF ZLATAR MOUNTAIN ON THE ENVIRONMENT

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Summary: *This paper presents the results of one-year study of antimicrobial effect of numerous forest species of Zlatar Mt.(Abies alba Mill., Pinus silvestris L., Acer platanoides L., Betula verrucosa Ehrh., Carpinus betulus L., Crataegus monogyna Jacq., Cornus mas L., Juglans regia L., Juniperus communis L., Mentha longifolia (L.) Huds., Ribes nigrum L., Rhus cotinus L., Quercus pubescens Willd., etc.) on the environment. The study reveals that all the aromatic species of trees, shrubs and herbaceous plants have an antimicrobial effect. The quantity of antimicrobial metabolites – essential oils is the highest in the species Juniperus communis (up to 3%) and Mentha longifolia (up to 4%). Their essential oils have an inhibitory activity on the majority of the most frequent bacterial species, and their fungicidal effects have not been recorded.*
Key words: *aromatic plants, forest, essential oils, antimicrobial effect, fungicidal effect*

INTRODUCTION

The plants have a fundamental significance for creating an attitude about the environment. One cannot determine the position of the man in the biosphere and his dependency of it without the knowledge about plants functions. The plants are the basic biologic and ecologic unit which supports the maintenance of the biosphere and also of the human population. Among the numerous functions of the plants, one of the most important is their antimicrobial activity. The antimicrobial activity of the plants is important for the existence of dynamical ecological balance which is the basic condition for the life on the Earth. As a result of this activity, very old and complex plant communities exist at present time. During their long evolution, the plants have created and improved an efficient biological system of antimicrobial activity. This system had supported their survival and also a successful distribution. The capability of antimicrobial protection is a result of the presence of secondary metabolites. Very important are essential oils that exist as secondary metabolites of aromatic plants (Adebajo et *al.* 1989; Diksit & Husain, 1984; Janssen et *al.* 1987; Morris et *al.*, 1979; Singh et *al.*, 1983).

The data from the study of autochtonous forest's protection had shown that the self-protection is not just the property of the plant species, but also the property of the plant communities (Mišić, 1981, 1982; Matović, 1983, 1986; Stevanović & Vasić, 1995).

MATERIALS AND METHODS

Plant material and isolation procedure

The aerial parts of numerous plants: *Abies alba* Mill., *Acer platanoides* L., *Betula verrucosa* Ehrh., *Carpinus betulus* L., *Crataegus monogyna* Jacq., *Cornus mas* L., *Juglans regia* L., *Juniperus communis* L., *Mentha longifolia* (L.) Huds., *Pinus silvestris* L., *Ribes nigrum* L., *Rhus cotinus* L., and *Quercus pubescens* Willd., have been collected at Zlatar Mt.

Dried aerial parts (200gr) of plants, were subjected to the hydro-distillation of 3h, using an all glass Clevenger-type apparatus, according to the method recommended by the European Pharmacopeia (European Pharmacopeia 1983) to produce oil. The oils were dried over anhydrous sodium sulfate and stored in sealed vials at low temperature before analysis.

Gas chromatography

GS analysis were performed using GC-FID (HP 5890 Series II, HP 5971 MSD, electron impact mode 70). Oven temperature was held at 50^{0}C for 5 min and then programmed to 240^{0}C at a rate of 3^{0}C/min. Detector (FID) temperature was 300^{0}C and injector temperature was 250^{0}C at the linear temperature program from 40 - 280^{0}C. The temperature of the transfer line was 280^{0}C. The identification of the components have been accomplished by comparation of their retention times and mass specters with the standards. The comparations of the mass specters have also been performed with the data available from the library of the mass specters (Wiley/NBC).

Antimicrobial activity

Antimicrobial activity of the investigated metabolites of *Juniperus communis* and *Mentha longifolia* was determined by the disc-diffusion method and by measuring the inhibition zone. The bacterial strains used in this study were: *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Esherichia coli*, *Pseudomonas flurescens* and *Pseudomonas syringae* var. *phaseolicola*. The fungal strains were: *Aspergillus niger*, *Mucor* sp., *Trichoderma viride* and *Trichoderma harzionum*. Test concentrations were two dilutions: a) 1g of the plant material in 10 ml of water and b) 1g of the plant material in 20ml of water. Bacteria strains were cultured in the melted feeding agar for 48 hours on 20^{0}C; fungi were cultured in the dextrose agar for 7 days at the same temperature. The results were compared with bactericide effect of the penicillin or the fungicide effect of the nistatyne.

The experiments have been performed as eight repetitions, and the results were statistically analyzed. The results of this analysis are presented in tables and chromatograms.

RESULTS AND DISCUSSION

The character of this paper is partially synthetic, and it includes the results of the previous studies. This refers to the numerous studies of the wide area of the former SSSR (Tokin, 1951) and to the results of the Bulgarian scientists research (Koleva, 1980), and at Zlatar Mt. (Matović, 1994, 1995a, 1995b, 1996, 1997, 1998; Matović et *al.*, 1995b).

Among numerous species with significant phytoncide activity we select several. Phytoncide activity was calculated as time (in minutes) which is necessary for destroying microorganisms. In the following Table 1. are the results of the previous studies of the antimicrobial plant metabolites and also the part of our recent studies results.

One fir during 24h release 30g of volatile compounds to the atmosphere. The fir forest can release 30 kg of the volatile compounds from the surface of 1 ha for the same time period. Dependently of the atmospheric conditions and the time of the year, 1 ha of the pine forest produces 154 to 392g of the phytoncides: the birch forest produces 28 to 310g of the phytoncides.

Mt. Zlatar has nearly 7.875 ha of forest space. The most dominant forest trees: fir, spruce and pine have also the bigger phytoncide activity. Fir, as dominant species in forest communities of Zlatar, occupies 2770,43 ha and can release 83,1 kg phytoncides during 24 h. Spruce occupies 2657,22 ha and can release 79,71 kg phytoncides during 24 h. Pine occupies 567,57 ha and can release 222,26 kg during 24 h.

We can apply this data to the whole planet; we will get a sum about 175 million tones of the essential oils that the planet's vegetation produce per year.

The quantity of antimicrobial metabolites – essential oils of *Juniperus communis* fruits from eight localities at Zlatar Mt. was 3,25% (Gradina), 2,70% (Tikva), 2,66% (Ceste), 2,53% (Pliješ), 2,47% (Osoje), 2,40% (Ravne), 2,20% (Muškovko Brdo) and 2,13% (Dolovi). In the *Juniperus communis* essential oil was registered 42 components. From these 42 components, 32 was identified and quantified. The previous study's data from the tables and graphs shows that the antimicrobial products of *Juniperus communis* have an inhibitory effect which is limited by its chemical content, concentration and by the taxonomical properties of the microorganisms. The antimicrobial effect of *Juniperus communis* essential oil is a result of the high content of α-pinene (30,763%), sabinene (19,372%), *p*-cymene (0,226%), l-limonene (4,904%) and α-terpinolene (1,318%). The biologically activity of this constituents have already been described by Ross et *al.* (1980), Adebayo et *al.* (1989), and Janssen et *al.* (1987). The content of the *Juniperus communis* essential oil depends of the geological, pedological, climate and other properties of their habitat.

This study established that the milled whole fruits, milled remains of the fruits after the alcohol extraction, milled remains of the fruits after hydro-distillation, water extract of the fruits, milled remains of the fruits after the distillation of the essential oils and essential oil of *Juniperus communis* are inhibiting the growth of *Agrobacterium tumefaciens, Bacillus subtilis* and *Pseudomonas fluorescens*. An alcohol extract and the milled leaves of *Juniperus communis* are inhibiting the growth of *Agrobacterium tumefaciens* and *Bacillus subtilis* (Figures 1 – 5). The intensity of the inhibitory effect is proportional to the concentration of the metabolites. Because of the *Juniperus communis* antimicrobial effect and attractive forms, the designers of the cities green areas should consider the higher frequency of this plant.

Table 1. Microbicidal time of metabolite exposition (min.)

Species	Tokin (1948)	Lunc (1974) after Koleva (1980)	Koleva (1980)	Matović (1994-2005)
Abies alba Mill.	-	-	18-20	19-22
Acer platanoides L. .	-	15	-	12
Acer tataricum L.	20	20	-	18-20
Betula verrucosa Ehrh.	20-25	20	-	20-24
Carpinus betulus L.	7	-	-	6-7
Cornus mas L.	7	-	-	7
Crataegus monogyna Jacq.	8	-	-	8-10
Juglans regia L.	18	18	-	17-19
Juniperus communis L.	-	-	-	10
Picea excelsa Link.	-	-	20-25	21
Pinus silvestris L.	5-15	-	7-10	6-9
Quercus pubescens Will.	6	6	-	5-7
Rhus cotinus L.	11	-	-	10-12
Ribes nigrum L.	10	10	10	9-11

Leaves of *Mentha longifolia* at Zlatar Mt. have up to 4% volatiles (essential oils). In the essential oil of *Mentha longifolia* was registered 56 components, and 15 of those components was identified and quantified. The main components of this oil are dihydrocarvone (46,49% from which was 15,89% in the *cis*-form and 30,60% in *trans* form), piperethine (20,33%), *cis*-dihydrocarveol (7,51%). The individually concentrations of the residual components was not exceeded 5%. In this sample were also identified α-pinene (0,61%), sabinene (0,54%), β-pinene (0,83%), 3-octanole (0,72%), 1,8- cineole (2,02%), limonene (4,26%), linalool (2,21%), 3,7- dimetyl-1,3,6- octatitriene (0,06%), β- burbonene (0,75%), *trans*–caryophyllene (3,43%), and γ-murolene (0,38%). The microbiological research that will continue, showed that essential oil of *Mentha longifolia* has an inhibitory effect to all investigated bacterial strains. The study of the antifungal effect of *Mentha longifolia* essential oil is still in progress.

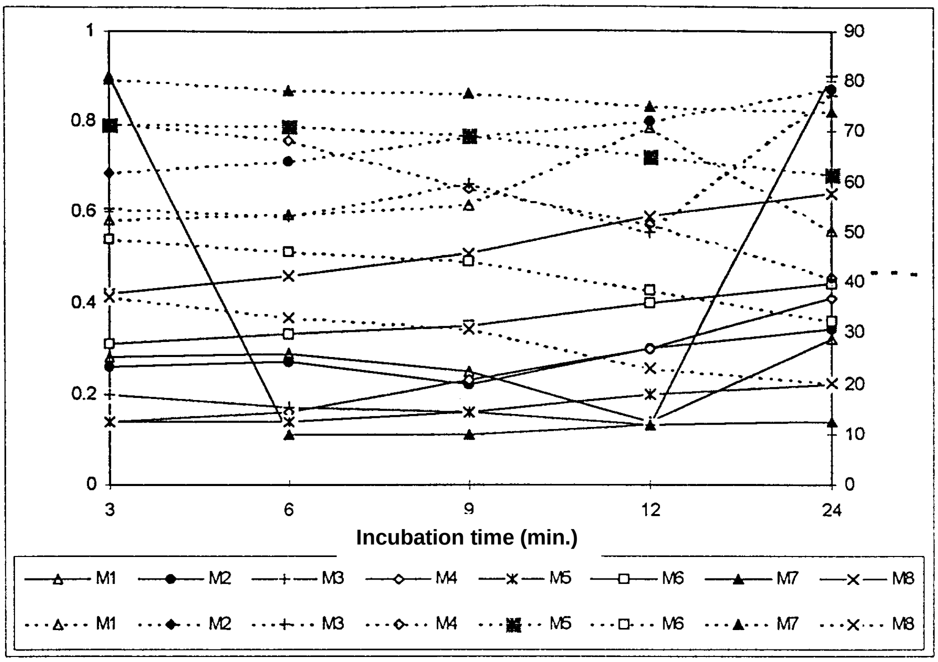

Figure 1. The effect of the *Juniperus communis* metabolite to the *Agrobacterium tumefaciens* population density

Legend for Figures 1 – 5: M_1-milled fruits; M_2-milled remains of the fruits after the alcohol extraction; M_3- alcoholic extract from the fruits; M_4-milled remains of the fruits after the water extraction; M_5-water extract of the fruits; M_6--milled remains of the fruits after the distillation of the essential oils; M_7-essential oil from the fruits; M_8-milled leaves of *Juniperus communis*

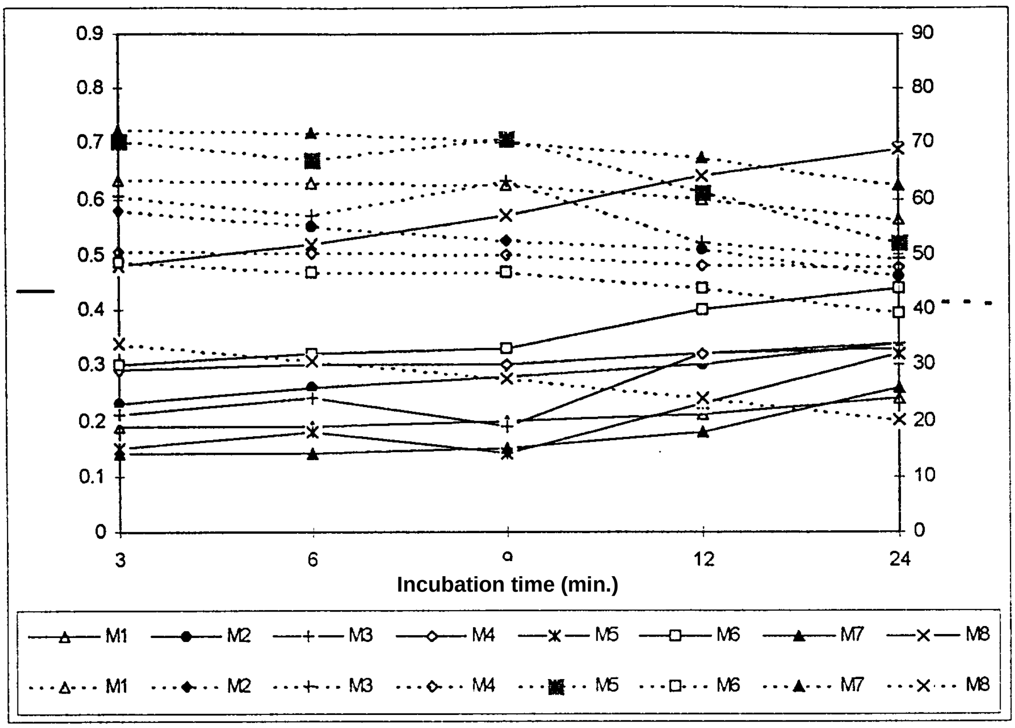

Figure 2. The effect of the *Juniperus communis* metabolite to the ***Bacillus subtilis*** population density

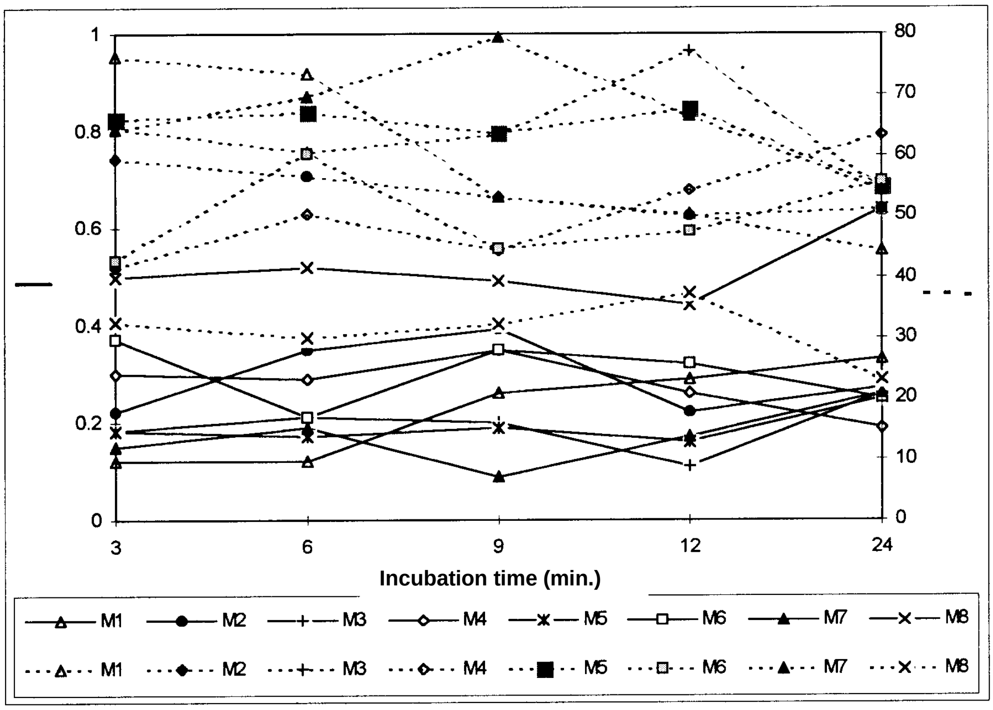

Figure 3. The effect of the *Juniperus communis* metabolite to the *Pseudomonas fluorescens* population density

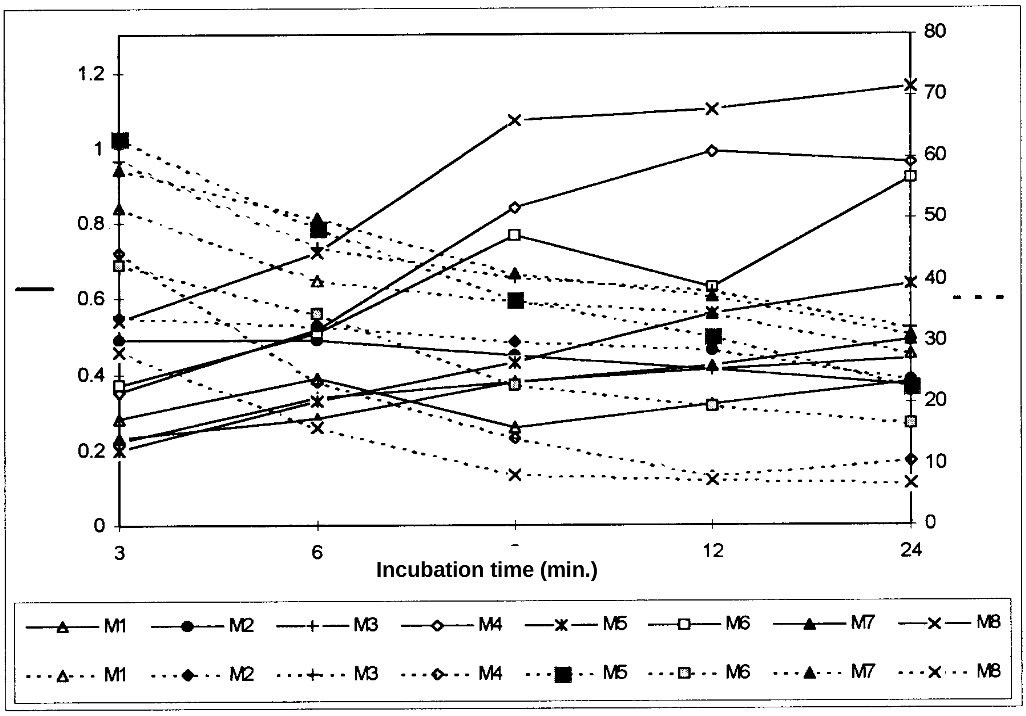

Figure 4. The effect of the *Juniperus communis* metabolite to the *Esherichia coli* population density

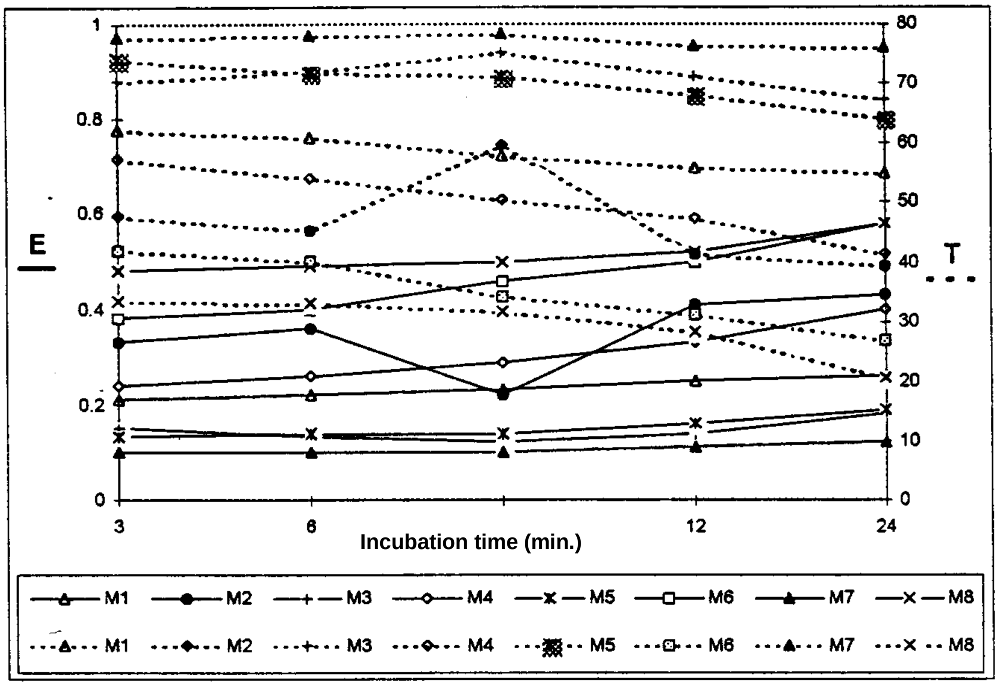

Figure 5. The effect of the *Juniperus communis* metabolite to the *Pseudomonas syringae* var. *phaseolicola* population density

CONCLUSION

The studies of essential oils antimicrobial effect on the area of Zlatar Mt. showed that metabolites of the plants that have been collected on various habitats has an inhibitory influence to almost all bacterial strains. The fungicidal effect of the *Juniperus communis* was not registered, and for this effect of the *Mentha longifolia* essential oil the experiments are still in progress. The intensity of the inhibitory effect depends of the metabolic material extract and it is proportional to the concentration of the metabolite content. The results from tables and graphs pointed out that investigated essential oils have more bacteriostatyc than bactericidal effect.

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CONTENTS OF Pb, Cd, Zn AND Ni IN THE SEDIMENTS OF THE RIVER DANUBE AND TISA AFTER FLOODING

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Abstract: *The great portion of heavy metal pollution is of antropogenic character. Heavy metals are considered as one of the main sources of the environmental pollution. The aims of this work were to determined the pollution of sediments after two great flooding on the river Danube and Tisa. The contents of the lead, cadmium, zinc and nickel were researched in the sediments after the flooding in the years of 2005 and 2006 on the river Danube and Tisa.*
The textural class of the sediments on the river Danube were sand, and on the river Tisa loamy sand. The reaction of soil solution were weak alkaline. The sediments were poor in humus. The contents of carbonates were in range of 10,41 to 13,79%.
The contents of lead were from 4,80 ppm on the sediments of river Danube to 9,72 ppm on the river Tisa. The contents of cadmium were 0,29 ppm on the river Tisa. In the sediments of river Danube were not determined. The contents of nickel were 4,80 ppm in the sediments on the river Danube and 11,66 ppm on the river Tisa. The contents of zinc were 32,77 ppm in the sediments on the river Danube and 39,81 ppm on the river Tisa.
The greatest contents fractions of lead, nickel and cadmium were determined for reducing fractions (for lead from 3,65 to 8,1 ppm, for nickel from 3,65 to 8,1 ppm and for cadmium from 0,00 to 0,16ppm).
Key words: *heavy metals, sediments, Danube, Tisa*

INTRODUCTION

Composition of alluvial plane is very specific, as well as the conditions under which it is formed. It has been known that alluvial plane is formed by moving away the courses of the river, and by river meandering. Sandbanks are formed during the first stage, and later on the terrain is raised due to deposition firstly of large, and than of smaller particles. Soil formation i.e. differentiation to evolution genetic series is conditioned by river transferring power, river deposits, and variation in the level of underground waters. Mentioned occurances are also the main causes of differences appearing among individual types of soils on the alluvial plane. Depending on the basic parameters which caused differentiation to evolution genetic series, poloj is differentiated to coastal, central pre-terrace part. Šumakov (1960) mentioned that these three genetic parts of poloj are characterized by specific relief, hydro-geographic, and vegetation traits.
Deposition of river sediments is a special problem under condition of greater pressure upon environment. Pressure on the environment is formed first of all as the consequence of human activities.

From the above mentioned reasons determination of heavy metals in soil, atmosphere, plants and sediments is very important in environment monitoring. However, total quantity of heavy metals in sediments is neither reliable indicator for estimation of biological activity, nor for potential risk (Chen et *al.*, 1997). In recent years methods for identification of bioactive forms of heavy metals in soil are being considered due to the above mentioned reasons (Wang et *al.* 2003). The most significant factors upon which biological heavy metals activity depends are physical and chemical soil properties, among others.

Heavy metals (HMs) are considered as serious inorganic pollutants because of their toxic effects on life in aquatic system, having a high enrichment factor and slow removal rate (Allowey and Ayres 1997). Sediments function as a sink for HMs from diverse sources, reflecting the natural soil composition of the surrounding areas, as well as human activities (Araina et all 2008). In BCR sequential extraction schemes (Gleyzes et all. 202), extractants are applied in order of increasing reactivity so that the successive fractions obtained correspond to metal association forms with lesser mobility. The extractants commonly used in BCR sequential extraction schemes fall generally within the following groups: acetic acid 0.11M (step 1); reducing agents (step 2); oxidizing agents (step 3).

Degree of lead, cadmium zinc, and nickel loads in the Danube and Tisa sediments, as well as exchangeable, oxidizing and reducible fractions of these heavy metals were analyzed in this paper.

MATERIALS AND METHODS

Studies were done on Danube and Tisa rivers on the recent alluvial deposits having morphology structure I – $(A)_b$ - IG_{so} (Škorić et al. 1985). Granulo-metric soil composition was determined according to international B pipette method, and the textural class according to Atteberg's classification (Hadžić et all. 2004). Following methods were used for determination of chemical traits: humus (%) according to Tjurin modified by Simakov (Škorić i Sertić 1966, and $CaCO_3$ (%) volumetrically using Scheiblerov's calcimeter (Hadžić et *al.* 2004). Heavy metal fractions were analyzed using modified BCR method (Rauret et *al.* 2000), and values were read on AAS purchased from Varian.

RESULTS WITH DISCUSSION

According to classification of soils of Yugoslavia (Škorić et all. 1985) soils were determined as recent alluvial deposits. Morphological profile structure of Danube was **I – $(A)_b$ - II G_{so} - ... G_r** of morphology prifile description:
I (0-115 cm): grey sand
$(A)_b$ (115-125cm): rusty-grey loamy sand with organic matter, full of plant roots
IIG_{so} (>125 cm): rusty-grey sand, wet full of plant root system

In comparison to Danube, deposit on river Tisa was 45 cm thinner. Morphological profile structure was **I – A_b - II G_{so} - ... G_r** of morphology prifile description
I (0 - 70 cm): grey-yellowish sand filled with organic matter, root system protruding through this layer, sharp transition into
A_b (70 - 90 cm): rusty-grey loamy sand, fossil horizon full of plant root system, with snail's shell, the sign of survival in this horizon
II G_{so} (90 – 150 cm and deeper): rusty-grey loamy sand to lamy sand

Since the aim of this paper was to analyze the load of alluvial deposits on rivers Danube and Tisa data relating to granulo-metric composition, textural class, humus content, carobnates, and heavy metals loads will be given only for alluvial deposits in tables 1, 2, 3, 4, 5 and 6. Fraction of fine sand prevailed in granulo-metric composition of both deposits (Table 1).

Table 1. Granulo-metric composition, textural class, humus and carbonate content in river sediments

	Granulo-metric composition %						Textural class	Humus	$CaCO_3$
	> 0,2	0,2 - 0,02	0,02 - 0,002	< 0,002	Total > 0,02	Total < 0,02		%	%
	mm	mm	mm	mm	mm	mm			
Danube	4.2	94.7	0.2	0.9	98.9	1.1	Sand	0.0	13.79
Tisa	9.6	76.0	9.2	5.2	85.6	14.4	Loamy sand	0.2	10.41

Greater participation of this fraction was determined in Danube deposits. Content of silt+clay fraction in Tisa alluvial deposits was 14.4%. Danube alluvial deposit was classifed according to its textural composition as sandy textural class, and that of Tisa as loamy sand.

Humus content was not determined due to the fact that alluvial deposit was in question. More expressed carbonate feature was determined in Danube sediments, which was in accordance with previous investigations (Živanov 1970, 1977, 1979; Živković et all. 1972). Total load of heavy metals is given in table below.

Sediments in river the Tisa were loaded with studied heavy metals content in relation to sediments in river Danube. Content of deposited lead was 9.72 ppm, cadmium 0.29 ppm, nickel 11.66 ppm, and zinc 39.81 ppm.

Sediment in Danube was significantly less loaded with heavy metals content than that of Tisa, so determined quantity for lead and nickl was 4.80 ppm, and for zinc 32.77 ppm.

Table 2. Sediments loaded with heavy metal

	Pb		Cd		Ni		Zn	
	ppm	%	Ppm	%	ppm	%	Ppm	%
Danube	4.80	4,8	0.00	0,0	4.80	9,6	32.77	10,9
Tisa	9.72	9,7	0.29	9,5	11.66	23,2	39.81	13,2
MAC	100		3.0		50		300	

*MAC maximal admissible concentration of heavy metals according to the Regulation for agricultural soils in Serbia

Cadmium was not found in Danube sediments. In comparison to maximum allowed quantity of heavy metals in agricultural soils, it was determined that sediments were not loaded with heavy metals. Analysis of exchangeable, reducible, oxidizing fraction for studied heavy metals was carried out, although sediments were not loaded with heavy metals.

Content of exchangeable, reducible, oxidizing lead fraction is given in table 3.

Table 3. Content of exchangeable, reducible, oxidizing lead fraction in sediment

	exchangeable	reducible	oxidizing	residue	Total
	ppm	ppm	Ppm	ppm	Ppm
Danube	0.25	3.65	0.90	0.00	4.80
Tisa	0.66	8.10	0.96	0.00	9.72

The greatest content in Danube sediment was determined for reducible lead fraction. Content of this fraction was presented as the total participation of lead of 75% in Danube sediment, and 83% in Tisa sediment. Total content of cadmium was small in both sediments, and the greatest content in Tisa sediment was determined for reducible fraction.

Table 4. Content of exchangeable, reducible, oxidizing cadmium fraction in sediment

	exchangeable	reducible	oxidizing	residue	total
	ppm	ppm	Ppm	ppm	ppm
Danube	0.00	0.00	0.00	0.00	0.00
Tisa	0.09	0.16	0.04	0.00	0.29

In total load of nickel of 4.80 ppm in Danube sediment i.e. 11.66 ppm in Tisa sediment, the greatest content belonged to reducible fraction (Table 5).

Table 5. Content of exchangeable, reducible, oxidizing nickel fraction in sediment

	exchangeable	Reducible	Oxidizing	residue	total
	ppm	ppm	Ppm	ppm	ppm
Danube	0.25	3.65	0.90	0.00	4.80
Tisa	0.66	8.10	0.96	1.94	11.66

Participation of reducible fraction in relation to total nickel load was 75% in Danube, and 71% in Tisa sediments.

Content of exchangeable, reducible, oxidizing zinc fraction in sediment is given in Table 6.

Table 6. Content of exchangeable, reducible, oxidizing zinc fraction in sediment

	exchangeable	Reducible	Oxidizing	residue	Total
	ppm	ppm	Ppm	ppm	ppm
Danube	2.46	12.76	3.15	14.4	32.77
Tisa	1.40	11.52	3.99	22.9	39.81

The greatest content in contrast to other heavy metals was determined in residue. Content of reducible fraction in Tisa was 28%, and in Danube sediment 38% from the total zinc content.These investigations included the analysis of heavy metals load in Danube and Tisa sediments after flooding during 2005 and 2006. Data revealed the fact that Danube and Tisa sediments were not loaded with heavy metals. Sediment characteristics and contaminant levels shows that the upper soil layer of the floodplains in river Rhine have (Klok and Kraak 2008) a moderate levels of cadmium and copper, but relativly high levels of zinc. Concentrations of all (Pb, Cd, Zn, Cu, Co, Ni, and Cr) elements on Vlatava after flooding (Navratil et all. 2008) were higher than appropriate “Target values” for soils (concentrations of contaminants in clean soils) but never exceeded the “Intervention values” for soils (criterion for potentially contaminated soils). The results from Nilufer (Guleryuz 2008) stream indicate that was a increase in sediment heavy metal content along the Nilufer stream depending on insiltrial activity. The contents of Zn and Ni was greater then in sediments of Danube and Tisa.

There is a big possibility that small heavy metals load in Danube and Tisa sediments was the consequence of high participation of total sand with small adsorption ability in contrast to sediment of Rajna river deposit. As in the case of Vltava sediment the load was below maximal allowed concentration.

CONCLUSION

Studies were carried out in Tisa and Danube rivers on systematic soil unit fluvisol f sandy morphological structure of profile I – $(A)_b$ - IG_{so}.

Thickness of river sediments was 45 cm greater on river Danube in relation to river Tisa. According to textural class sediment of river Danube was sand, and of river Tisa loamy sand. Content of silt+clay fraction in Danube sediment was lower in relation to Tisa. Tisa sediment was more loaded with content of all studied heavy metals in relation to Danube. Lead content in sediment was 9.72 ppm, cadmium 0.29 ppm, nickel 11.66 ppm, and zinc 39.81 ppm. Danube sediment was significantly less loaded with heavy metals in relation to Tisa sediment, so determined quantity for lead and nickel was 4.80 ppm, and for zinc 32.77 ppm. Cadmium was not detected in Danube sediment. It was determined that sediments were not loaded with heavy metals in relation to maximal allowed quantity of heavy metals in agricultural soils. The greatest content was determined for reducible lead, cadmium, and nickel fractions in the sediments of Danube and Tisa.

There is a big possibility that this small load with heavy metals in sediments of Danube and Tisa was the consequence of high participation of total sand content with small adsorption ability.

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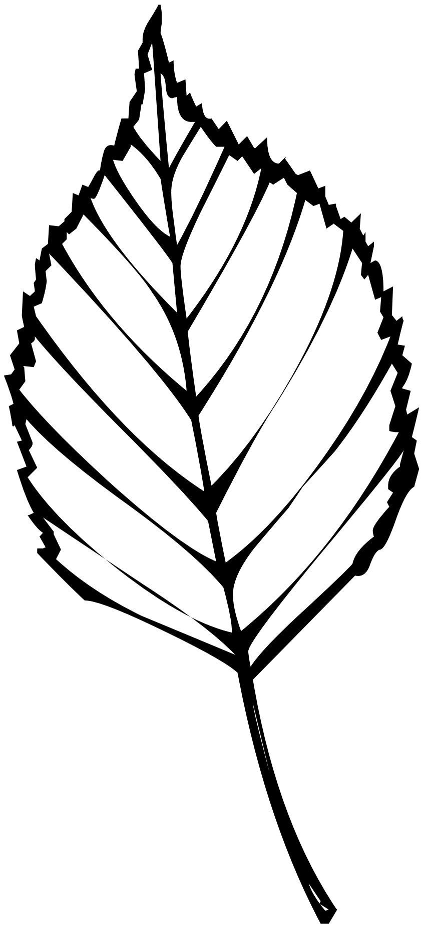

PRELIMINARY RESEARCH OF TRITROPHIC RELATIONSHIPS BETWEEN COMMON OAK, MILDEW AND GYPSY MOTH

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Summary: *Common oak forest dying is caused by the action of three harmful biotic factors: the gypsy moth, oaк mildew and honey fungus. During the gypsy moth outbreaks which last for 3-5 years, the first oak leaves are destroyed by caterpillar defoliation. However, oak soon gets new leaves and the second oak leaves are destroyed by mildew. During 2005, intensive mildew infestations occurred at some localities in the surroundings of Belgrade (Velika Moštanica, Besni Fok) and caused the defoliation of the first common oak leaves in early spring. Another intensive attack in the same year caused the defoliation of the second common oak leaves. At both sites, there was no presence of the gypsy moth, although the outbreak was in culmination in all larger complexes in the surroundings. The density of early season oak defoliators was symbolic, so that the possibility of defoliation of the first oak leaves followed by mildew infestation of the second leaves was excluded at these sites..*

This phenomenon has motivated the initiation of the research of the gypsy moth and mildew inter-relationship, as very important biotic factors with detrimental effects on oak foliage. We wanted to know if the gypsy moth caterpillars can be reared on the foliage which was already infested by mildew and how it will be reflected on their development. In this aim, we established an experiment with the gypsy moth 4th instar caterpillars which were, after hatching, fed on the foliage with and without mildew symptoms. The experiment lasted for 96 h. Leaf weight was measured before and after feeding, the quantity of excrements and the larval mass before and after the observation. The parameters of nourishment and growth (CR, GR and AD) were calculated by Waldbauer's formulas. Caterpillars fed on the diseased foliage consumed 3 times more food per mg of their mass per day, than those fed on the foliage without mildew symptoms. There were no differences in the growth of the gypsy moth caterpillars (GR) between the groups fed on the foliage with and without mildew symptoms. The efficacy of assimilation (AD) was more than twice higher in caterpillars fed on the foliage without mildew symptoms.

Oaks are characterised by a high level of constitutive defence, reflected in the increased content of tannin to which the gypsy moth has become well adapted during its evolution by the increased alkalinity of its midgut. The higher C/N ratio in the leaves with mildew symptoms, as the reaction to the attack of this pathogen, points to the presence of chemical compounds of the induced defence to which the gypsy moth caterpillars are not adapted.. This fact can explain the differences between the experimental groups of the gypsy moth caterpillars in the consumption and efficacy of food assimilation.

Keywords: *common oak, mildew, gypsy moth performance, tritrophic relationships*

INTRODUCTION

Common oak forest dying is caused by the action of three harmful biotic factors: the gypsy moth, oak mildew and honey fungus (Škorić, 1926, Josifović, 1929). During the gypsy moth outbreaks which last for 3-5 years, the first oak leaves are destroyed by caterpillar defoliation. However, oak soon gets new leaves and the second oak leaves are destroyed by mildew. After several years of successive infestations, the trees weaken physiologically and are infested by honey fungus (*Armillaria mellea*), causing root rot. This results in tree dying. Numerous authors, however, claim that in the process of oak decline and dying, a far greater significance is that of the so called "tracheo-mycoses", i.e. fungi developing in the vessels.

Mildews are obligatory parasites occurring in great numbers on broadleaf species of forest trees, but serious damage is caused to oaks. Oak mildews are widely distributed worldwide and they are identified from all the regions supporting *Quercus* species. However, the severity of infestation depends on the species of mildew and the species of oak. Infestation severity also depends on the climate factors, especially humidity. The conidia formed in dry conditions are more infective and keep their vitality for a longer time compared to those formed in moist conditions (Hammarland, 1925, Yarwood, 1978). For this reason, mildews cause epiphytotics also in the regions with very warm summers. Frequent rains, especially heavy showers, are unfavourable for mildew development, because they wash down the conidia and epiphytic mycelium from the infested plant parts.

During 2005, intensive mildew infestations occurred at some localities in the surroundings of Belgrade (Velika Moštanica, Besni Fok) and caused the defoliation of the first common oak leaves in early spring. Another intensive attack in the same year caused the defoliation of the second common oak leaves. The same occurrence was also recorded in 2008. It should be noted that at both sites, there was no presence of the gypsy moth, although the outbreak was in culmination in all larger complexes in the surroundings. The density of early season oak defoliators was symbolic, so that the possibility of defoliation of the first oak leaves followed by mildew infestation of the second leaves was excluded at these sites. This phenomenon has motivated the initiation of the research of the gypsy moth and mildew inter-relationship, as very important biotic factors which have detrimental effects on oak. We wanted to know if the gypsy moth caterpillars can be reared on the foliage which was already infested by mildew and how it will be reflected on their development.

MATERIALS AND METHODS

The gypsy moth caterpillars, from the hatching till the 4th instar, were reared in groups of 5 individuals per one Petri dish of 15 cm diameter, in controlled conditions (T=23+/-0, 1 C, Rh= 65+/-5 %, L /D = 15/9). Till the 4th instar, caterpillars were fed on artificial food (gypsy moth wheat germ diet MP Biomedicals, Inc., lot No. 7227). In the 4th instar, caterpillars were divided into two groups and they were individually fed on common oak foliage. The first group of caterpillars (15 individuals) was fed on the foliage with severe mildew infestation symptoms (LP). The second group (15 individuals) was fed on common oak foliage without mildew symptoms. The experiment lasted for 72 h. The leaf weight was measured before and after the feeding, quantity of excrements, larval mass at the beginning and after the end of the experiment. The nourishment parameters (CR, GR and AD) were calculated by Waldbauer's formulas (Waldbauer, 1968).

CR (consumption rate) = m_c / t_3
GR (growth rate) = (m_4 - m_3) / t_3
AD (assimilation efficiency) = ((m_c – m_e) / m_e) x 100
m_c – consumed food weight
m_e – feces weight
t_3 – duration of experiment
m_3 – weight at the beginning of observation
m_4 – weight at the end of observation

The measured parameters (leaf and excrement mass) were converted into dry mass. The leaves were taken from two different trees in the Arboretum of the Faculty of Forestry in Belgrade.

Chemical analyses included the determination of total carbon by Astett's method modified by Ponomareva and Plotnikova (1975), and total nitrogen, by Kjeldlahl's method (Dzamić *et al.*, 1966).

The statistical data processing included the analysis of variance in which arcsine transformed values were applied for AD.

RESULTS AND DISCUSSION

Altogether nine species of mildew were identified on oak (Table 1). *Microsphaera alphitoides* Griff. and Muab. (n.f. *Oidium quercinum*) is the most significant fungus causing oak mildew. It was introduced to Europe from North America and identified for the first time from Portugal in 1887. However, the undoubtedly confirmed finding in Europe was that in 1907 when this fungus was identified from France. Until the finding in France, this mildew was almost unnoticed, but very soon it spread throughout the continent.

Table 1. The most frequent oak mildews and their significance

Name of fungus	Significance
Erysiphe trina Hark.	+
Microspheara alphitoides Griff. and Maub.	+++
M. extensa Cooke and Peck.	++
M. hypophilla Nevodskij	+
M. penicillata (Wallr. Fr.) Lév.	+
M. silvatica Vlasov	+
Phyllactina coryela (Pers.) Karst.	+
Ph. roboris (gahet) Blum.	++
Sphaerotheca lanestris Hark.	+

Oak species in Serbia can be classified by susceptibility as follows: *Quercus robur* (the most susceptible). *Q, pubesven*s, *Q. frainetto*, *Q. petraea. Q. cerris*, *Q. ilex*, *Q. suber* and *Q. coccifera* (Карaџић & Лазарев, 1995, Карaџић, 2000, 2001). Common oak foliage with mildew symptoms used in the experiment was infested by the species *M. Alphitoides*. This fungus is an obligatory parasite and it causes great problems in the nurseries, juvenile plantations and to oak natural regeneration.

The results of chemical analyses of the leaves used in the experiment on tritrophic relationships between common oak, mildew and the gypsy moth are presented in Table 2.

Table 2. Percentage of carbon, nitrogen, proteins and C/N ratio in common oak foliage

common oak leaves	C (%)	N (%)	% Prot	(C/N)
without mildew symptoms	47.91	1.66	10.38	28.86
with mildew symptoms	48.97	1.44	9.00	34.09

Carbon content in the infested foliage, fed to 4th instar caterpillars, was higher than in the uninfested common oak leaves, and nitrogen content was lower (Table 2). Because of the content of the above two elements, their ratio (C/N) in the infested foliage was lower. Based on the results of chemical analysis of the leaves, it can be concluded that the infested foliage was less favourable food for the gypsy moth caterpillars. This was confirmed by the higher content of proteins in the uninfested leaves than in the infested common oak leaves.

Table 3. Growth and nutritional parameters for IV instar of gypsy moth caterpillars fed on infested and uninfested common oak foliage

Parameter	infested			uninfested			F	p
	N	Means	Std.Err.	N	Means	Std.Err.		
CR (mg/day)	15	41.22	2.8187	15	12.99	1.0892	63.13	0.0000
GR (mg/day)	15	5.52	1.4185	15	5.3594	1.1532	0.01	0.9318
AD (%)	15	36.1445	2.53580	15	83.0472	1.73677	214.43	0.0000

During the co-evolution of plants and insects, plants developed the capacity to respond to the attacks of pathogens and herbivores (Baldwin and Preston, 1999) by changing the chemical composition of the leaves and by different morphological and physiological characters. The mechanisms of plant defence can be divided by origin into constitutive and induced (Slansky, 1990). Constitutive mechanisms include the development of defence strategies regardless of the fact whether the plant was attacked by herbivore or pathogenic organisms. Such defence mechanisms are characteristic of perennial plants, such as woody plants, and they are efficient in the struggle against the generalists, such as the gypsy moth. Induced defence includes all plant reactions to attacks, directed to plant defence against pathogens and herbivores. Induced defence means the production of chemicals and/or physical structures, or the elimination of essential substances for herbivores, as the response to pathogen or herbivore attack. Plants contain a great number of compounds, products of secondary metabolism (allelochemicals) which include alkaloids, glycosides, tannins, flavonoids, essential oils, saponins and organic acids (Southwood, 1973). All these compounds are included in plant defence against insects and pathogens. Allelochemicals based on carbon, used by plants against folivorous insects, have a key position in the theory of plant and insect relationships (Tuomi *et al.*, 1988). These substances do not contain nitrogen and phosphorus and their use in insect control is referred to as the carbon-based defence (resistance). The increase in the content of these compounds in foliage reduces the possibility of herbivore nutrition. As carbon is a key element in the composition of these compounds, its higher content in the foliage indicates the higher presence of defence substances.

The parameters which characterise the nourishment, growth, and efficacy of the consumed food are presented in Table 3. The quantities of consumed food expressed in absolute form (CR) show a significant statistical difference ($F= 63.13$, $p=0.0000$) among caterpillar groups reared on common oak foliage without and with mildew infestation symptoms. Caterpillars reared on the diseased foliage consumed 3 times more food per mg of their mass per day, than those fed on the foliage without mildew symptoms. The higher quantity of consumed food is a compensation response to the consumption of food of lower nutritive value (Slansky & Wheeler, 1989, Lindroth *et al.*, 1991). This conclusion is supported by the results obtained by the chemical analysis of nitrogen content in the foliage of healthy and diseased trees (Table 2). Namely, nitrogen and protein contents in the foliage with mildew symptoms are lower, and carbon content is somewhat higher. The increase of the quantity of consumed leaves (CR) is a typical response of Lepidoptera larvae to the shortage of nutrients in their food (Mattson, 1980).

There were no statistically significant differences ($F= 0.01$, $p=0.9318$) in the growth of the gypsy moth caterpillars in absolute form (GR) between the caterpillar groups fed on the foliage with and without mildew symptoms (Table 3). The compensating response of caterpillars on the food of lower nutritive value was adequate.

The efficacy of assimilation (AD) shows that the leaves without mildew symptoms provide the nutritively richer food for caterpillar growth. It was more than twice higher in caterpillars fed on the foliage without mildew symptoms.

The defined differences in the values of nutritive parameters in the gypsy moth caterpillars fed on common oak foliage with and without mildew symptoms can be the consequence of the presence of chemical compounds characteristic of various defence mechanisms. Namely, oaks are characterised by a high level of constitutive defence, reflected in the increased content of tannin to which the gypsy moth has become well adapted during its evolution by the increased alkalinity of the midgut.(Berenbaum, 1980; Govenor *et al.*, 1997). However the common oak leaves with mildew symptoms contain also the defence substances induced by the previous mildew infestation to which the gypsy moth is not adapted. This fact is essential for the explanation of the high percentage of assimilation (AD) in caterpillars reared on uninfested common oak foliage.

CONCLUSIONS

The gypsy moth 4th instar caterpillars can be fed on the common oak foliage which was previously infested by mildew.

The compensatory response of the gypsy moth caterpillars on the food of lower nutritive value (the leaves infested by mildew) is manifested by the increase of the quantity of consumed food (CR).

There were no statistically significant differences in the growth of the gypsy moth 4th instar caterpillars in absolute form (GR) between the caterpillar groups fed on the foliage with and without mildew symptoms.

The efficacy of assimilation (AD) shows that the leaves without mildew symptoms provide the nutritively richer food for caterpillar growth. It was more than twice higher in caterpillars fed on the foliage without mildew symptoms.

The higher C/N ratio in the leaves with mildew symptoms, as the reaction to the attack of this pathogen, points to the presence of chemical compounds of the induced defence to which the gypsy moth caterpillars are not adapted.

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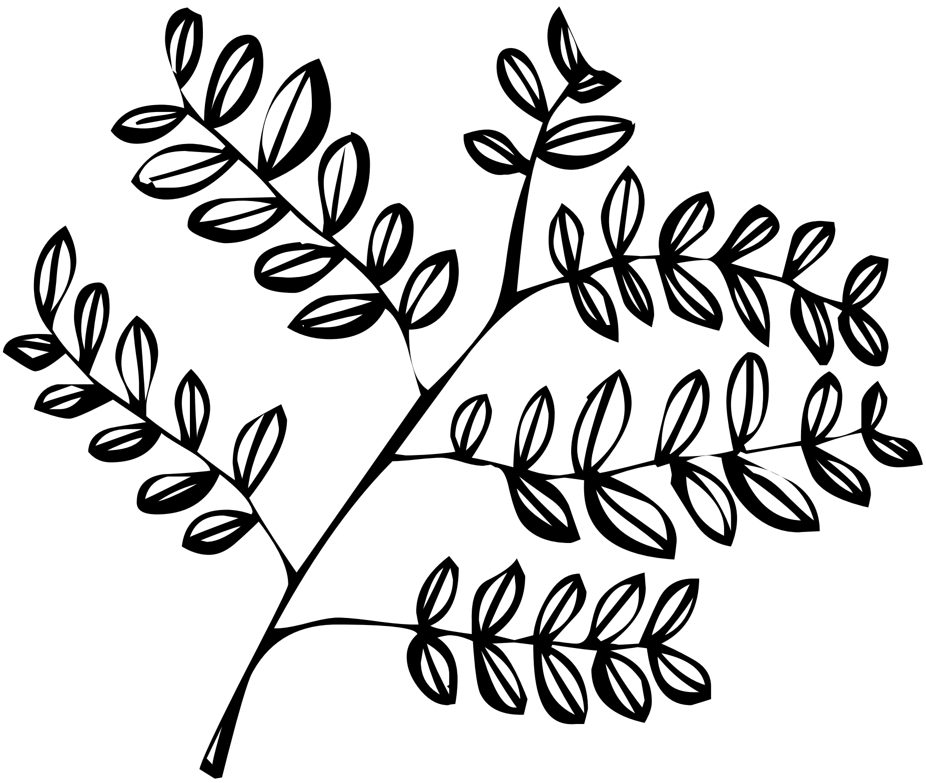

ANALYSIS OF THE EFFECT OF 2,4-D STIMULATIVE CONCENTRATIONS ON THE DEVELOPMENT OF SOME WOODY SPECIES IN THE EARLY STAGE OF JUVENILE DEVELOPMENT

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Abstract: *Modern plant production has been increasingly based on the application of stimulative substances and measures aiming at the increase in seed germination percentage, uniform germination and faster early growth of juvenile plants. The stimulative effect of 2,4D low concentrations on the initial growth of juvenile plants of woody species was assessed by the experiments with ginkgo and Austrian pine seeds. The technical substance 2,4D (2,4 dichlorophenoxy) acetic acid used in the experiments was produced by BDH Chemicals Ltd. England, purity 98%, diluted in deionizing water to the concentration of* $5x10^{-9}$ *mol* L^{-1} *. The research points to the stimulative effect of 2,4D low concentrations on plant development in the earliest stage of ontogenesis, which is reflected in the increase in seedling shoot and root length and mass, development of root system and intensified proliferation. The application of 2,4D hormetic concentrations, as the compound with double effects, toxogenic and/or stimulatory, should be based on the experiments of the concrete species, thus enabling the manipulation of the direction of biochemical reactions. It aims at the stimulation of seed germination percentage and/or juvenile plant growth, which can have the practical significance.*
Key words: *2,4 dichlorophenoxy acetic acid, low concentration, stimulative effect, ginkgo, Austrian pine, seedlings*

INTRODUCTION

Modern plant production has been increasingly based on the application of stimulative substances and measures aiming at the increase in seed germination percentage, uniform germination, and faster early growth of juvenile plants. The stimulation process and numerous stimulatory substances have been studied by toxicology for centuries, in which hormesis holds a significant position. Hormesis is defined as the stimulative effect of low doses of some substances (which are toxic at high concentrations) on growth, longevity and other metabolic and physiological reactions (metallothionein synthesis, DNA, RNA synthesis, mitosis, photosynthesis, oxygen consumption, tissue regeneration, stress protein synthesis, etc. (Calabrese & Baldwin, 1998; http://hormoesissociety.org.)

The issues of hormesis in plant production (mainly agriculture) have been studied since the end of the 20th century thanks to the resistance of some plant species to the particular herbicides (Schabenbergeral et *al*., 1999; Duke et *al*., 2006). Due to the complex effect of the substances that could be designated as hormetics (depending on the applied dose and the effect on cell metabolism), it is necessary to conduct more detailed research of numerous natural and synthetic substances, regardless of the fact that their primary use is the suppression of living organisms, i.e. pest control.

The previous research shows the stimulatory effect of some procedures or chemicals such as: antioxidants (Gorecki & Harman, 1987), individual inhibitors (Chowdhinry & Choudhuri, 1994), hormone regulators (Richa & Sharma, 1994) or the procedure known as priming (McDonald, 1999) based on controlled seed hydration which enables the activation of reparation mechanisms and the so-called removal of germination inhibitors. Pursuant to the above, Brajnceva (1972), Dejeva & Šeleg (1976) studied the stimulative effect of 2,4 D (active substances of herbicides Monosan, Deherban, etc.) on the growth of dicotyledonous and monocotyledonous plants, and concluded that low concentrations increase the intensity of respiration and phosphorylation (higher ATR content), with higher accumulation of new-synthesised proteins, thiol proteins and nucleic acids, which is related to the intensified proliferation. Such a mechanism results in the increased growth, with the decrease in antioxidant levels: GSH and ascorbic acid. For this reason, 2,4 D could be designated as hormetic. In Serbia, Dragičević et *al.* (2004, 2005, 2007) reported the stimulative effect of

2,4D low concentrations on maize seeds exposed to accelerated aging, which is reflected in the increase in the percentage of germinated seeds, increase in fresh weight, root and shoot length, promoted hydrolysis and biosynthesis of dry substance. Seedling growth is stimulated by the increase in their redox and energy potentials.

MATERIAL AND METHOD

The stimulative effect of 2,4D low concentrations on the initial growth of juvenile plants of woody species was assessed by the experiments with ginkgo (Šijačić-Nikolić et *al.* 2006, 2006a) and Austrian pine seeds The technical substance 2,4D (2,4 dichlorophenoxy) acetic acid used in the experiments was produced by BDH Chemicals Ltd. England, purity 98%, diluted in deionizing water to the concentration of $5x10^{-9}$ mol L^{-1} (Dragičević, 2007).

Ginkgo seeds were sown in the spring 2006 after soaking in the prepared solution of 2,4D low concentration in which it remained for 30 min (treatment A), 60 min (treatment B) and 180 min (treatment C). Austrian pine seeds were soaked before sowing in the same solution and kept for 60 min (treatment A), 30 min (treatment B) and 15 min (treatment C). As the control (K), the sown ginkgo seeds and Austrian pine seeds were not treated before sowing. Initial development of juvenile plants was monitored in controlled environmental conditions, Figure 1, after which the plants were exposed to outdoor environmental conditions.

The analysis of plants in the early stage of juvenile development included the following morphometric parameters: shoot length (mm), root length (mm), fresh shoot and root mass (g), i.e. number of leaves and leaf mass (g) of ginkgo. The study data were processed by computer programme «Statistica». Statistical justification of the differences among the different treatments and the control was determined by Student's t-test.


a

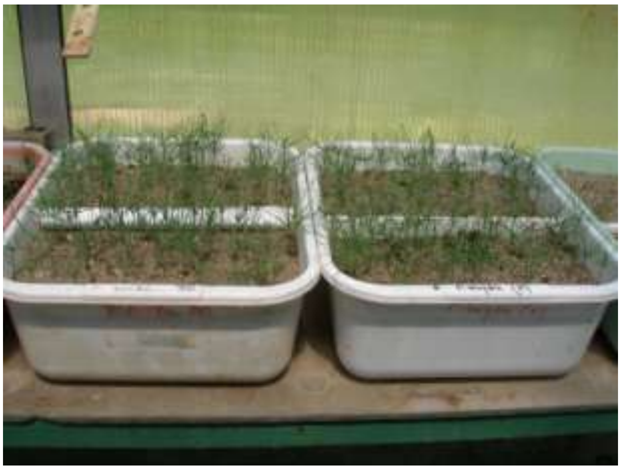
b

Figure 1. Experiments with ginkgo (a) and Austrian pine (b) in controlled environmental conditions

RESULTS AND DISCUSSION

The results of the analysis of ginkgo and Austrian pine seedling morphometric traits, in different treatments and the control, are presented in Tables 1 and 2.

Based on the study results, it can be concluded that 2,4D low concentrations have a stimulative effect on the development of ginkgo seedlings, Figure 2. Also, it can be concluded that the plants react to different treatments, best shown by the values of Student's t-test. The highest shoot length, the highest number of leaves, the highest leaf mass and shoot mass was observed in the plants in treatment A, and the highest root length and root mass was observed in the plants in treatment B. The lowest values of the analysed morphometric parameters of ginkgo seedlings were found in treatment C, or in the control.

The stimulative effect of 2,4D was also recorded in Austrian pine seedlings, Figure 3. The highest shoot and root length and mass were measured in treatments B and C, and the lowest values were observed in the control, i.e. treatment A.

Table 1. Morphological characters of ginkgo seedlings in different treatments (A, B and C) and the control (K)

Treat-ment	Mean value	Standard deviation	t-value			
Shoot length (mm)						
			A	**B**	**C**	**K**
A	223.07	15.64	-			
B	185.33	32.52	-5.58*	-		
C	174.37	41.77	2.78*	1.03	-	
K	205.33	16.28	3.82*	-2.78*	-3.82*	-
Root length (mm)						
			A	**B**	**C**	**K**
A	101.43	9.07	-			
B	106.60	17.11	-1.50	-		
C	98.10	18.42	1.04	2.37*	-	
K	91.46	18.98	2.66*	3.59*	1.42	-
Number of leaves						
			A	**B**	**C**	**K**
A	5.33	0.55	-			
B	4.63	0.85	4.18*	-		
C	4.70	0.70	3.47*	-0.44	-	
K	4.86	0.57	3.12*	-1.15	-1.04	-
Leaf mass (g)						
			A	**B**	**C**	**K**
A	0.46	0.15	-			
B	0.37	0.15	0.18*	-		
C	0.25	0.16	5.28*	3.60*	-	
K	0.34	0.15	2.86*	0.80	-2.13*	-
Shoot mass (g)						
			A	**B**	**C**	**K**
A	1.28	0.19	-			
B	1.08	0.22	0.31*	-		
C	0.91	0.28	0.33*	2.81*	-	
K	1.08	0.15	4.06*	-0.11	-3.02*	-
Root mass (g)						
			A	**B**	**C**	**K**
A	0.54	0.09	-			
B	0.61	0.17	-2.12*	-		
C	0.55	0.14	-0.28	1.79	-	
K	0.45	0.12	3.25*	4.73*	2.33*	-

Table 2: Morphological characters of Austrian pine seedlings in different treatments (A,B and C) and the control (K)

Treat-ment	Mean value	Standard deviation	t-value			
Shoot length (mm)						
			A	**B**	**C**	**K**
A	57.66	7.98	-			
B	66.80	9.95	-2.95*	-		
C	67.00	9.60	-2.96*	-0.06	-	
K	62.53	9.25	-1.55	1.31	1.48	-
Root length (mm)						
			A	**B**	**C**	**K**
A	99.66	37.20	-			
B	106.53	43.66	-0.47	-		
C	99.00	42.72	0.04	0.41	-	
K	81.00	25.99	1.62	1.96	1.45	

Shoot mass (g)						
			A	B	C	K
A	0.31	0.12	-			
B	0.46	0.11	-5.19*	-		
C	0.48	0.15	-3.54*	-0.42	-	
K	0.36	0.14	-1.10	2.11	2.84*	-
Root mass (g)						
			A	**B**	**C**	**K**
A	0.02	0.009	-			
B	0.03	0.02	-2.16*	-		
C	0.02	0.02	-0.84	0.71	-	
K	0.02	0.02	-0.19	1.38	0.70	-

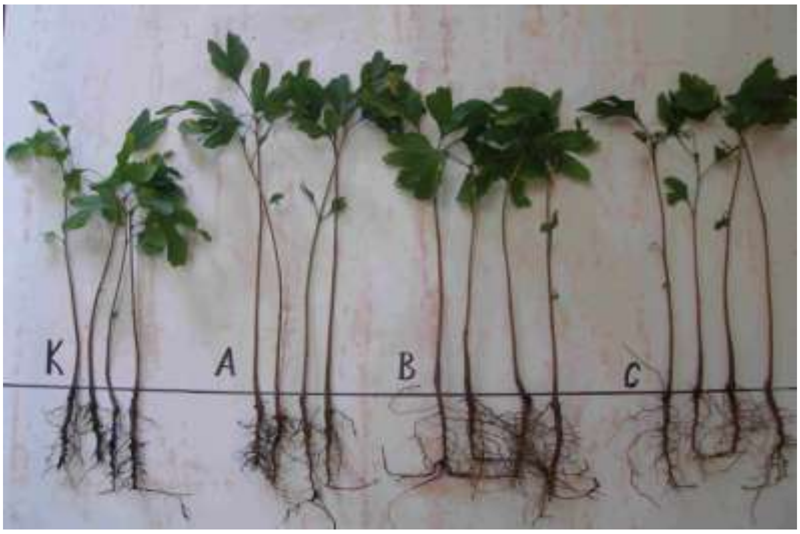

Figure 2. Variability of ginkgo seedlings in the control (K) and treatments (A,B and C)


Figure 3. Variability of Austrian pine seedling in the control (K) and treatments (A, B and C)

CONCLUSION

The research of ginkgo and Austrian pine seeds points to the stimulative effect of 2,4D low concentrations on plant development in the earliest stage of ontogenesis, which is reflected in the increase in seedling shoot and root length and mass, development of root system, and intensified proliferation. The magnitude of the stimulative effect depends primarily on the length of pre-sowing treatment, in which significant factors are most probably the seed size, i.e. the seed coat hardness. In ginkgo seeds, which are larger-sized and with harder seed coats, the highest values of analysed morphometric traits are shown by the seedlings whose seeds were soaked before sowing in 2,4D: 30 solution (treatment A), i.e. for 60 minutes (treatment B). As for Austrian pine seeds, which are smaller and with softer seed coats, the highest values of the analysed morphometric traits are shown by the seedlings whose seeds were soaked before sowing in the solution 2,4D: 30 (treatment B), i.e. 15 minutes (treatment C). In both cases, seed treatment for a longer time period (ginkgo 180 minute-treatment C and Austrian pine 60 minute-treatment A) has an adverse effect on seedling growth, which is the consequence of toxicity of this substance realised in longer treatments.

Based on the study results, it can be concluded that the application of hormetic concentrations of 2,4D as the compound with double effects, toxogenic and/or stimulatory, should be based on the experiments with the concrete species, which will enable the manipulation with the direction of biochemical reactions in the aim of stimulation of seed germination percentage and/or juvenile plant growth and can have a practical significance. The undertaken research should be extended also to other woody species, especially to those with large seeds and hard seed coats.

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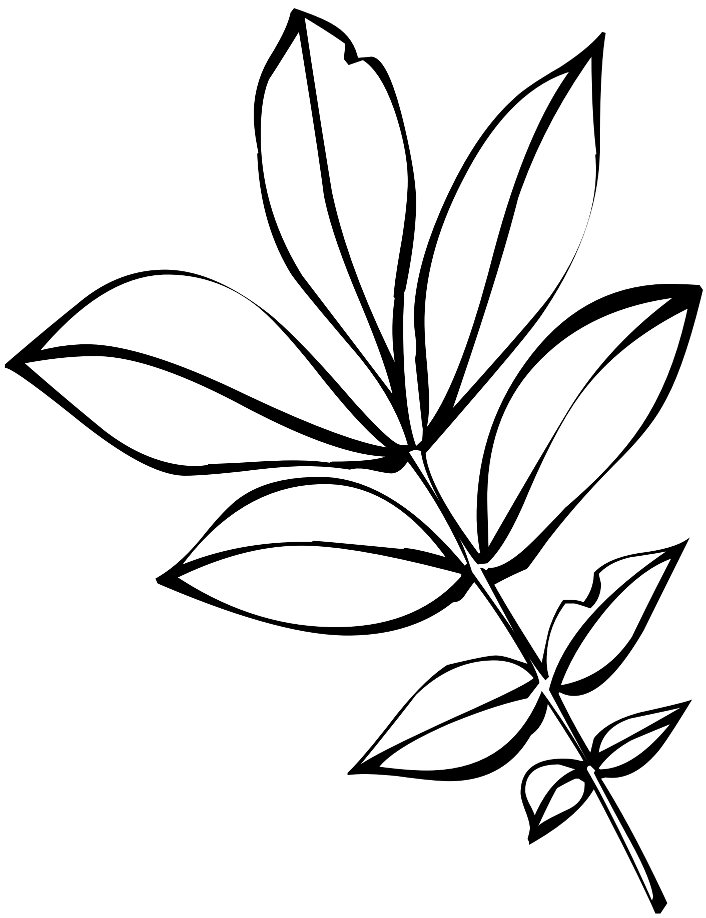

MOESIAN BEECH A SIGNIFICANT GENETIC RESOURCE FOR THE CONVERSION OF CONIFEROUS FORESTS IN SERBIA

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Abstract: *In Serbia, beech belongs to the most widespread and economically most important broadleaf species. Beech forests are the most significant part of the growing stock, because in the form of pure or mixed stands, they occupy about 45 % of the total forest area. They include a wide belt starting from the montane beech, which occurs in the zone of oak forests, mountainous beech forests, forests with maple, fir and spruce, to the subalpine beech forests at higher altitudes. In the complex of beech forests, there are 9 coenoecological groups of forest types.*

The strategy of conservation of biodiversity and genetic resources of the economically most valuable tree species, imposed the conservation of national resources of natural forests as the primary task of Serbia forestry. As Moesian beech (Fagus moesiaca), an autochthonous species, is the most significant economic potential of forestry in Serbia, 19 seed stands, total area 137.52 ha, were singled out by selection from the best natural populations.

Planned tasks for the conversion of forests, as well as the aim of meeting the needs of afforestation plans, require the ensured sufficient quantities of good-quality, healthy and selected forest seed and planting material.

Based on the analysis of the phytocoenological features of forest communities in Serbia, it was determined that the East and the largest part of the Central Serbia belongs to the Moesian Province. In this part of the Balkan Peninsula, a special species of beech is described as Moesian beech (Fagus moesiaca Domin, Maly/Czeczott): It is considered to be formed by hybridisation of European beech (Fagus silvatica L.) and Caucasian beech (Fagus orientalis Lipsky), at the border of their ranges (Jovanović, 1985). At first, it was described as Fagus silvatica ssp moesiaca, and then it was considered as a «transient» species between the two mentioned beech species, i.e. that it can be considered as a subspecies which has its own range of distribution. Today, it is accepted that all the three species of beech are distributed in the region of the Balkan Peninsula: in the south and east Fagus orientalis, in the west Fagus silvatica, and Fagus moesiaca in the space between the two mentioned ranges.

Of more than 100,000 ha of coniferous plantations in Serbia, many were established at unsuitable sites, especially during the campaign of coniferisation. For this reason, the health of such plantations deteriorated with time (especially in Austrian pine plantations).

This paper includes the economically significant biotic agents of damage in coniferous plantations in Serbia, especially in those established at the natural beech sites. In such plantations, along with other parameters, the plantation health condition should be the major factor that affects the decision on the conversion of conifers with autochthonous broadleaf species (in this case beech).

Key words: *Moesian beech ,conversion, Serbia, genetic potential, seed stands*

INTRODUCTION

The strategy of conservation of biodiversity and genetic resources of the economically most valuable tree species, imposed the conservation of national resources of natural forests as the primary task of Serbia forestry. As Moesian beech (*Fagus moesiaca*), an autochthonous species, is the most significant economic potential of forestry in Serbia, 19 seed stands, total area 137.52 ha, were singled out by selection from the best natural populations.

The planned forest conversion and afforestation require the sufficient quantities of good-quality, healthy and selected forest seed and planting material.

The area of forests and other woodland in Serbia is 2,654,577 ha, percent forest cover is 26.7%. (The European percent forest cover is 28,7% and the world forest cover is about 30.0%).

Forest area and percentage

On the basis of ecological classification of forests, there are eight forest complexes in Serbia:

1. Alluvial hygrophilous forests 0.11 %
2. Xerothermophilous Hungarian oak-Turkey oak forests 15.41 %
3. Xeromesophilous sessile oak-Turkey oak-hornbeam forests 12.49%
4. Mesophilous beech forest and beech-coniferous forests 52.58%
5. Thermophilous pine forests 12.92 %
6. Frigoriphilous coniferous forests 6.03 %
7. Subalpine shrubby conifers and broadleaves 0.07 %
8. Grass communities on sand 0.79 %

Species percentage in the total growing stock in Serbia:

♣ Beach 47 %
♣ Oak 25 %
♣ Poplar 1 %
♣ Other broadleaves 16 %
▲Spruce 5 %
▲ Fir 3 %
▲ Pines 2 %
▲ Other conifers 1 %

Total broadleaves 89 %
Total conifers 11 %

MATERIAL AND METHOD

The state of conifer plantations in Serbia

The most significant issue of these stands is that they were frequently established on "non-native" sites, e.g.:

-on the sites of various productivity, which is sometimes far above the demands of the planted species;

-often on the sites of other, mostly broadleaf species, in which the sprouts and suckers of autochthonous and undesirable species endanger the new established plantations;

-different methods of soil preparation and planting technique;

-different planting density (seedling number per ha);

-different practice of restocking;

-a smaller part of conifer plantations was established on the natural site of the concrete tree species.

All the above is very significant both for the wood volume (current increment), and for the wood quality (technological properties). Conifer plantations were often established with numerous mistakes: on various sites of different ecological productivity and value, from the sands, riparian sites along the large rivers to alpine spruce sites. There were cases with the wrong selection of species, e.g. Austrian pine on oak and beech sites of high site class, on beech-fir sites, at high altitudes above 1500 m etc. or introduction of exotic species to various sites without any preliminary experience of their «behaviour» at such sites. Plantation establishment at the sites with different degrees of soil degradation (most often erosion) led to very different conditions for growth and development. Also, their development depended on the degree of canopy cover or the process of succession of the autochthonous vegetation.

The second important issue in conifer plantations is their present age and planting density. The majority of these stands were established after the Second World War. The most intensive afforestation occurred in the period 1960-1980, but it was considerably reduced in the past 10-15 years. Therefore, most of the plantations are about 30-50 years old, i.e. they are in the stage when their tending should be intensive. Special problems are different practices of tending and maintenance, i.e. silvicultural neglect, i.e. the absence of the adequate tending. Special problems are also the plantations established at the sites where the removed autochthonous broadleaf vegetation was in an unsatisfactory condition – severely degraded forests, multiannual suppression of the primary broadleaf species, i.e. removal of suckers and sprouts.

The plantations were established with different numbers of seedlings per ha, often disregarding the bioecological data. Dense planting (about 10,000 seedlings per ha) was applied in the initial period of conifer plantation establishment, while during the last decades afforestation was performed less densely, by about 2,500 seedlings per ha. The too high or too low number of seedlings per ha had mostly the unfavourable effect on the plantation development. Under dense planting, due to the absence of tending, the stands are unstable, the trees are too thin with reduced crowns. Under thin planting, due to too slow canopy closure, the trees are of lower quality (low, branchy, etc.).

Another issue is forest composition. Many times various authors emphasised that the most utilised species were Austrian pine and Scots pine, much less spruce, and the least - other species of conifers (about 80% of the area are pines, 10% are spruce and the remaining part 10% are all other conifers).

Plantation productivity is as follows: In Austrian pine and Scots pine plantations, based on sample plot data, wood volume ranged from 100 to 400 m^3/ha, mostly 150-250 m^3/ha. Current volume increment varies from 4.0-14.0 m^3/ha, most often 6.0-9.0 m^3/ha. In other conifer species (spruce, Douglas-fir, Weymouth pine, larch) wood volume and current volume increment at the same age attains much higher valued, so that the increment exceeds 15 m^3/ha. This indicates that the site productivity is very high, and often the tree species with low demands of mineral nutrients in the soil are not capable of using the very high soil production potential. The above values of wood volume and current increment refer to sample plots and the homogeneous fully stocked stands, meaning that for the whole area under artificially established stands, the values should be lowered for about 20-30%, to get the real value

The above data point to some shortages during the artificial establishment of conifers. First of all it is the selection of tree species and exaggerated introduction of conifers even where the autochthonous broadleaf species could be planted. To conclude, the present state of plantations and artificially established stands is characterised by various degrees of stocking, quality, health, productivity, succession of vegetation by introduction of autochthonous vegetation.

The main conversion problems in Serbia

- Inadequate forestry policy
- Insufficient experience
- Poor health of allochthonous coniferous plantations
- Planting material

- *Inadequate forestry policy* in Serbia (Ex Yugoslavia) is the consequence of the Second World War.

During the War, the growing stock was severely damaged and during the post-war period it was exploited without control. The consequences are the bare lands at the sites of autochthonous species (beech, oak, maple, ash,...). The Government decision recommended the afforestation by fast growing conifers (Austrian pine and Scots pine) in order to:

- complement the devastated growing stock
- restock the degraded sites
- enable the autochthonous species in the second layer to regenerate naturally

More than 100 000 ha of coniferous plantations were established in Serbia of which:

- about 80 % are Austrian pine and Scots pine plantations
- about 10 % are spruce plantations
- 10 % are other conifers (Douglas-fir, cedar, larch, etc.)

- *Insufficient experience* in conifer plantation establishment during the post-war period showed its results after many years. It was caused by:
- wrong selection of species (the provenance of introduced conifers was not taken into account)
- dense planting, high number of seedlings per ha
- wrong method of plantation tending - cleaning.

- *Health* -Of more than 100,000 ha conifer plantations in Serbia, about 72 % are Austrian pine and Scots pine. Many of them are established on the degraded broadleaf forest sites. Some of these plantations have protection function and they are mainly tended and maintained. Those outside the protected regions are neglected and their tending was not started on time. As planting density in most of the established plantations was 10,000 seedlings per ha, and their age is now over 30 years, each measure of plantation thinning would have adverse consequences on the remaining trees (breaking, uprooting, bending) which would lead to the loss of production area.

Due to the great planting density, the condition is also weakened by the biotic agents, most often pathogenic fungi causing needle diseases

- In Austrian pine plantations they are most often *Mycosphaerella pini, Sphaeropsis sapinea*, and in
- Scots pine plantations *Lophodermium pinastri, Cyclaneusma sp.* and *Lophodermella sulcigena.*
- The common problems in the plantations of both species are: *Cenangium ferruginosum, Armillaria spp., Heterobasidion annosum* and other less represented pathogenic species.

The health of Austrian pine and Scots pine plantations is also threatened by numerous insect pest species in the orders *Homoptera, Coleoptera*, *Lepidoptera*, *Hymenoptera* and *Diptera*.

Taking into account the condition of pine plantations established on the sites of autochthonous broadleaf species, the justification of their further existence is questionable. These species do not have the prospect of further successful development, and occupy the production area where autochthonous broadleaf species could be reintroduced, first of all beech (Lazarev, V. 1983, 2001)

- *Planting material* is one of the current issues.
  - insufficient number of seed stands and seed orchards
  - easier production of conifer seedlings compared to broadleaf seedlings
  - conifer seedlings are more resistant in the field, their adaptation is faster
  - conifer seed is more readily available.

Why beech for conversion ?

Common beech (*Fagus silvatica* L.) is the most widely distributed economically important broadleaf tree species in Europe. The extent of beech forests in Europe and Asia represents approximately 10% of European forests (between 17-20 million ha).

In southeast Europe beech forests are valued for their quality and high natural diversity.

In Serbia, beech is one of the most widespread and economically most important broadleaf species. Beech forests are the most significant part of the growing stock, because in the form of pure or mixed stands, they occupy about 47% of the total forest area.

Why Moesian beech?

Based on the analysis of the phytocoenological features of forest communities in Serbia, it was determined that the East and the largest part of the Central Serbia belongs to the Moesian Province. In this part of the Balkan Peninsula, a special species of beech is described as Moesian beech (*Fagus moesiaca* Domin, Maly/Czeczott): It is considered to be formed by hybridisation of European beech (*Fagus silvatica* L.) and Caucasian beech (*Fagus orientalis* Lipsky), at the border of their ranges (Jovanović, 1985). At first, it was described as *Fagus silvatica ssp moesiaca,* and then it was considered as a «transient» species between the two mentioned beech species, i.e. a subspecies which has its own range of distribution. Today, it is accepted that all the three species of beech are distributed in the region of the Balkan Peninsula: in the south and east *Fagus orientalis,* in the west *Fagus silvatica,* and *Fagus moesiaca* in the space between the two mentioned ranges.

Beech seed stands in Serbia

The key of successful afforestation is a circular chain for which it is not know whether it begins or end with seed.

The genetic quality of planting material can be produced only by the seed from seed stands - "normal seed" and seed orchards - "selected seed".

According to the data of the Register of Seed Stands of the Ministry of Agriculture, Forestry and Wather management of the Republic of Serbia, 310 seed stands are registered with 73 tree species on the total area of 2,135 ha, which is 0.095 % of the total woodland area in Serbia (2, 360, 400 ha).

Of altogether 310 seed stands:

137 seed stands are with 29 coniferous tree species and

172 seed stands are with 44 diferent species of broadleaves

As an autochthonous species and one of the most commercial species, beech (*Fagus moesiaca*) constitutes the most significant economic potential of forestry in Serbia. 19 seed stands were designated by selection from the best natural populations, total area 137.52 ha, and 10 reserves of pure and mixed beech stands in Serbia (Table 2).

Table 1. Beech seed stands in Serbia

TREE SPECIES	SEED STAND LOCATION	AREA (ha)
1. Fagus silvatica	GJ ”Lomnička reka” od.73 a, Kruševac	4.00
2. Fagus silvatica	GJ “Dajićke planine” od.23 a, Ivanjica	2.89
3. Fagus silvatica	GJ “Rudnik” od.62 a, Kragujevac	19.35
4. Fagus silvatica	GJ ”Crna reka” Goč	4.00
5. Fagus moesiaca,Abies alba	GJ “Goč-Gvozdac” od. 11a, Goč	5.16
6. Fagus moesiaca, Abies alba	GJ “Goč-Gvozdac” od.43a, Goč	12.56
7. Fagus moesiaca, Abies alba	GJ “Goč-Gvozdac” od.34/1a Goč	9.62
8.Fagus moesiaca, Abies alba	GJ “Goč-Gvozdac” od.18a, 19a Goč	17.23
9. Fagus silvatica	GJ “548 Ravne”, od 18 a, Fruška Gora	4.00
10. Fagus moesiaca	GJ “Kamenička reka I”, 34a Boljevac	2.50
11. Fagus moesiaca	GJ “ Zlotske šume” od. 36a,35 c,Boljevac	3.60
12. Fagus moesiaca	GJ “Jelova Gora”,35a, Užice	15.00
13. Fagus moesiaca	GJ “ Majdan-Kučajna“ 22a, Kučevo	4.61
14. Fagus moesiaca	GJ ”Istočna Boranja“,149a, Loznica	6.30
15. Fagus moesiaca	GJ ”Istočna Boranja“,119a, Loznica	8.35
16. Fagus moesiaca	GJ “Gobeljska reka“44a, Kopaonik	13.95
17. Fagus moesiaca	GJ ”Crni vrh-Kupinovo“25a, Boljevac	1.20
18. Fagus moesiaca	GJ ”Južni Kučaj 3 “,68d, 71f,Boljevac	2.00
19.Fagus moesiaca	GJ ”Troglan bare“76a, Despotovac	1.20
Total area 137.52 ha		

Table 1. presents beech seed stands based on the data by the Ministry of Natural Resources and Environment Protection of the Republic of Serbia.

Table 2: Reserves of pure and mixed beech stands in Serbia

No.	RESERVE	AREA (ha)	MUNICIPALITY
1	VITANOVAČA	37.43	DESPOTOVAC
2	KUKAVICA	78.43	VLADIČIN HAN
3	ZELENIČJE	41.70	CRNA TRAVA
4	VELIKI ŠTURAC	8.00	GORNJI MILANOVAC
5	ZELENIKA l	3.71	UŽICE
6	ZELENIKA ll	0.12	UŽICE
7	IZNAD TATALIJE	2.16	B. BAŠTA
8	TESNA JARUGA	2.92	B. BAŠTA
9	DANILOVA KOSA	6.00	KRUPANJ
10	FELJEŠANA	45.00	MAJDANPEK
	Σ = 225.47 ha		

The beech seed stands are significant genetic potential, which will ensure sufficient amounts of good quality beech seed from different parent rocks and altitudes (range of altitudes is from 100 to 1568m).

Conversion technique

I. Direct conversion - in pure plantations

- Begins with clear cutting
- soil preparation consists of removal of the remnants the original stands
- beech seed sowing is not applied because it used to have poor results
- afforestation with containerised beech seedlings.

II. Indirect conversion - in stages - in mixed broadleaf and conifer plantations

- preparatory felling - gradual thinning of the stand canopy and preparation for abundant seed crop, thinning up to 30%
- regeneration felling - in seed years, stand canopy is reduced to 0.5-0.6
- final felling - when the seedlings of autochthonous species appear.

If the regeneration is not satisfactory, artificial restocking is necessary

CONCLUSION

- Continue the management of conifer plantations (on non-native sites) until the realisation of the planned target (production of small technical timber)
- Intensify the action of restoring broadleaf species (beech) to their original sites
- Preserve the coniferous plantations as the permanent forest vegetation at the sites which are not suitable for broadleaf species,

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PRODUCTIVITY OF *Populus x euramericana* I-214 ON SITES WITH MAXIMUM POTENTAIL FOR POPLAR TREE GROWTH IN THE CENTRAL DANUBE BASIN

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Abstract: *In this paper physical and chemical properties of three forms of fluvisol (sandy-loam, loam and with fossil soil) in the central Danube Basin and their influence on productiviity were analyzed.*
The greatest variation among physical properties was found for silt+clay fraction which caused different texture class per profile depth (sand to silty loam). Chemical properties of studied soil were very similar on the average, with the greatest difference being determinated for humus content.
Variation of humus content in humus-accumulative horizon ranged from 0.67 to 6.90%. Average humus content per profile depth ranged from 0.71 to 2.91%.
Volume of the average trunk stand ranged from 2.88 to 3.09 m^3 of the studied soil-systematic units at the end of rotation period. The greatest stand volume was determined when fraction of silt+clay was 37.4%. The mentioned data confirmed results of previous investigation that dependence of volume of Populus x euramericana cl. I-214 in relation to fraction of silt+clay fraction had the form of parabola with the silt+clay fraction content ranging from 30-45%
Rotation period of maximal production of wood mass on fluvisol with fossil soil and on sandy loam fluvisol was determined in the 21st, while on loamy fluvisol in the 23rd year of stand age. The greatest participation in the structure of selection was determinated for F logs, so the participation of F logs ranged from 50 on loamy fluvisol to 56.5 on fluvisol with fossil soil. The greatest income at the end of rotation period was obtained on sandy-loamy fluvisol.
Key words: *poplars, fluvisol, productivity, Populus x euramericana cl. I-214*

INTRODUCTION

Production characteristics of poplar stands are predetermined by the high quality habitat, variety and intensive cultivation (Stanturf et *al.* 2001). Fertility level in physiologically active profile part on the soils of alluvial plane is mainly determined by: content of silt and clay fraction, humus content, quantity of water useful for plants and airy soil (Ivanišević , 1991; Ivanišević et *al.*., 1991; Ivanišević, 1995; Guzina et *al.* 1995, Galić 2000, Galić et *al.* 2006). The second factor for successful cultivation is poplar variety with maximal production of wood mass on highly productive sites. The third factor is an intensive cultivation i.e. establishment of stand by applying full technology, which means total preparation of soil, as well as the care, and all measures of stand protection from diseases and pests during the whole rotation period.

However, data on stand productivity of poplar clones at the end of rotation period were not sufficient. The aim of this study was to define the production on stands with maximal potential for black poplar cultivation.

MATERIALS AND METHODS

Investigations were carried out in the Central Danube Basin on *Populus x euramericana* cl. I-214 stands at the end of rotation period (aged 30 years) on three trial areas. Trial area 1: G.J. »Šajkaška« department 37, section k on systematic soil unit: sandy-loamy fluvisol, morphology profile structure:A_{mo}- IG_{so} – II G_{so} – $G_{r'}$
Trial area 2: G.J. »Topolik« department 56, section f on systematic soil unit: loamy fluvisol, morphology profile structure: A_a- I G_{so} – II G_{so}
Trial area 3: G.J. »Topolik« department 17, section d on systematic soil unit: fluvisol with fossil soil, morphology profile structure: A_a- I – II - A_b - IIIG_{so}

Granulo-metric composition and soil chemical properties (Reaction of soil solution, carbonate content, and humus content) were determined using standard laboratory methods (Group of authors 1997, 1971).

Analysis of growth elements of *Populus x euramericana cl.* I-214 was done using method of mean trunk section at the end of rotation period.

RESULTS WITH DISCUSSION

Soil characteristics

According to the soil classification of Yugoslavia studies were done on three forms of fluvisol soil: sandy-loamy, loamy and with fossil soil. Sandy-loamy fluvisol (trial area 1) per profile depth is characterized by texture class of sandy loam (Table 1).

Table 1. Granulo-metric composition and texture class

Horizon	Depth	Granulo-metric composition %						Texture class
		> 0,2	0,2 - 0,02	0,02 - 0,002	< 0,002	Total > 0,02	Total < 0,02	
	cm	mm	mm	mm	Mm	mm	mm	
A_{mo}	0-25	5,4	49,8	32,5	12,3	55,2	44,8	Sandy loam
I Gso	25-115	1,0	62,8	23,9	12,3	63,8	36,2	Sandy loam
II Gso	> 115	8,3	60,6	21,8	9,3	68,9	31,1	Sandy loam
Average		4,9	57,7	26,1	11,3	62,6	37,4	

The greatest participation was determined for fraction of fine sand particles. Content of this fraction ranged from 49.8 to 62.8%, which pointed out to favorable soil water-air characteristics. Chemical soil characteristics (Table 2) were characterized by light alkaline to alkaline reaction of soil solution, and expressed carbonic feature of soil per depth profile. Humus-accumulative horizon of this from of fluvisol was rich in organic matter (6.90%).

Table 2. Content of humus, carbonate and reaction of soil solution

Horizon	Depth (cm)	pH u H_2O	Humus (%)	$CaCO_3$ (%)
A_{mo}	0-25	7,43	6,90	11,81
I Gso	25-115	7,71	1,11	19,58
II Gso	> 115	7,38	0,74	17,51
Average		7,50	2,91	16,3

Texture class of silty loam (Table 3) was determined on loamy form of fluvisol (trial area 2) in humus-accumulative horizon.

Table 3. Granulo-metric composition and texture class

Horizon	Depth	Granulo-metric composition %						Texture class
		> 0,2	0,2 - 0,02	0,02 - 0,002	< 0,002	Total > 0,02	Total < 0,02	
	cm	Mm	mm	mm	mm	mm	mm	
Aa	0-20	0,8	31,5	48,0	19,7	32,3	67,7	Silty loam
IGso	20-80	4,5	71,7	16,0	7,8	76,2	23,8	Sandy loam
IIGso	80-120	1,0	61,5	23,8	13,7	62,5	37,5	Sandy loam
Average		2,1	54,9	29,3	13,7	57,0	43,0	

In remaining two layers prevailing texture class per depth profile was sandy loam. Humus accumulative horizon 20 cm in depth was characterized by heavier texture composition, which could have negative influence on airy traits of this systematic soil unit (smaller capacity for air). Chemical properties were very similar to the former profile (Table 4), with the basic difference in humus content decrease (2.10).

Table 4. Content of humus, carbonate, and reaction of soil solution

Horizon	Depth (cm)	pH u H_2O	Humus (%)	$CaCO_3$ (%)
Aa	0-20	7,36	2,01	17,12
IGso	20-80	7,59	0,37	15,48
IIGso	80-120	7,60	0,43	21,99
Average		7,51	2,81	18,19

Two textural classes prevailed on fluvisol with fossil soil (trial area 3) per profile depth: loamy up to 85 cm in depth, and sandy below depth of 85 cm (Table 5).

Table 5. Granulo-metric composition and texture class

Horizon	Depth	Granulo-metric composition %						Texture class
		> 0,2	0,2 - 0,02	0,02 - 0,002	< 0,002	Total > 0,02	Total < 0,02	
	cm	mm	mm	mm	mm	mm	Mm	
Aa	0-8	0,6	44,7	39,1	15,6	45,3	54,7	Loam
I	8-50	0,2	40,8	40,8	18,2	41,0	59,0	Loam
II	50-85	0,7	45,9	30,6	22,8	46,6	53,4	Loam
Ab	85-110	1,9	91,7	2,7	3,7	93,6	6,4	Sand
III Gso	>110	29,4	64,5	1,5	4,6	93,9	6,1	Sand
Average		6,6	57,5	12,9	13,0	64,1	35,9	

Mentioned content pointed out to unfavorable water properties for development of poplar below 85 cm due to sand content ranging from 64.5 to 91.7%. Chemical traits of fluvisol with fossil soil on average were very similar to other studied forms of fluvisol (Table 6). Difference in humus content (0.71%) per profile depth was even smaller.

Table 6. Content of humus, carbonate, and reaction of soil solution

Horizon	Depth (cm)	pH u H_2O	Humus (%)	$CaCO_3$ (%)
Aa	0-8	7,24	0,67	13,44
I	8-50	7,47	1,33	20,80
II	50-85	7,57	0,10	17,53
Ab	85-110	7,58	1,34	20,39
III Gso	>110	7,68	0,13	17,54
Average		7,51	0,71	17,94

Average content of silt+clay fraction per profile depth ranged form 35.9 to 43%. The least average content per profile depth was determined on fossil soil, and the greatest one on loamy fluvisol. Average carbonate content per profile depth ranged from 16.30 to 18.19%, while average value of pH was 7.5. The greatest difference per profile depth was determined for humus content which approx. ranged from 0.71 to 2.9%. On the basis of the given soil traits the sandy loamy fluvisol was found to be the most favorable for cultivation of highly productive poplar clones.

Stand production

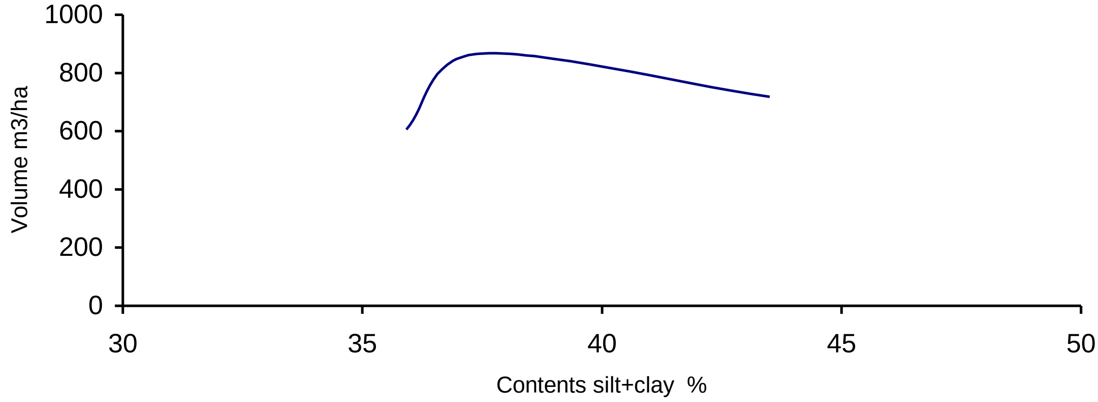
Production of stands on three fluvisol forms was determined at the end of rotation period. Analysis of mean tree stand volume and stands was done at the end of rotation period on trial areas.

Table 7. Mean tree stand volume m^3

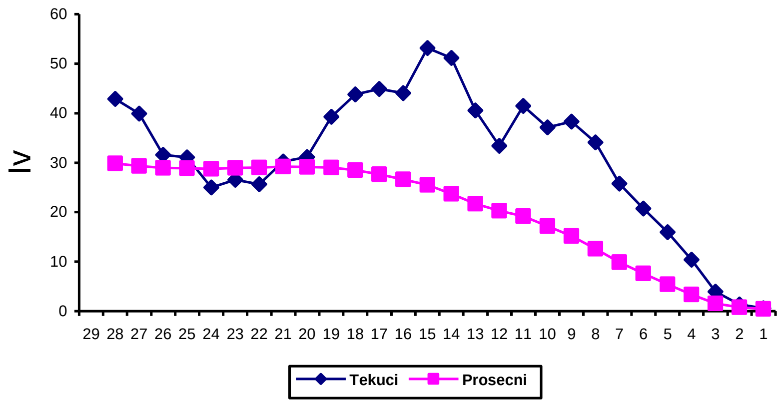
Soil type	Mean tree stand volume	Stand volume
	At the end of rotation	At the end of rotation
Sandy loamy fluvisol	3.09	865,20
Loamy fluvisol	2.99	717,60
Fluvisol with fossil soil	2.88	605,50

From the above mentioned it can be seen that the mean tree stand volume at the end of rotation period on studied systematic soil units ranged from 2.88 to 3.09 m^3. Mean tree stand volume, as well as the stand volume was the greatest on sandy loamy fluvisol, and the lowest on fluvisol with fossil soil. On the basis of previous investigation it was determined that dependence of stand volume in relation to siltt+clay fraction had the form of parabola, and that the greatest stand volume was achieved when content of fraction silt+clay ranged from 30 to 40% (Graph 1).

The greatest stand volume was determined when content of silt+clay fraction was 37.4%. Mentioned data confirmed previous investigations that dependence of stand volume in relation to silt+clay fraction had the form of parabola when content of silt+clay fraction ranged from 30 to 45% (Živanov 1970, 1977, 1979; Ivanišević, 1991; Ivanišević, 1995; Galić 2000, Galić 2006). Mentioned statements pointed out to conclusion that the most favorable conditions for the growth of *Euroamericana* black poplar cl. I-214 were sandy and loamy soils with moderate wetting obtained by using technology of greater turnovers – longer rotations (Galić 2000). Immediate proof was dynamic of average and current increase of volume stand on systematic soil units (Graphs 2, 3 and 4).

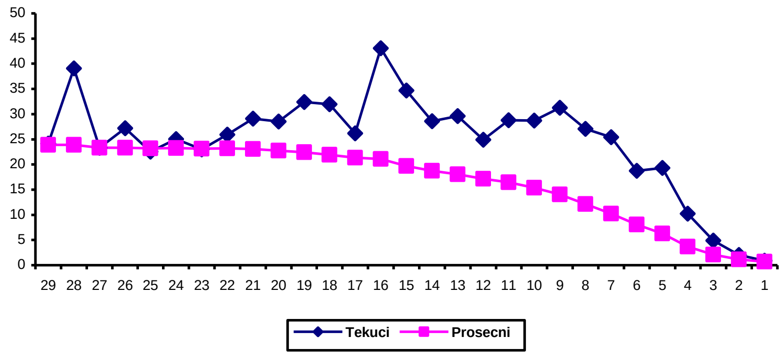


Graphic 1. Dependence of stand volume and silt+clay fraction



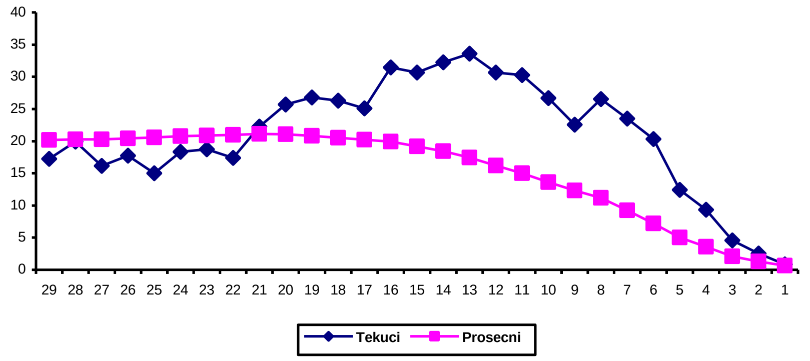
Graphic 2. Average and current increase of stand volume m^3/ha on sandy loamy fluvisol

Maximal current increase on sandy loamy fluvisol was determined at the stand age of 17 years (Graph 2), while maximal current increase in volume of clone *Populus x euramericana cl.* I-214 was determined on loamy fluvisol at the stand age of 16 years.



Graphic 3. Average and current increase in stand volume m3/ha on loamy fluvisol

Maximum current volume increase on fluvisol with fossil soil was determined in the youngest stands - 13 years of age (Graph 4)



Graphic 4. Average and current increase in volume stand m³/ha on fluvisol with fossil soil

Rotation of maximal production of wood mass on fluvisol with fossil soil and on sandy loamy fluvisol was determined at the stand age of 21 years, while on loamy fluvisol it was determined at the stand age of 23 years.
Sortiment structure is given in Table 8.

Table 8. Average Sortiment structure of wood mass %

	F	L	I	II	Prm	Celullose
Sandy loamy fluvisol	56,3	18,7	17,2	0,9	3,9	3,0
Loamy fluvisol	50,0	13,5	14,5	7,8	13,2	1,0
Fluvisol with fossil soil	56,8	13,2	12,0	4,5	12,2	1,3

The greatest participation in sortiment structure was determined for F logs, so the participation of F logs ranged from 50 on loamy fluvisol to 56.8% on fluvisol with fossil soil. Total participation together with L logs in volume ranged from 63.5% on loamy fluvisol to 75% on sandy loamy fluvisol.

The greatest income at the end of rotation period was obtained on sandy-loamy fluvisol (Table 9).

Table 9. Stand volume at the end of rotation period dinar/ha

Sistematic soil unit	Achieved income
Sandy loamy fluvisol	3.948096,82
Loamy fluvisol	2.723.685,50
Fluvisol with fossil soil	2.139.787,50

- 1 euro = 86 dinars; VAT not included (tax on added value)

In comparison to loamy fluvisol the stand volume was 31% greater, and on fluvisol with fossil soil it was 45% greater. It can be seen from the given indicator that systematic soil unit - sandy loamy fluvisol was the most favorable for stand production.

CONCLUSION

Investigation was carried out in three stands of *Populus x euramericana* cl. I-214 in the Central Danube basin on systematic soil unit: sandy-loamy fluvisol, morphology profile structure: A_{mo}- IG_{so} – II G_{so} – $G_{r'}$; on systematic soil unit: loamy fluvisol, morphology profile structure: A_a- I G_{so} – II G_{so}**;** and on systematic soil unit: fluvisol with fossil soil, morphology profile structure: A_a- I – II - A_b - $IIIG_{so}$.

The greatest variation in physical soil properties was found in silt+clay fraction, which caused formation of different textural classes per profile depth (sandy to loamy). Chemical properties of studied soils were on average very similar, with the greatest difference in humus content. Humus content variation in humus-accumulative horizon ranged from 0.67 to 6.90%

Mean tree stand volume on studied systematic soil units ranged from 2.88 to 3.09 m³ at the end of rotation period. The greatest stand volume was determined when silt+clay fraction content was 37.4%. Mentioned results confirmed previous findings that dependence of stand volume in relation to silt+clay fraction had the form of parabola when silt+clay fraction content ranged from 30 to 45%.

Rotation of maximum wood mass production on fluvisol with fossil soil and on sandy loamy fluvisol was determined in the 21st year, while on loamy fluvisol it was determined in 23rd year of stand age.

The greatest participation in sortiment structure was determined for F logs, so participation of F logs ranged from 50 on loamy fluvisol to 56.8% of fluvisol with fossil soil. The greatest income at the end of rotation period was obtained on sandy loamy fluvisol.

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MODERN TECHNOLOGIES IN BURNT AREA REFORESTATION

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Summary: *The effects of organic polymers on the survival and growth of Scots pine seedlings was analysed in the reforestation of burnt areas. The study results, bearing in mind the extreme climate conditions, degraded, sandy soils, as well as the economic gain, point out the justification of mass application of organic powder polymers in the reforestation of difficult terrains degraded by wildfires.*
Key words: *burnt areas, reforestation, seedlings, Scots pine, organic polymers, survival, plant growth*

INTRODUCTION

For Serbia to attain 41 percent of its territory under forest in 2050, as it is planned, instead of the present 29.1%, it is necessary to re/afforest another million of hectares. The realisation of such an ambitious plan is all the more difficult if it is known that the areas intended for re/afforestation are mainly difficult, degraded terrains, often damaged by wildfires. Such soils, without the humus horizon, ecologically unfavourable because of the intensive insolation and lack of moisture, which are without forest cover and in which forest mycoflora is disturbed, require the application of modern reforestation technologies in combination with the latest scientific knowledge.

During the eighties of the twentieth century, the American scientists, aiming at the intensified agricultural production based on polymers (acrylamide which is not toxic), defined the substance called *Super Absorbent Polymer*, today known under different names (superabsorbent, hydrogel, water-absorbing crystals), and trade names (Horta-Sorb®, Super-Hydro-Grow,...), depending on the type and the manufacturer. The first polymer formulations were based on inorganic substances, and today organic polymers are increasingly used because of definite residual substances which remain in the soil after their decomposition. This is especially topical when it is taken into account that plant production is increasingly directed towards organic production which excludes the application of chemical and inorganic substances.

The functional principle of this substance is that each free monomer which is present in the supeabsorbent absorbs the contacted water, retains it and releases it when it is not available in the environment. In this way, moisture is constantly available to plants, regardless of the periodicity of watering or precipitation, which is positively reflected on plant survival, growth and development and has the concrete commercial effects. The polymer efficiency remains even after many cycles of swelling and shrinking of its particles. In natural environment, they are available to plants through the period from about 12 months to several years, which primarily depends on the soil microorganisms which cause biodegradation. One of the main polymer components, starch, is the source of food for the soil microorganisms, i.e. by decomposition it is transformed into starch sugars, thus increasing the nutritive value of the soil. After polymer biodegradation, whatever remains in the soil, functions as soil additive to improve the aeration and other soil properties (creating the optimal conditions for the more intensive development of the root system). Biodegradation transforms the polymers into practically the same material as the soil itself.

Nowadays, the application of polymers in agriculture is becoming increasingly diverse (Dragičević *et al.*, 2008, 2008a), and the positive experience is the impetus for their application also in the field of forestry: as an addition to substrate mixtures for plant production and cultivation (Henderson & Hensley, 1986; Kjelgren *et al.*, 1994; Kahl *et al.*, 2000; Vilotić *et al.,* 2006), for seed germination (Henderson & Hensley, 1987, 1987a; Woodhouse & Johnson, 1991), for soaking naked-root seedlings in transport, for soil stabilisation (Aly & Letey 1989, 1990; El-Hady *et al.*, 1981, 1991; Barvenik, 1994; Bouranius *et al.*, 1995), for the establishment of tree rows, shelterbelts and for reforestation of difficult, degraded terrains in climatically modified environmental conditions caused by drought and high temperatures (Cook & Nelson, 1986; Callaghan *et al.,* 1989; Huttermann *et al.*, 1990).

MATERIAL AND METHOD

Under the auspices of the Ministry of Environmental Protection and Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, the establishment of the network of field experiments started in the spring 2008, in the aim of testing the effect of polymers in the re/afforestation of cinder dumps, burnt areas and degraded areas in climatically modified conditions, conditioned by global warming.

The analysis of polymer effect on seedling survival and development in the first year after transplanting, in the reforestation of burnt areas, was performed in a part of the Sands Deliblatska Peščara, where a pine plantation was destroyed by wildfire in the summer 2007, at the age of about 60 years. Experiments were established with Scots pine seedlings aged 2+0, produced in Nisula rolls. The seedlings were planted in holes formed in furrows, with 2.5m distance between rows and 1.6m distance between seedlings, Figure 1. The following polymers were tested: *Water Retainer/Polymers - Hydro Absorption Rate between 250 and 350* (type A) and *Stockosorb (Hydrogel)* (type B), in powder and gel state, designated and monitored as:

Treatment 1 - seedlings planted in holes with added 2.5 grams of powder polymer type «A»
Treatment 2 - seedlings planted in holes with added 5 grams of powder polymer type «A»
Treatment 3 - before planting, seedling roots were soaked in polymer type «A» gel-solution in water
Treatment 4 - seedling roots were soaked in polymer type «B» gel-solution in water
Control - seedlings were planted in holes without the addition of polymers.

The basic characteristics of these polymers are organic origin, ability of absorbing and retaining water about 300 times more than their own weight, inactivity from the chemical aspect, neutral pH value, capacity of remaining in the soil up to three years and capacity of decomposition into organic elements available to plants.

In the autumn 2008, seedling survival and development was analysed in different treatments and in the control. The collected data were processed by computer programme «Statistika». The differences between the treatments and control were statistically justified by Student's t-test. Form the beginning of the experiment, the daily weather conditions were monitored in the region of Bela Crkva, They are summarised in Table 1.

Table 1. Average values of the minimal and maximal temperatures with the number of rainy days in the area of Bela Crkva for the period April-August 2008 (according to data collected by Sretko Munćan, B.Sc.)

Month	**Average minimal temperature ^{0}C**	**Average maximal temperature ^{0}C**	**Number of rainy days**		
			Rain throughout the day	Light rain in a part of the day	Showers
April	3-10	13-20	2	7	1
May	7-14	20-26		1	
June	12-19	24-30		2	1
July	13-20	25-32	1	3	1
August	15-20	27-33		3	

Figure 1. Reforestation of burnt areas in Deliblatska Peščara in the spring 2008 with the application of polymers

RESULTS WITH DISCUSSION

The effect of polymer types A and B on the success of reforestation of burnt areas in Deliblatska Peščara was assessed based on: recording the number of survived seedlings after the first growing season, analysis of the total seedling height, analysis of height increment in the first year, and analysis of seedling root development in different treatments and the control.

Survival of seedlings after the first growing season

The analysis of Scots pine seedling survival after the first growing season, was performed on the sample of 50 plants, Table 2.

Table 2. Results of the analysis of Scots pine seedling survival after the first growing season

	Mean value	Standard deviation	t - value				
			K	**T1**	**T2**	**T3**	**T4**
K	0.64	0.48	-				
T1	0.76	0.43	-1.63	-			
T2	0.70	0.46	-0.62	0.72	-		
T3	0.66	0.47	-0.20	1.09	0.50		
T4	0.80	0.40	-1.83	-0.46	-1.15	-1.55	-

The results obtained on the sample of 50 plants show that seedling survival with the application of polymers was higher than that without polymers. For example, if we compare treatment 1 and the control, it can be concluded that, with polymer application 38 plants survived out of 50 plants, whereas 32 plants survived out of 50 control plants. The difference of 6 plants is not statistically significant in the sample of 50 plants, but if it is taken into account that the reforestation of one hectare of Deliblatska Peščara requires 2500 seedlings, then the study results acquire a different significance. This means in fact that, with the polymer application, the number of seedling survival per hectare will be by 300 seedlings higher than seedling survival without polymer application, it is 600 seedlings per two hectares, etc. The commercial effects are not difficult to calculate if the costs of site preparation, labour force and seedling procurement are also taken into account.

Seedling height after the first growing season

Seedling height after the first growing season was measured on the sample of 35 plants. The collected data, Table 3, should be accepted with reserve, bearing in mind the planting depth, which cannot be considered as constant.

Table 3. Results of the analysis of Scots pine seedling height after the first growing season

	Mean value	Standard deviation	t - value				
			K	**T1**	**T2**	**T3**	**T4**
K	20.06	6.69	-				
T1	18.34	6.15	1.05	-			
T2	19.83	7.50	0.13	-0.87	-		
T3	20.14	5.90	-0.06	-1.34	-0.21	-	
T4	19.85	7.38	0.12	-1.35	-0.02	0.20	-

Seedling height increment after the first growing season

Seedling height increment after the first growing season was measured on the sample of 35 plants. The collected data are presented in Table 4. Based on the data, it can be inferred that polymer application had different effects on height increment of plants in the first year after planting. The positive effect, occurred only in treatment 1, while in all other polymer treatments reduced seedling height increment compared to the control, which was also reported in previous researches (Šijačić-Nikolić *et al.,* 2006, 2006a, 2006b).

Table 4. Results of the analysis of Scots pine seedling height increment after the first growing season

	Mean value	Standard deviation	t - value				
			K	**T1**	**T2**	**T3**	**T4**
K	12.26	4.15	-				
T1	12.89	3.87	-0.64	-			
T2	11.74	3.65	0.59	1.12	-		
T3	9.05	3.27	4.83*	4.32*	3.34*	-	
T4	9.82	4.36	2.98*	2.80*	2.05*	-1.03	-

This is especially true in polymer application by root soaking in gel solution before planting (Treatments 3 and 4), which was also confirmed by the values of Student's t-test.

Analysis of seedling roots after the first growing season

The analysis of seedling roots after the first growing season was performed on the sample of ten lifted seedlings. The following morphometric parameters were measured: diameter in root collar (mm) and the mass of seedling shoot/root in air dry state (g).

Based on the study results, it can be concluded that polymer application had a positive effect on root development of Scots pine seedlings in the first growing season after transplanting, Figure 2, especially in treatment 2 with higher quantities of polymers in powder state added directly to the planting holes.

CONCLUSION

Based on the study results of the effect of organic polymers on the survival and growth of Scots pine seedlings in the areas degraded by wildfires, it can be concluded that the application of polymers:

- had a positive effect on seedling survival after the first growing season;
- retarded the seedling height increment;
- had a positive effect on root collar diameter and seedling root development.

The tested concentrations and the methods of polymer application, in powder and gel state, show that the application of powder polymer type «A» in greater quantities (T2) had the best effect, while root soaking in polymer gel, obtained by dissolving polymer type «A» in water, was not a good solution. Somewhat better results were obtained by seedling roots soaked before planting in the gel obtained by dissolving polymer type «B» in water.

	K	T1	T2	T3	T4
Root collar diameter (mm)	3.6	6.3	10.0	4.9	6.6
Shoot mass (g)	6.02	21.02	40.81	10.81	24.02
Root mass (g)	0.72	3.71	6.39	2.28	5.24

Figure 2. Variability of Scots pine seedling roots in the control (K) and treatments (T1, T2, T3 and T4)

Based on the above, it can be concluded that the application of powder organic polymers, by adding directly to planting holes in the concentrations of 2.5 grams and higher, had a positive effect on Scots pine seedling survival and growth in burnt areas, in the first year after transplanting. In the following years, intensified seedling height growth should be expected, based on well developed root system, which should contribute to the reforestation success in degraded sites, such as burnt areas. The above facts are all the more significant, if we consider the climate conditions through the growing season, as in five months there were only three days with heavy rain, sixteen days with light rain during a part of the day and three days with showers, while the average maximal daily temperatures ranged up to 33^{0}C.

The study results, taking into account the extreme climate conditions, degraded, sandy soil and the economic gain, point to the justification of mass application of organic powder polymers in the reforestation of difficult terrains degraded by wildfires.

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ECO-PEDOLOGICAL CHARACTERISTICS OF PODZOLISED ACID BROWN SOILS IN BEECH FORESTS IN SERBIA

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Abstract: *The systematized data on the existence, properties, genesis and distribution of podzolised acid brown soils in Serbia are very few. In the Soil Classification (Škorić, et al., 1985), this soil is defined as the subtype of acid brown soil.*
Podzolised acid brown soil has its typical morphology and its own evolution. The genesis, as well as the formation of the profile morphology, is performed under the effect of the corresponding constellation of site factors. Acid siliceous rocks, very much dissected relief, steep slopes, scanty vegetation and often the sunny exposures, water and wind erosion, specific microclimate, are the main agents of this soil formation.
Podzolised acid brown soil in acidophilous beech forests (suballiance: Luzulo-Fagenion moesiacae B. Jov. 1976) is a very interesting soil subtype, which occurs fragmentarily on small areas throughout Serbia. The principal characteristics of this soil: small depth, very acid or extremely acid soil reaction, transition of sequioxides, specific group-fraction composition of humus, are the properties that make it approaching to brunipodzols and podzols.
In harmony with the modern approach to forestry development, the degraded and devastated area of the coeno-ecological group of acidophilous beech forests (Luzulo-Fagenion moesiacae B. Jov. 1976) on podzolised acid brown soil, especially the communities of acidophilous forests of beech with mosses (Musco-Fagetum B. Jov. 1953) on very steep slopes, should be designated as special management entities and a special management treatment should be applied. Such entities occur in beech forests throughout Serbia and their total area is not at all to be disregarded.
Key words: *podzolised acid brown soil, ecological conditions, acidophilous beech forest.*

INTRODUCTION

Podzolised acid brown soil, according to the Classification criteria by Škorić *et al.* (1985), is defined as a subtype of acid brown soil. In Serbian pedological literature there are not many systematised data on the existence, properties, genesis and distribution of this soil subtype in the area of Serbia. The main reason of insufficient study of podzolised acid brown soil is in the fact that previous study was focused primarily on the soils of the most important forest ecosystems. Podzolised acid brown soil, as the soil in "uninteresting" acidophilous beech stands, from the forest-economic aspect, occupying relatively small areas within the large forest complexes, was the subject of only partial researches.

Podzolisation as the pedogenetic process, in previous researches in Serbia, is mainly related to high mountainous areas, perhumid climate, coniferous and mixed broadleaf-coniferous forests, acid siliceous rocks rich in quartz. Ćirić (1982) and Burlica (1982) report that podzolised soils, in our regions, have very limited elementary ranges and they most often occur in combination with dystric brown soils, series type or mosaic series. According to Jović (1965, 1981) the most widely distributed podzolised soils in Serbia are brown podzolic soils, with about 1,079.00 *ha*, and the most frequent zone of their occurrence is above the altitude of 1400*m*, in coniferous forests (60.2%) and mixed forests of beech and conifers (39.8%).

Antić and Bunuševc (1952) report on the fragmentary presence of "highly acid brown soils, of poor properties" in acidophilous forests of beech with mosses throughout Serbia, and the inferior quality and quantity characteristics of wood volume are explained by unfavourable edaphic conditions. Jovanović (1953) emphasises that "the association *Fageto-Muscetum* B. Jov. 1953 is related to the soils with the podzolisation process", and the process itself is explained by the "relief, always siliceous bedrock, the altitude, as well as by the vegetation growing on it".

Filipovski and Ćirić (1963) report the "highly acid brown soils" formed on quartz sandstones, hornfels and quartzites at the lower altitudes (below 900 *m*), while brown podzolic soil alternate with podzols at the higher altitudes on the same bedrocks. The "extremely acid brown soil of specific morphology and chemical properties" was described in the researches on the slopes of the mountain Gučevo (Vukićevićand Avdalović, 1972), in the acidophilous forest of beech with moss, at the altitude of 300-400 *m*, on north aspects, on rhyolites. Hartmann (1974) describes a great number of associations in the wider area of Central Europe, among them also *Luzulo-Fagetum montanum* obd. subas. with *Leucobryum glaucum*, on the “dry, especially quartz soils, and the soil type corresponds to highly podzolised brown soil“. Avdalović (1975) reports that podzolised acid brown soil has its typical morphology and its own evolution. Extremely acid brown soil occurs in the beech forest with blueberry (*Vaccinio-Fagetum moesiacae* Fukarek 1969), on west, northwest and north aspects and on steep slopes (20-35°) in the forest management unit “Crni Vrh-Kupinovo-Bor” (Jović and Knežević, 1990). Jović (1996) claims that podzolised acid brown soils in beech - fir forests in Serbia are more rarely found, compared to dystric brown soils.

The podzolic subtype of dystric brown soil was studied in the area of the mountain massif Kukavica, by ecological-vegetation studies in beech forests (Tomić *et al.*, 2000). The formation of “podzolic brown soil“ is linked to acidophilous forests of beech with woodrush (*Luzulo-Fagetum moesiacae montanum* Jov. 1976), which occurs on south, warm slopes or on prominent ridges and peaks, i.e. “at places where there are conditions for faster soil degradation“, at the altitudes of 870-1170 *m*.

Knežević and Košanin (2005) report that podzolised acid brown soils as well as eroded, shallow to medium deep acid brown soils, occur in acidophilous beech communities on steep and narrow ridges of the hilly and mountainous regions in Serbia, which are often exposed to soil loss processes. Podzolised acid brown soil was studied in the acidophilous forest of beech with moss on Veliki Jastrebac (Milošević, 2006), and also in the area of the mountain Čemernik (Ćirković, 2006).

There are practically no data on the podzolisation in beech forests. The occurrence of mainly severely and extremely acid brown soil is reported on the extreme sites in beech forests in Serbia. Podzolised acid brown soil is mentioned in the latest literature sources. In harmony with modern approach to forestry development, and based on the fact that beech stands account for the greatest percentage of the growing stock in Serbia, this paper presents the study results of eco-pedological characteristics of podzolised acid brown soils in acidophilous forests of beech with moss (*Musco-Fagetum* B. Jov. 1953). Such entities occur in beech forests throughout Serbia, the total area of which is not insignificant.

MATERIALS AND METHODS

The research program was realised by field and laboratory methods adopted and defined by the Yugoslav Soil Science Society. The field study phase on the profiles included the detailed morphogenetic study of the soil and the description of environmental conditions. In the end, the samples were taken in the disturbed state, and the following was determined: standard physical and chemical properties of the soil, group-fraction composition of humus, and the dynamics of sesquioxides as diagnostic indicators of podzolisation.

As it is logical that forest soil should be studied in relation to vegetation, the vegetation was studied in detail in the phase of field study. The vegetation was studied on phyto-coenological releves by *Braun-Blanquet*’s method.

Laboratory research included a set of standard physico-chemical analyses adopted and accepted by YSSS. Special attention was focused to quantitative and qualitative characteristics of humus. Humus study included the following determination:

1. Content of humus and carbon (*%*) by *Tjurin I. V.* (1960) method in modified by *Simakov*;
2. Group-fraction composition of humus by *Ponomareva, V. V.* (1957) method after *Škorić, A.* and *Racz, Z.* (1966):
   - Determination of the percentage of organic substances bound to R_2O_3;
   - Determination of the percentage of organic substances bound to calcium;
   - Determination of groups of the most mobile organic substances soluble in 0.05 *M* H_2SO_4 during soil decalcification (*Ponomareva, V. V.* 1957) - fulvic acids fraction 1a, after *Tjurin*;
   - Simultaneous with the determination of organic carbon in the acid solution, we also determined the bases of iron and aluminium readily soluble or extracted from the adsorptive state. The content of mobile Fe_2O_3 was determined complexometrically (*Škorić, A.* and *Sertić, V.* 1963), and the content of Al_2O_3 was calculated from the difference of R_2O_3 content, determined gravimetrically by deposition with ammonia by *Ponomareva, V. V.* (1957) method, and the content of Fe_2O_3;

- Calculation of the ratio R_2O_3/Fk, where R_2O_3 is the total quantity of mobile sesquioxides, and Fk - fulvic acid fraction 1a. The value of Fk is obtained by multiplying the percentage of carbon fulvic acid fraction 1a by two, i.e. it is taken that the percentage of carbon in this fraction is 50% (*Martinez, F. M.* 1965);
- Calculation of the ratio of mobile Al_2O_3/Fe_2O_3.

3. The content of free, mobile (amorphous) forms of iron and aluminium in the oxalate extract was determined by *Tamm's* method (1934). In this, total sesquioxides were determined after the deposition of ammonia, and the mobile forms of Fe_2O_3 from the extract were determined complexometrically.

RESULTS AND DISCUSSION

In agreement with the idea on the "integral nature", special new methods of soil study have been developed. One of them is the profile-genetic method, which observes the changes of bedrock properties influenced by a series of environmental factors in the process of soil formation, i.e. it studies the soil genesis. Gerasimov (2004) reduces soil formation, or pedogenesis, to the following three-member formula: soil properties←processes of pedogenesis←factors of pedogenesis. The genesis and the properties of the formed soil depend on the constellation of pedogenetic factors: relief, altitude, aspect, slope, climate, parent rock and vegetation. For this reason, special attention in this paper is focused on site conditions.

In the hilly belt of Serbia, in the communities of submontane forests of beech (*Fagetum submontanum*), occurring up to the altitude of 600 *m* in its northeast part, i.e. 800 *m* in other regions, the climate is temperate to mild continental. In the lower part of the belt, forests are not in their climate-physiological optimum, but they are in the optimum in the upper part of the belt. The climate-regional belt of beech montane forests (*Fagetum moesiacae montanum Jov.*) which in north-east Serbia occurs at the altitude of 600 - 1100 *m*, i.e. from 800 to 1300 *m* in west Serbia and south-east Serbia, and from 800 to 1500 *m* on Kopaonik, is characterised by humid to perhumid climate, and beech forests are in their biological optimum.

A very significant role in the genesis of podzolised acid brown soil is assigned to highly dissected relief and steep slopes. At lower altitudes, in the zone of submontane beech forests (*Fagenion moesiacae submontanum* Jov. 1976), podzolised acid brown soils are mainly formed fragmentarily on steep slopes at colder aspects. In the belt of montane beech forests (*Fagenion moesiacae montanum* Jov. 1976), on the majority of mountains in Serbia, podzolised acid brown soils occupy the south, warmer slopes or the prominent ridges, where soil degradation is faster, while acid brown soils remain in the valleys and in the cooler aspects.

Podzolised acid brown soils in beech forests in Serbia are formed on siliceous substrates composed of acid rocks: granodiorites, granites, schists, gneisses, mica schists, conglomerates, phyllites, cornites, etc. Bedrock disintegration is performed by the processes of physical and chemical decomposition, which cause essential changes in bedrock and mineral composition. By the decomposition of acid siliceous rocks, coarse sandy material is released, predominantly of loamy-sandy particle size composition, which enables a good aeration and water permeability to these soils. The main characteristic of acid siliceous rocks is that they are poor in bases, or their relatively low content of bases which are rather readily washed (K, On). Thanks to the above substrate properties, already the first soil development phases are poor in bases and they are acid.

The communities on podzolised acid brown soils belong to the sub-alliance of acidophilous forests of Moesian beech *Luzulo-Fagenion moesiacae* Jov. 1976, more exactly, they are formed in the associations: acidophilous forests of beech with woodrush (*Luzulo-Fagetum moesiacae submontanum* Jov. 1976.), acidophilous forests of beech with moss (*Musco-Fagetum moesiacae* Jov. 1953.) and acidophilous forests of beech with blueberry (*Fagetum montanum myrtilletosum* Jov. 1973). Acidophilous beech forests are mainly represented by the stands with low heights and broken canopy, with curved, thin, branchy beech stems without major technical value. They consist of a lower number of species, compared to typical beech forests. The characteristic species in the sub-alliance are: *Vaccinium myrtillus L., Luzula luzuloides, Luzula sylvatica, Luzula pilosa, Veronica officinalis, Pyrola rotundifolia* and numerous mosses - *Musci spp.*

This paper presents the study results of podzolised acid brown soil formed in acidophilous forests of beech with moss (*Musco-Fagetum moesiacae* Jov. 1953). Profile morphology of podzolised acid brown soil is characterised by some specificities. The most frequent profile structure of podzolised acid brown soil is: OhA – (B) – C. The classical horizon of forest litter in the communities of beech with moss, is practically completely replaced by moss “cushions“ and “patches“. Already by spring, the leaf litter which is found on the soil surface in the autumn, is completely removed by wind and water down the steep slopes. Therefore, the decisive role in soil genesis is that of moss facies.

Because of the more or less developed moss cover, there is not a sharp transition between O and A horizons, because of the specific humification character. This part of the profile can be marked as OhA, it is at the same time the humus accumulation horizon and it has some characteristics of the eluvial horizon.

Podzolised acid brown soils are mainly shallow to medium deep. The OhA horizon is usually not very deep, only 3-5 cm. The colour of OhA horizon varies from grey-brown, brown, brown-black to black. The colour mostly depends on the quantity of the accumulated humus. The middle and the lower parts of the podzolised acid brown soil profile show the morphological match with acid brown soil. Namely, the cambic horizon retains all morphological characteristics of (B)-horizon of acid brown soils: rusty brown colour and thickness ranging from 25 to 55 *cm*.

As acid siliceous rocks are subject to the processes of intensive physical decomposition, podzolised acid brown soils inherit the lighter particle-size composition, mainly sandy loamy, silty-loamy to loamy. These soils, also often contain a high percentage of skeletal material, the content of which usually increases with depth.

The predominant fraction in the particle-size composition of the greatest number of the analysed soils is total sand - i.e. they have a high content of coarse and fine sand fractions. Regardless of the bedrock type, the percentage of the fraction of fine sand is mainly the highest and the percentage of silt fraction is rather high, which cannot be considered as a favourable property. Podzolised acid brown soils, taking into account the particle-size composition, are characterised by good aeration and excellent water permeability. The exception is, to some extent, the soil formed on phyllites, with somewhat more clayey variants.

The coarse-grain system of these soils affects their liability to podzolisation processes, i.e., causes the easier migration of the colloid fraction, as well as the migration of organomineral compounds from the higher to the lower parts of the soil.

Table 1 presents the chemical properties of three representative profiles of podzolised acid brown soil. Its chemical properties are characterised, primarily by a high content of humus in the surface part of the soil (OhA-horizon), which decreases sharply with depth, during the transition to (B)-horizon. However, disregarding the high content of humus, the content of nitrogen is relatively low. The C:N ratio in OhA-horizon is relatively wide and points to the formation of dystrophic moder humus. The high content of humus results also in the high total capacity of adsorption for cations and it ranges up to 109.98 cmol/kg. The sum of bases, as well as the degree of soil saturation with bases, is low and it does not exceed 20.61%.

Soil reaction in water ranges from 3.38 to 4.80 in OhA-horizon, which is also the most acid horizon. In the cambic horizon, soil reaction increases with depth, and ranges from 4.00 to 5.40. Hydrolytic acidity is in harmony with soil reaction, as well as the total quantity of acid cations. In OhA-horizon, podzolised acid brown soil hydrolytic acidity is up to 4 times higher compared to that in acid brown soil and ranges up to 162.83 ccm n/10 NaOH. The content of readily available phosphorus in podzolised acid brown soil is low, and the content of potassium is medium to high in OhA-horizon. All these values decrease with depth and approach the properties of the acid brown soil cambic horizon.

The group-fraction composition of humus of the podzolised acid brown soil in acidophilous beech forest with mosses is distinguished by the ratio Ch:Cf<1. The superficial horizon has all characteristics of podzol with the ratio Ch:Cf amounting to 0.50-0.80, while the lower part of the profile retains all the characteristics of acid brown soils. Fulvic acids, i.e. fraction 1, are dominant among humus components. In OhA-horizon, the content of fulvic acid fraction 1 accounts for 13.83-26.09% of the total carbon. The content of this fraction mainly decreases with depth and accounts for 3.40-11.76% (exceptionally 29.25%). The content of fulvic acid fraction 1a is found immediately after the fraction 1.

Table 1: Chemical propertis of podzolised acid brown soil

Locality and profile number	Depth (cm)	Horizon	pH		Y1 ccm n/10 NaOH	Adsorptive complex				Humus	C	N	C/N	Available	
						(T-S)	S	T	V					P_2O_5	K_2O
			H_2O	KCl		cmol/kg			(%)	(%)	(%)	(%)		mg/100g	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
GUČEVO 25/71 *Musco-Fagetum Altitude: 390 m Aspect: N Slope: 25° Supstrate:rhyolite*	0 - 3	OhA	3,38	2,50	102,77	66,70	3,14	69,84	4,49	13,05	7,36	0,15	49,10	0,60	7,30
	3 - 35	(B)	4,00	3,12	66,10	42,96	1,93	44,89	4,29	2,84	1,60	0,11	14,80	2,00	6,70
	35 - 70	(B)	4,00	3,28	47,15	30,64	1,74	32,38	5,37	0,60	0,43	0,00	0,00	0,80	6,30
	70 - 110	(B)C	4,40	3,45	38,42	24,95	4,24	29,19	14,52	0,41	0,23	0,00	0,00	0,80	7,00
N.P.ĐERDAP 22a/02 *Musco-Fgetum Altitude: 430 m Aspect: NW Slope: broad crest Supstrate: gneiss*	0 - 5	OhA	3,82	3,20	162,83	105,84	4,14	109,98	3,76	33,64	19,51	1,31	14,90	2,60	40,00
	5 - 10	A	4,20	3,95	26,54	17,25	0,00	17,25	0,00	4,97	2,88	0,25	11,50	0,50	6,80
	10 - 40	(B)	4,30	4,02	21,14	13,74	0,00	13,74	0,00	1,91	1,11	0,13	8,50	0,00	3,40
GOČ 8/00 *Musco-Fagetum Altitude: 950 m Aspect: crest Slope :20-25° Supstrate:phyllite*	0 - 5	OhA	3,76	3,06	110,44	71,79	8,00	79,79	10,03	24,93	14,46	0,70	20,60	6,00	34,50
	5 - 40	(B)	4,20	3,65	61,89	40,23	3,20	43,43	7,37	3,89	2,26	0,24	9,40	0,00	10,50

The content of this fraction is the lowest in OhA horizon and accounts for 2.98-7.82% of total carbon, in (B)-horizon the content of this fraction increases with depth up to 25.58% of total carbon.

In the solution of genetic and classification tasks, soil scientists traditionally select the determination of the quantity of free compounds of iron (Водяницкий, 2004). According to Зоонн (1982), iron capacity of changing the valence (Fe^{+3}, Fe^{+2}) is of special significance for soil genesis. Iron can migrate in the soil only in ionic colloid and complex-chelate state.

Total quantity of released iron and aluminium greatly depends on the content of these oxides in bedrock, and their percentage increases from sandstones, through granites to basalts (Дюшофур, 1964). According to Avdalović (1971), the soils formed on granodiorites, phyllites and especially on cornites, differ from the soils formed on mica schists, rhyolites and gneisses, by the contents of iron and aluminium oxides. The quantity of iron oxides is not so significant for the genesis of podzolised acid brown soil, its dynamics in the profile is more significant.

The migration of iron and aluminium oxides is significant for the soils with podzolisation processes. As opposed to acid brown soils, in podzolised soils aluminium oxides migrate to the substantially deeper parts of the profile. Table 2 presents the content of free forms of iron and aluminium bound to the mineral part of the soil - clay, which were obtained by oxalate method after T a m m (1934), from the samples of the representative profiles of podzolised acid brown soil formed in the community *Musco-Fagetum.*

Table 2. Contents of free iron and aluminium oxides in % (in the extract after Tamm) from the samples of podzolised acid brown soil

Locality and profile number	Dept (cm)	Horizon	% in soil sample			Fe B/Fe A	Al B/Al A
			Fe_2O_3	Al_2O_3	R_2O_3		
CER Profile: 13/05 *Musco-Fagetum* Altitude: Ekspozicija: NW Slope: 20° Supstrate: granit	0 - 5	OhA	0.27	0.83	1.10		
	5 - 27	(B)	0.32	1.23	1.55	1.19	1.48
	27 - 50	(B)	0.36	1.39	1.75	1.33	1.68
N.P.ĐERDAP Profile: 22a/02 *Musco-Fgetum* Altitude: 430 m Aspect: NW Slope: broad crest Supstrate: gneiss	0 - 5	OhA	0.36	0.56	0.92		
	5 - 10	(B)	0.41	0.70	1.11	1.14	1.25
	10 - 40	(B)C	0.20	0.63	0.83		
GOČ Profile: 8/00 *Musco-Fagetum* Altitude: 950 m Ekspozicija: greben Slope:20-25° Supstrate:phyllite	0 - 10	OhA	0.62	0.33	0.95		
	10 - 45	(B)	0.76	0.83	1.59	1.23	2.52

In addition to the contents of free Fe and Al oxides, a closer classification requires also the value of Fe-quotient and Al-quotient after Kundler, 1962 (cited after Avdalović, 1971). This quotient represents the ratio of free Fe and Al oxides in B-horizon compared to A-horizon ($Fe\ B_{hor}/Fe\ A_{hor}$). According to Avdalović (1971), the values of Fe-quotient in acid brown soils in Serbia range from 0.6 to 1.1, mean value 0.8. In brown podzolic soils in Serbia, the values of Fe-quotient range from 1.2 to 1.4 (Jović, 1966), and in podzol the values range up to 80 (Antić *et al.* 1971).

In podzolised acid brown soils on Mt. Goč, (Avdalović, 1971), the values of Fe-quotient range within 1.8-2.0, and the values of Al-quotient from 1.6 to 3.1. The occurrence of podzolised acid brown soil on Mt. Goč is conditioned by the acidophilous community, on the one hand (association *Abieto-Fagetum luzuletosum*), and on the other hand, by highly acid parent rock quartzite, which is very rich in quartz, and poor in siliceous minerals. In the community *Myrtillo-Abietum leucobryetosum* on podzol, at the same site and on the same bedrock, the values of Fe-quotient amounted to 52.3-86.0, and the values of Al-quotient were lower and ranged from 2.8 to 21.5. These data confirm that the vegetation species affects the values of Fe and Al quotients.

In the samples of podzolised acid brown soil, which are presented in this paper the values of Fe-quotient range from 1.14-1.33, while the values of Al-quotient are somewhat higher and amount to 1.25-2.52. There are differences between the study results presented in Table 2, and the results reported in previous research in our country. The differences are conditioned by biological and climate differences, quantity and type of formed organic and humus acids, moisture intensity and character, and other factors. With the increase of the percentage of broadleaf species in the communities, in this case beech, the degree of forest litter decomposition also increases. With the percentage of broadleaf species, the degree of podzolisation decreases, because the quantity of produced organic and fulvic acids decreases and also their role in Fe mobilisation, the effect of surface water decreases, which leads to the change of Fe^{+3} in Fe^{+2}, which is mobile. Therefore, in the conditions of lower acidification, conditioned by the chemical composition and structure of the organic waste in beech forests and fast water percolation through the soil profile, reduced production of free acids, it is siliceous forms of Fe that prevail in the soil and free forms are characteristic only of the upper part of B horizon.

CONCLUSIONS

Podzolised acid brown soil has a simple profile structure: OhA – (B) – C, and compared to the related acid brown soil, the differences occur in the surface horizon which is also the horizon of accumulation of the dystrophic form of humus, and it has some characteristics of the eluvial horizon.

A closer definition of podzolised acid brown soil has been required for a long time in our soil science literature. Different authors classified this soil as “highly acid brown soil”, “extremely acid brown soil”, “podzolised acid brown soil” and the like. The extremities of this soil, which approach it to brunipodzols and podzols, are primarily the high acidity, low degree of saturation of the adsorptive complex with bases, formation of moder humus, and the domination of fulvic acids over humic acids (Ch:Cf<1).

The migration of iron and aluminium oxides is significant for the soils with podzolisation processes. In the samples of podzolised acid brown soil, which are presented in this paper, the values of Fe-quotient range from 1.14-1.33, while the values of Al-quotient are somewhat higher and amount to 1.25-2.52. There are significant differences in the properties of podzolised acid brown soil formed in different ecological conditions (vegetation, climate, bedrock, etc.), which conditions the differences in quantity and type of formed organic and humus acids, moisture intensity and character, and other factors. With the percentage of broadleaf species, in this case beech, the degree of podzolisation decreases, because the quantity of produced organic and fulvic acids and their role in Fe mobilisation decreases, the effect of surface water decreases, which leads to the change of Fe^{+3} into Fe^{+2}, which is mobile. In this way, iron migration to the lower parts of the profile decreases.

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APHID INFLUENCE TO CONTEST OF PHOTOSYNTETIC PIGMENTS OF DIFFERENT POPLAR CULTIVARS

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Abstract: *The influence of aphids on the content of photosynthetic pigments was researched on the foliage of different poplar clones. The analysed physiological parameters are important parameters of the plant physiological status and they are parts of the complex process of photosynthesis. Even the slightest changes of some of the study parameters can decrease the process of photosynthesis significantly. In July 2001, the leaf samples (with and without the presence of aphid colonies) were taken randomly from ten poplar clones. The content of photosynthetic pigments was determined according to W e t s t e i n's method.*
The study results indicate that the aphids on poplar foliage have a significant impact on the content of photosynthetic pigments in the leaves and that there are differences among the study clones. In one group of clones, there was an increased content of photosynthetic pigments, while in the other group of clones the content was decreased. Although in the study clones there was no regularity regarding the aphid influence on the content of photosynthetic pigments, it can be concluded that aphids have a significant influence on the content of photosynthetic pigments in the leaves.
Key Words: *Aphids, photosynthetic pigments, poplars clones*

INTRODUCTION

The family of plant lice (Aphididae) attracts attention of both entomologists and scientists interested in plant production, due to a great number of widespread species, number of host plant species which they attack, specificity of development and life style, specific morphological and anatomical characteristics, as well as the consequences of their feeding on host plants.

Numerous plant lice species are mentioned in the literature, which are developing and feeding on parts of different tree species, along with their deleterious influence. According to available literature data, previous studies did not quantify the effect on growth elements (diameter and height), which are the most important in the economic sense. Growth elements (diameter and height) are not reliable parameters for evaluation of destructive effects of insect feeding on the host plant, because they depend on other external and internal factors, such as availability of mineral nutrients and water in soil, temperature, light, possibility of using light and space, specific genotype characteristics under actual conditions, etc. All these factors could also influence the host plant negatively, and «mask» disturbances resulting from lice feeding. Therefore, it is very interesting to analyze and find direct relation between lice infection and photosynthesis, respiration and transpiration, which will enable evaluation of their destructive effects using the degree of these processes disturbance.

MATHERIAL AND METHODS

The content of photosynthetic pigments was determined in acetone extract (15). Leaves were obtained from poplar clones PE 19/66, 54/76-2, 88/82, S_{11}-8, 181/81, S_1-8, 88-82, 182/81 and 265/81 (which belong to *Populus deltoides* species) and clone Pannonia (*Populus x euramericana* hybrid), grown at the Institute's experimental field. Fully formed leaves were randomly selected and sampled in July 2001, with or without lice colony. Leaves were cut into pieces and homogenized using the pestle, with addition of 5-10 ml of acetone. This homogenized content was poured into a glass cylinder using the vacuum pump. The obtained filtrate presents the pigments extract. Concentration of chlorophylls a and b, and carotenoids were calculated after absorbance measurement using spectrophotometer, at wavelengths of 662, 644 and 440 nm.

RESULTS AND DISCUSION

Data related to content of photosynthetic pigments are summarized in Tables 1-5.

Content of chlorophyll a

Content of chlorophyll a was shown in Table 1.

Table 1. Content of chlorophyll a in healthy and infested leaves (mg/g dry matter)

Clone	**PE 19/66**		**54/76-2**		**88/82**		**S_{11}-8**		**181/81**	
	BV	SV	BV	SV	BV	SV	BV	SV	BV	SV
Content of chlorophyll a	3,808	4,036	3,27	4,956	3,427	5,636	3,638	5,089	4,946	2,586
Clone	**Pannonia**		**S_1-8**		**88/22**		**182/81**		**265/81**	
	BV	SV	BV	SV	BV	SV	BV	SV	BV	SV
Content of chlorophyll a	6,452	4,722	5,539	3,992	3,230	3,038	1,884	3,334	3,305	4,488

BV- healthy ; SV- infested

Variations of chlorophyll a content between healthy (uncolonized) and leaves colonized with *Chaitophorus leucomelas* as related to genotype were found. The highest content of chlorophyll a in uncolonized leaves was recorded in *Populus x euramericana* »Pannonia« (6,45 mg/g dry matter), while the lowest in *Populus deltoides* clone 182/81 (1,854 mg/g dry matter), which is in accordance with results of Orlović (1996).

In colonized leaves, content of chlorophyll a was the highest in *Populus deltoides* clone 88/82 (5,639 mg/g dry matter), and the lowerst in *Populus deltoides* clone 182/81 (2,586 mg/g dry matter).

Considering reaction of clones to the lice presence, surveyed clones could be seperated into two groups (Fig. 5). First group consists of clones which responded by chlorophyll a increase (clones PE 19/66, 54/76-2, 88/82, 182/81 and 265/81). The highest increase of chlorophyll a content was recorded in 182/81 clone (by 76.92%), while the lowest in PE 19/66 (5.99%). Another group consists of clones whose response to lice presence was mirrored in decrease of chlorophyll a content in leaves (clones 181/81, Pannonia, S_1-8, 88-22). The strongest response was found in clone 181/81 (decrease by 47,73%), while response of 88-22 clone was week, and its chlorophyll a content was lowered by 5.94%.

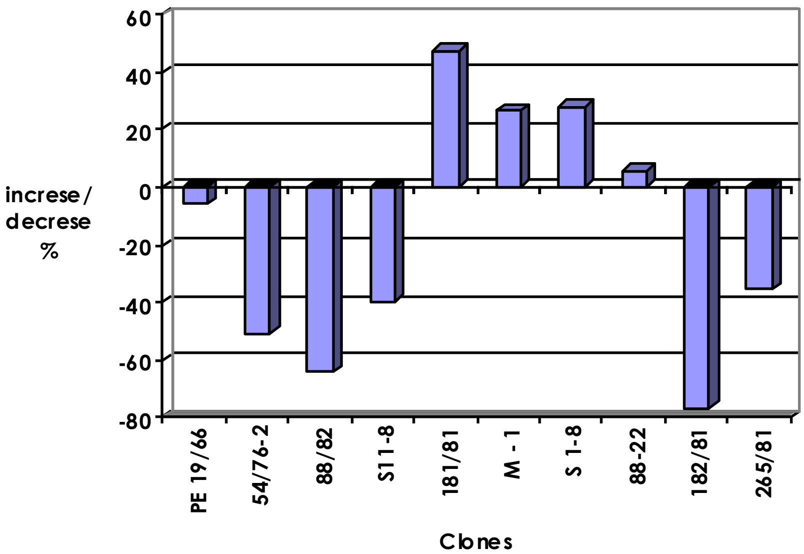

Figure 1. Ratio of chlorophyll a and content in uncolonized and colonized leaves of poplar clones

Content of chlorophyll b

Content of chlorophyll b, shown in Table 2, was the highest in uncolonized leaves of Pannonia clone (2.007 mg/g dry matter), while the lowest in 182/81 clone (0.54 mg/g dry matter). Considering values obtained for infested leaves, the highest chlorophyll b content was recorded in 88/82 clone, while the lowest in 88-82 (1.179 and 0.347 mg/g dry matter, respectively).

Table 2. Content of chlorophyll b in healthy and infested leaves (mg/g dry matter)

Clone	**PE 19/66**		**54/76-2**		**88/82**		**S_{11}-8**		**181/81**	
	BV	SV	BV	SV	BV	SV	BV	SV	BV	SV
Content of chlorophyll b	1.128	0.900	0.864	0.872	1.035	1.179	0.931	0.954	1.221	0.363
Clone	**Pannonia**		**S_1-8**		**88/22**		**182/81**		**265/81**	
	BV	SV	BV	SV	BV	SV	BV	SV	BV	SV
Content of chlorophyll b	2.007	0.886	1.473	0.757	0.786	0.347	0.547	0.656	0.884	0.711

BV- healthy ; SV- infested

Taking into consideration this parameter, clones were separated into two groups (Figure 2). The chlorophyll b increase was found in PE 19/66, 54/76-2, 88/82, S_{11}-8 and 182/81 clones, while reduction in 181/81, Pannonia, S_1-8, 88-22 and 265/81. The highest percentage of increase was observed in 182/81 (19,79), while the lowest in 54/76-2 (0,90). Chlorophyll b reduction was the most pronounced in 181/81 (70,24) while the least in 265/81 (19,58).

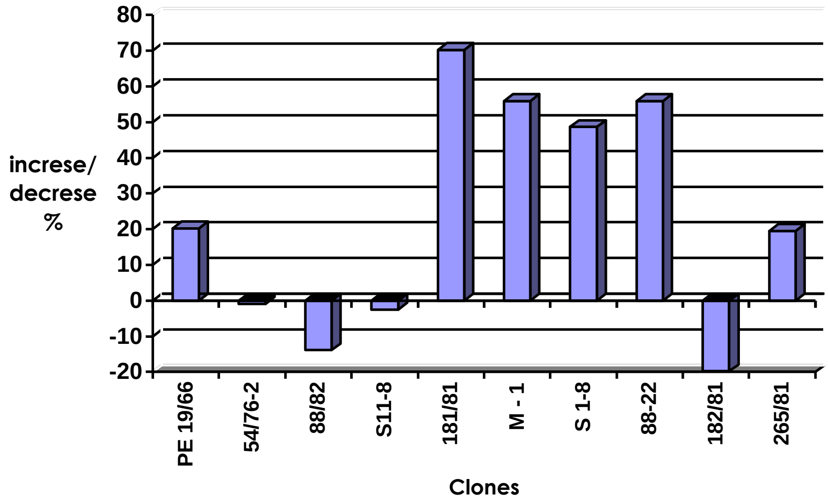

Figure 2. Ratio of chlorophyll b content in uncolonized and colonized leaves of poplar clones

Clorophyll a+b content

The highest chlorophyll a+b content of healthy leaves was found in clone Pannonia (8.459 mg/g dry matter) while the lowest in 182/81 (2,432 mg/g dry matter) (Table 3). The highest amounts of chlorophyll a+b in colonized leaves were recorded in 88/82 (6,514), while the lowest in 181/81 clone (2,43).

Table 3. Content of chlorophyll a+b in healthy and infested leaves (mg/g dry matter)

Clone	**PE 19/66**		**54/76-2**		**88/82**		**S_{11}-8**		**181/81**	
	BV	SV	BV	SV	BV	SV	BV	SV	BV	SV
Content of chlorophyll a+b	4.936	4.936	4.143	5.828	4.462	6.814	4.569	6.043	6.167	2.949
Clone	**Pannonia**		**S_1-8**		**88/22**		**182/81**		**265/81**	
	BV	SV	BV	SV	BV	SV	BV	SV	BV	SV
Content of chlorophyll a+b	8.459	5.608	7.012	4.749	4.016	3.385	2.432	3.989	4.189	5.199

BV- healthy ; SV- infested

Increase of chlorophyll a+b content was the response of 54/76-2, 88/82, S_{11}-8, 182/81 and 265/81 clones to *Chaitophorus leucomela* infection, while in 181/81, Pannonia, S_1-8 and 88-22 values were decreased. Reaction of clone PE 19-66 was not evident (Fig. 3), which could be explained by its lower adaptability (Orlović *et al.*, 1998).

The highest decrease of chlorophyll a+b content was recorded for 181/81 clone (52,18), while the lowest for 88-22 clone (15.70).

The percentage of increase was the highest in 182/81 (64,06), while the lowest in 265 (24,11).

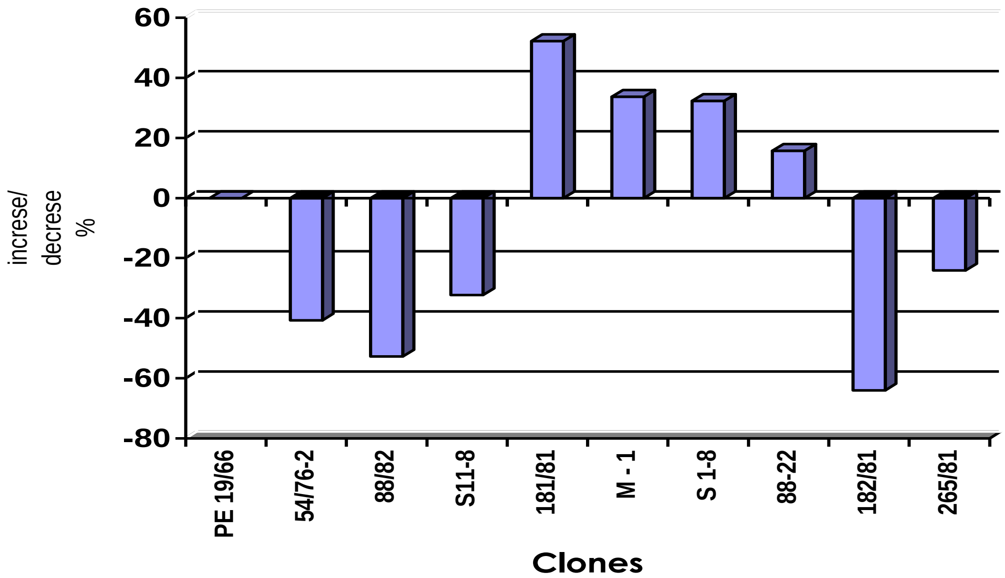

Figure 3. Ratio of chlorophyll a+b content in uncolonized and colonized leaves of poplar clones

Chlorophyll a/b ratio

Ratio of chlorophyll a and b contents varied due to certain clone and *Chaitophorus leucomelas* infection of leaves. According to literature data, this ratio ranges from 3:1 to 5:1 in uncolonized leaves (Orlović, 1996). In the present study, ratio of chlorophyll a and b contents varied from 4,11:1 (clone 88-22) to 3,21:1 (clone Pannonia). The highest value of colonized leaves was found in clone 88-22 (8.76:1), while the lowest in clone PE 19/66 (4.48:1) (Table 4).

Table 4. Ratio of chlorophyll a and b contents in healthy and infested leaves

	Clone	a/b healthy	a/b infested
1.	PE 19/66	3.374223	4.481953
2.	54/76-2	3.793034	5.68244
3.	88/82	3.309947	4.780982
4.	S11-8	3.90877	5.333555
5.	181/81	4.051645	7.1177
6.	Pannonia	3.215183	5.330388
7.	S 1-8	3.758929	5.275547
8.	88-22	4.111683	8.760277
9.	182/81	3.443427	5.085741
10.	265/81	3.736293	6.309221

Content of carotenoids (mg/g dry matter)

Contentof carotenoids is shown in Table 5. Variability of this parameter as related to surveyed clone and lice attack was found.

The highest amounts of this pigment in uninfested leaves were recorded for clone Pannonia (2.625), while the lowest for 88-22 clone. Concentration of carotenoids in colonized leaves was the highest in 54/76-2 clone (2.103), while the lowest in 182/81 (0.644).

Table 5. Content of carotenoids in healthy and infested leaves (mg/g dry matter)

Clone	PE 19/66		54/76-2		88/82		S_{11}-8		181/81	
	BV	SV	BV	SV	BV	SV	BV	SV	BV	SV
Content of carotenoids	1,916	1,457	1,620	2,103	1,492	2,003	1,718	1,828	2,029	1,439
Clone	**Pannonia**		**S_1-8**		**88-22**		**182/81**		**265/81**	
	BV	SV	BV	SV	BV	SV	BV	SV	BV	SV
Content of carotenoids	2,625	1,737	2,265	1,767	1,308	1,113	1,433	0,644	1,875	1,115

BV- healthy ; SV- infested

Ratio of carotenoids content in healthy and infested leaves varied in dependance on clone.

Group of clones, consisting of PE 19/66, 181/81, Pannonia, S_1-8, 88-22, 182/81 and 265/81, responded by reduction of carotenoids content between 55.06 and 14.92%, while in clones 54/76-2, 88/82 and S_{11}-8 values were increased. This increment was the highest in 88/82 clone (34.20%), while the lowest in S_{11}-8 clone (6,37%).

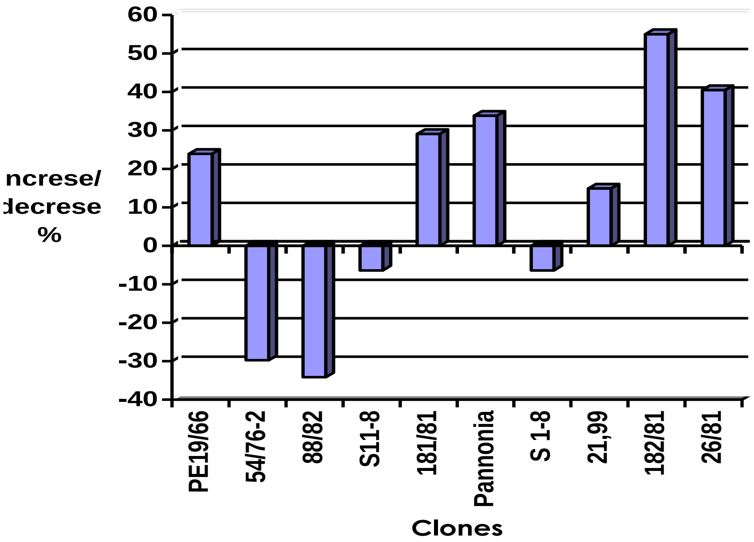

Figure 4. Ratio of carotenoids content in uncolonized and colonized leaves of poplar clones

CONCLUSIONS

According to available data, a small number of scientists have been studying effects of plant lice upon some basic physiological processes in plants. Mechanical injury of plant tissue occurs during the stylet penetration, as well as during the lice feeding, by the injection of their saliva. The aphids saliva contains phenol substances, plant hormones and enzymes, which affect plant metabolism (Miles, 1965). Plants respond to these damages by phenol accumulation, which is oxidised to quinones, which are known to be toxic (Miles, 1987). Surveyed physiological parameters represent important indicators of physiological condition of plants, being involved in complex photosynthetic process. Even the smallest change of some of the surveyed parameters could reduce, while in extreme situations stop photosynthetic process. Obtained results and values of studied physiological parameters of uninfected plants are in accordance with results of Orlović (10), and Ceulemans and coworkers (1991). Results obtained for some physiological parameters showed regularity regarding to plant reaction to plant lice attack. Divergences were recorded only for photosynthetic pigments content: cultivars were separated into two groups that exhibited different responses to the lice presence. Being the parameter characterizing plant water regime, stomatal diffusive resistance is influenced by numerous external factors, such as light intensity (1), number of stomata (2,6). The lice saliva is transferred throughout the vascular conduits of plants and affects all its parts. Miles (1987) reported that chloroplast degradation is one of the most frequent plant reactions to the lice feeding, which could be reflected in number of chlorotic spots on leaves. Investigation of *Pemphigus bursarius* and

Pemphigus phenax influences upon photosynthetic pigments content in *Populus nigra* "Italica" (3) revealed that their presence causes a high degree of reduction. In this work, the photosynthetic pigments content was lowered, which may be the consequence of the lice saliva presence. Our results are in agreement with the study of *Pemphigus bursarius* and *Pemphigus spyrotecae* effects upon photosynthesis, respiration and transpiration, published by (3,4,5). The lice influence on plants depends on cultivars, plant age and lice stage of development. Certainly, a great abundance of plant lice affects plant physiological status. Furthermore, it is known the smallest change of plant water regime results in stopping photosynthetic process. In infected plants, the rate of photosynthesis was decreased from 25 to 75 % in relation to lice species, cultivars and plant (leaf) age, which is in agreement with results of Miles (8). At the same time, respiration was increased by 15-56.77 % in infected plants. Stomatal diffusive resistance was greater in infected leaves, indicating that the presence of lice reduced stomatal conductivity and, by doing so, decreased other physiological processes. It may be supposed that changed values of surveyed physiological parameters also influenced growth parameters, as well as plant vigor.

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MIKROBIOLOGICAL ACTIVITY OF SOIL IN REGENERATION OF PEDUNCULATE OAK FOREST

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Abstract: *Soil is a complex and dynamic environment in which microflora and and microfauna coexist and form biocenosis. Favourable conditions of the soil enable microorganisms to survive and multiply in enormous quantities giving the soil characteristics of "living" environment. Since microorganisms play indispensable role in providing soil fertility, the aim of this paper was to investigate the number and dehydrogenase activity of soil microorganisms in regeneration in pedunculate oak forests. Results obtained by studying the number and enzimic activity of soil microorganisms in regeneration of pedunculate oak forests pointed out to the fact that the number of studied groups of microorganisms, as well as dehydrogenase activity of the soil depended on soil type and vegetation period.*
Key words: *microorganism, forest soil*

INTRODUCTION

Soil is a complex and dynamic environment in which microflora and microfauna coexist and form biocenosis. Favourable conditions of the soil enable microorganisms to survive and multiply in enormous quantities giving the soil characteristics of "living" environment.

Agricultural soil was formed and maintained by the conscious influence of man, as an important ecological factor. Man's activities directly alter soil ecological conditions which are greatly reflected in the activity of microorganisms (Jarak & Colo, 2007).

However, forest land is almost completely out of reach of man, or his influence is very little, and microbiological processes are undisturbed developed under given ecological conditions. These microbiological processes have great influence not only on nature of forest land, but also on cultivation, maintenance and nutrition of forest. What is distinguishing forest soils from agricultural soil is the soil coverage, which can not be found outside the forest. Forest coverage is very interesting for microbiologists to study it, and it is not only the "dead coverage" but also the extremely vivid and dynamic environment.

Microbiological processes have great influence not only on the nature of the forest soils, but also on cultivation, maintenance and nutrition of the forest. Under the influence of microbiological activity forest coverage turns into humus material, which is then transformed into plant assimilative used for forest plants nutrition (Tesic, 1968).

Forest areas in our country contain several species of oak trees, and the most widely spread, and the most valued are the pedunculate oak stands (*Quercus robur* L.). From the total area under oak forest in Serbia, less than half, but the most valuable are located in Srem, i.e. on the areas under the forest menagament of Sremska Mitrovica (Bobinac, 1999). Since micro-organisms have an indispensible role in providing soil fertility, the aim of this paper was to study the number and dehydrogenase activity of soil microorganisms in regeneration of pedunculate oak forests.

MATERIALS AND METHODS

Soil samples for microbiological analysis were taken during 2007 at the beginning of vegetation (in May), and in the middle of vegetation (in July). Total number of bacteria, participation of azotobacter, actinomycetes, fungi, and aminoheterotrophs was determined using method of dilution on appropriate nutritive media (Poshon & Tardieux, 1962), and dehydrogenase activity using Thalmann method (1968). Soils samples for microbilogy analysis were taken using shovel previously sterilized with ethanol at the depth of 0 - 30 cm. Samples were placed into sterilized plastic bags, kept at 4^{o}C (five days), and then needed laboratory analyses were done. Soils samples were taken on three localities in the region of lumber camp of Sremska Mitrovica. Trial area Varadin was located in the region of forest management Morovic, and trial areas Naclo in Vinichna in the region of forest management Visnjicevo.

RESULTS AND DISCUSSION

There are number of factors influencing microbiological and enzyme activity of soil. The greatest number and activity of microorganisms was found in the soil layer of up to 40 cm, where there were most organic matters, and enough moisture and oxygen (Jarak & Colo, 2007). Number of microorganisms in soil also changed depending on climatic conditions, and height above sea level. Ivanova *et al.*, (2006) and Lejon *et al.*, (2005) mentioned that micobiological activity in the forest soil depend of forest types and Highland (2001) mentioned that micobiological soil activity was also influenced by forest spacing, i.e. that number of fungi and bacteria, and enzimyc soil activity was higher in forests with dense spacing. Phisico-chemical soil characteristics were the most important trait influencing the activity of microorganisms (Govedarica *et al.*, 1993; Sessitsch *et al.*, 2001; Milosevic *et al.*, 2003). The most significant phisico-chemical characteristics of soil at depth of 0-30cm are given in table 1. Obtained results revealed that reaction of studied soils were acidic to slightly acidic. The lowest pH value was recorded in the locality of Varadin (pH u H_2O 5,24), and highest in the locality of Naklo (pH u H_2O 6,05). Studied soils differed in $CaCO_3$ content, and were well provided with humus. On the basis of total sand and clay content studied localities belonged to loamy soils according to textural class (Varadin and Vinichna), and locality of Naklo belonged to sand-loam textural class.

Table 1. Fundamental characteristics of soil

Localities	Total sand	Total clay	Soil type	pH u H_2O	$CaCO_3$ (%)	Humus (%)
Naklo	57.68	42.32	sand - clay	6.05	0.42	4.61
Varadin	37.56	62.44	clay	5.24	0.84	6.21
Vinichna	46.44	53.56	clay	5.74	2.09	6.26

Number of bacteria in these studies was great and there were millions of them in 1 g of soil (table. 2). Number of bacteria was even greater in the middle of vegetation period in all three localities. In that period, during July plants were in the stage of intensive growth and development, the quantity of root exudates was increased, which favorized the increase of bacterial number (Jarak & Colo, 2007). The basic significance of bacteria in the soil was reflected in the increased synthesis and decomposition of organic matter providing plant assimilatives (Jarak & Govedarica, 1995).

Table 2. Total number of bacteria 10^6 ha^{-1}

Localities	Biginning of vegetation	Middle of vegetation
Naklo	10.2569	46.7900
Varadin	23.0167	136. 4244
Vinichna	6.1399	78.4307

Azotobacter is a specific family of microorganisms which is able to reduce elementary nitrogen transferring it into form of ammonia. It belongs to a group of free azotofixators, and can be found in the soil, rhizosphere, or on the surface of the root. Although azotobacter exists freely in the soil, its number is higher in plant rhizosphere, where there is higher concentration of organic matters which plants intake through the root (Jarak & Colo, 2007). In tested soil samples azotobacter was present only in locality Naklo at the beginning of vegetation (table 3), which can be explained by acidic reaction of the soil (table 1). The pH value of the soil in locality Naklo was one of the reasons why azotobacter was present only in this locality.

Table 3. Abundance of azotobacters 10^1 ha^{-1}

Localities	Biginning of vegetation	Middle of vegetation
Naklo	55.8422	-
Varadin	-	-
Vinichna	-	-

Species of azotobacter family were numerous in soils with greater quantity of easily degradable carbon hydrates. Azotobacter was sensitive to acidic reaction of surrounding (so its number and activity was greatest in the neutral soils and ranged from several hundreds to several millions in 1 g of soil). Azotobacter was present in significantly greater number in agricultural soil, but the same could not be said for the forest soils. Although azotobacter was also present in the forest soil, its activity was significantly lower due to unfavourable i.e. acidic reaction of the environment (Tesic, 1968).

Table 4. Abundance of actinomycetes 10^4 ha^{-1}

Localities	Biginning of vegetation	Middle of vegetation
Naklo	3.3030	14.3403
Varadin	9.8395	29.1349
Vinichna	1.2171	8.9988

Actinomycetes are heterotrophic radial bacteria which run the process of humification and mineralization of organic matter in the soil. They decompose the most resistant humus components, and in that way form assimilative for plants (Jarak & Colo, 2007). In studied samples of forest soils there were tens of millions of actinomycetes in 1 g of soil (table 4), but that number was significantly lower in comparison to agricultural soil (Marinkovic *et al.*, 2007). Number of actinomycetes in studied localities was greater in the middle of vegetation period.

Results obtained by testing the number of fungi in the samples of forest soil showed that it was greater in the middle of vegetation period, i.e. in July (table 5). Number of fungi was greater in the middle of vegetation due to greater content of organic matters originating from lysised root residues, microbiological residues (Jarak & Govedarica, 1995).

Table 5. Abundance of fungi 10^4 ha^{-1}

Localities	Biginning of vegetation	Middle of vegetation
Naklo	1.0205	5.6311
Varadin	17.3170	41.5232
Vinichna	14.4662	17.5968

Number of aminoheterothrops at the beginning of vegetation period was the greatest in the locality of Varadin, while this number was significantly decreased in the middle of vegetation period. Number of aminoheterothrops in localities of Naklo and Vinichna increased in the middle of vegetation period (table 6). Such different number of aminoheterothrops in studied localities was the result of difference in the quantity of easily degradable proteins, but also of the participation of individual groups of microorganisms participating in the process of amonification. Number of bacteria and fungi in the locality Naklo was greater in relation to other two localities at the beginning of vegetation period (table 2, 5).

Table 6. Abundance of aminoheterotrophs 10^6 ha^{-1}

Localities	Biginning of vegetation	Middle of vegetation
Naklo	17.0393	22.7650
Varadin	109.0569	1.1097
Vinichna	22.5734	75.7827

Besides testing numerous microorganisms in forest soil, these investigations were also aimed at testing enzymatic i.e. dehydrogenase activity of soils in chosen localities. Since dehydrogenase is the constitutive enzymes of all microorganisms, the estimation of general microbiological soil activity can be given on the basis of dehydrogenase activity (Milosevic *et al.*, 1993). In these studies the greatest activity was noticed at the beginning of vegetation period in the locality of Naklo, and it was higher in the middle of vegetation period in localities of Varadin and Vinichna (table 7).

Enzymatic soil activity was in correlation with number of microorganisms, however on nutritive media only small number of micro-organisms population present in the soil was registered, so the image obtained under laboratory conditions sometimes deviates from the expected results. It can be seen from the example of locality Naklo where the number of micro-organisms was not the greatest at the beginning of vegetation period, while dehydrogenase activity in this locality was the highest.

Table 7. Dehydrogenase activity (μg 10 TPF g-1 soil)

Localities	Biginning of vegetation	Middle of vegetation
Naklo	938	530
Varadin	99	351
Vinichna	96	246

CONCLUSIONS

Results obtained by studying participation and dehydrogenase activity of micro-organisms in the soil in regeneration of pedunculate oak forests showed that number of studied groups of micro-organisms and dehydrogenase activity depended on type of soil and period of vegetation.

Total number of micro-organisms, bacteria, fungi and actinomycetes was higher in the middle of vegetation period in all three localities due to increased content of organic matter and root exudates.

Number of aminoheterotrophs was higher at the beginning of vegetation period in locality of Varadin, while in localities Naklo and Vinichma it was higher in the middle of vegetation period.

Azotobacter was registered only in locality Naklo at the beginning of vegetation, which can be explained by acidic reaction of soil.

Dehydrogenase activity was higher at the beginning of vegetation in locality Naklo, while in localities Varadin and Vinichna it was higher in the middle of vegetation period.

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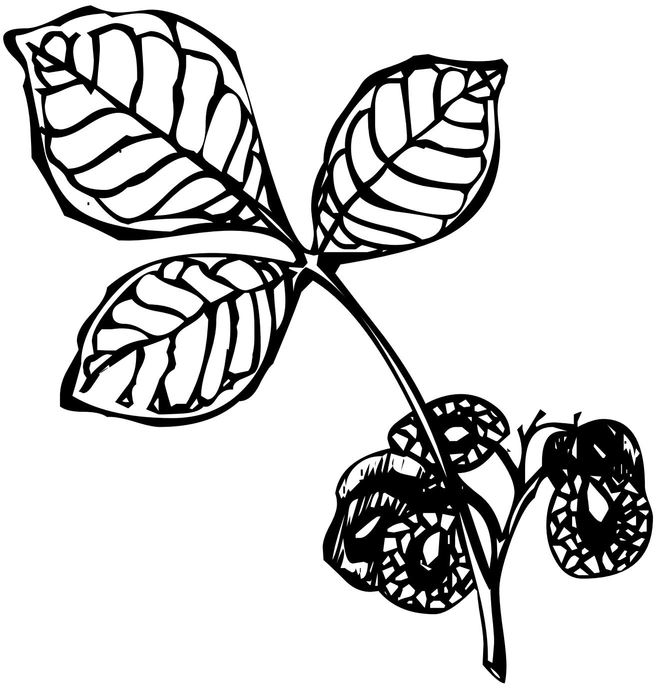

THE INFLUENCE OF NICKEL, CADMIUM AND LEAD ON THE GROWTH OF THE WHITE POPLAR CLONES' SHOOTS *IN VITRO*

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Summary: *Heavy metals pollution has become serious environmental problem. Since heavy metals can not be degraded, they should be extracted from contaminated sites. Poplars are used very often for this purpose for these tree species are successful in heavy metals phytoremediation of contaminated areas.*
This research deals with the effect of particular heavy metal (nickel, cadmium and lead) on some growth parameters and development of four clones from section Populus cultivated in vitro. Shoots of white poplar (Populus alba L.) clones L-12, L-80, L-111/81 and LBM were grown on solid ACM medium with 20 mg/l adenine-sulphate, 100 mg/l myo-inozitole, 0.5 mg/l benzylaminopurine (BAP), 0.02 mg/l α-naphthaleneacetic acid (NAA). The heavy metal was added in different concentrations: 0, 0.1 mM and 0.5 mM. Effects of each heavy metal on growth parameters: height of shoots, biomass and multiplication were investigated compared to control. Also, differences among clones in their responses to heavy metals are analysed.
In the treatment with lower concentration cadmium increased and nickel decreased biomass of shoots. In higher concentration both heavy metals decreased biomass and height of investigated clones' shoots compared to control. In both concentrations lead decreased height, but biomass decrease of clones' shoots was not statistically significant. Investigated clones differed among themselves in their reactions on heavy metals presence. Clone L-111/81 was the most successful in heavy metals tolerance and this clone could be used for phytoremediation of soils contaminated with lower concentration of nickel, cadmium or lead. On the other hand, clone LBM could be used on sites contaminated with higher concentration of these heavy metals.
Key words: *Poplars, phytoremediation, heavy metals, clone, in vitro*

INTRODUCTION

Pollution with heavy metals becomes one of the most serious environmental problems nowadays. Most of lead and cadmium contamination results from anthropogenic activities: burning liquid and solid fuels, smelting and foundry works, sewage high in Pb and Cd and soil-applied chemicals, including fertilizers (Seregin & Ivanov, 2001).

Toxic effect of heavy metals may result from its binding to SH groups in proteins, leading to inhibition of activity or disruption of structure or from displacement of an essential element, resulting in deficiency effects (van Assche & Clijsters, 1990). Most enzyme activities decline when they are affected by lead and cadmium. This fact is crucial for understanding the inhibitory effects of these metals on diverse aspects of cell metabolism, mineral nutrition, water regime, respiration etc. The decline of photosynthetic rates results from the distorted chloroplast ultra structure, the restrained synthesis of photosynthetic pigments and the obstructed electron transport. The effect of same concentration of particular metal on chlorophyll content varies among different plant species. Growth inhibition by heavy metals results from metabolic disorders and direct effect on growth, e. g. due to the interactions with cell wall polysaccharides decreasing cell wall plasticity. It is noted, however, that in low concentrations lead promote the growth of root systems (Seregin & Ivanov, 2001).

High quantities of nickel are released to the environment, as well. This is conditioned by the development of industry, activities in mines, smelters, production of alloys, use of fertilisers, pesticides, as well as by dumping the destructive waste (Alloway, 1990).

Nickel holds a special place among the heavy metals. This metal is an essential, biogenic element, necessary for plant life. Its concentration in dry plant mass is about 0.001μmol/g and hence it is classified as microelement (Epstein, 1972). Nickel is considered an essential element primarily because of its function as an irreplaceable component of urease which is responsible for the hydrolysis of urea obtained by the metabolism of amino acids and nucleotides (Gerandas *et al.*, 1999; Seregin & Kozhevnikova, 2006). It was found that in tomato plants cultivated in tissue culture, urease was not active in the substrate without nickel, whereas the addition of nickel activated this enzyme. Also, the level of urease activity depended on the dose of nickel and it was reduced with the addition of cobalt (Witte *et al*, 2002).

In very low concentrations, nickel can have a positive effect on the growth of some species, such as wheat, cotton, tomato, pepper and potato (Mishra & Kar, 1974). Zornoza *et al.* (1999) reported that, in a test with sunflower in water culture, the simultaneous supply of plants with ions NO^{3-} and NH^{4+} not only reduces nickel toxicity, but also the growth is stimulated by nickel if the plants are supplied with ions NO^{3-} and NH^{4+}. Exceedingly low levels of $NiSO_4$ (10^{-4} M) strongly stimulate urea-supported growth in suspension cultures (Polacco, 1977).

High concentrations of Ni^{2+} in growing medium may turn toxic to plants. Visual symptoms of nickel toxicity are foliar necrosis. Excess Ni^{2+} affects mineral nutrition, as well as photosynthesis and respiration (Carlson, 1975).

Heavy metals can not be degraded, and thus should be extracted from contaminated areas. In the last decade of 20th century in the USA were conducted researches on plant-affection to the sites contaminated by heavy metals (EPA, 2001; Barcelo & Poschenrieder, 2003; Ghosh & Singh, 2005). Researches showed that plants are able to decrease concentration of various contaminants in soil, surface and groundwater. Technology that uses various plant species in these purposes is called phytoremediation.

Poplars are very often used in phytoremediation due to their characteristics of growth vigour. They tolerate low fertility soils, have well developed root system which can reach the ground water and they can transpire high quantities of water (Aitchison *et al.*, 2000). They can, directly or indirectly, phytoremediate several types of pollutants in several ways, such as: phyto-extraction, phyto-degradation, phyto-volatilisation and rhizo-degradation (Pilipović *et al.*, 2002; Kališova - Špirochova *et al.*, 2003; Bojarczuk, 2004; Pilipović *et al.*, 2005, 2006).

The aim of this study is to determine the effect of nickel, cadmium and lead on the growth of four white poplar clones' shoots in tissue culture, depending on the applied concentration of particular heavy metal in the growth medium.

MATERIAL AND METHODS

Four white poplar clones: L-12, L-80, L-111/81 and LBM (section *Populus*) were selected for the test because their growth vigour in tissue culture has already been proved (Guzina & Tomović, 1989; Kovačević *et al.*, 2005). Axillary buds from the trees of different ages in the dormant period were placed in tissue culture. Shoots were multiplied on Aspen Culture Medium (ACM) (Ahuja, 1984; Kolevska-Pletikapić & Tomović, 1988) with the addition of 20 mg/l adenine-sulphate, 100 mg/l myo-inozitole, 0.5 mg/l benzylaminopurine (BAP), 0.02 mg/l α-naphthaleneacetic acid (NAA). The cultures were kept at 26±2 °C in the white fluorescent light with a 16 hour photoperiod. Terminal shoots were cultivated on the same medium with addition of heavy metal in different concentrations: 0, 0.1 mM and 0.5 mM. For each heavy metal separate experiment was set up. There were five jars per treatment with five microshoots planted. Experiment with cadmium lasted 30 days. Heavy metal was used in the form of cadmium chloride monohydrate. Nickel was added to the medium in the form of nickel sulphate hexahydrate and cultivation lasted 28 days. Lead was added in the form of Pb EDTA and *in vitro* growth lasted 35 days. Effects of each heavy metal on growth parameters: height of shoots, biomass and multiplication (number of axillary shoots per explants) are investigated compared to control. Statistical analysis was based on jar average value using Duncan's multiple range test.

RESULTS AND DISCUSSION

The influence of nickel, cadmium and lead on height of poplar clone shoots is shown in Table 1. It can be seen that clones L-12 and L-111/81 achieved statistically significant increase of height in the treatment with 0.1 mM of nickel. In the same concentration lead significantly decreased height of shoots compared to control in general and in clones L-80 and L-12. However, effect of cadmium was not statistically significant.

In higher concentration all investigated heavy metals had inhibitory effects on shoots growth, which resulted in achieved lower height compared to control.

It can be noticed that in lower concentration nickel had stimulatory effect on shoots growth, but in higher concentration it was even more toxic to shoots of investigated clones compared to two other heavy metals. Based on achieved results, it can be concluded that effect of particular heavy metal on the same plant species could vary in accordance to its concentration.

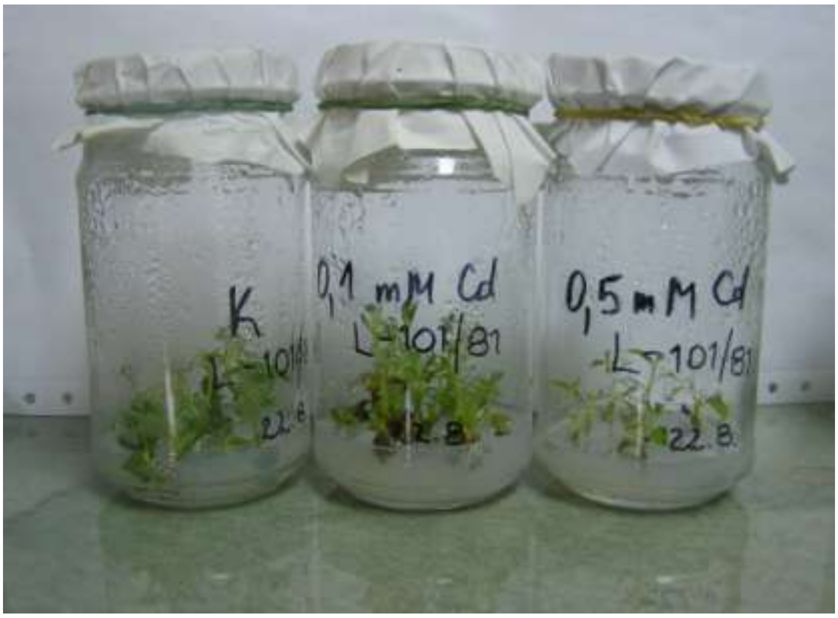

Figure 1. Shoots of clone L-111/81 grown on different concentrations of cadmium

Applied in lower concentration, heavy metals had different effect on biomass of investigated clones of white poplar. Nickel had inhibitory effect on shoots biomass accumulation in total and in all investigated clones, except in clone L-111/81. In this clone the effect on biomass was positive, but not statistically significant. In concentration of 0.1 mM cadmium increased biomass of clones shoots in general and especially in clone L-111/81 (Figure 1). Effect of lead in both investigated concentrations, on biomass accumulation was not significant. On the other hand, in higher concentration nickel and cadmium significantly decreased shoots biomass. This indicates that in concentration of 0.5 mM these two heavy metals were more toxic than lead (Table 2).

The influence of different concentration of nickel, cadmium and lead on shoots multiplication is presented in Table 3. It can be seen that nickel in both applied concentrations inhibited multiplication of shoots and it was especially expressed in the treatment with higher concentration where the number of axillary shoots decreased for 99% compared to control. In lower concentration effect of cadmium on shoots multiplication was not statistically significant. On the other hand, in the treatment with higher concentration of cadmium number of axillary shoots significantly decreased compared to control. Also, lead in lower concentration had no influence on multiplication, but in higher concentration decrease was statistically significant. Compared to other two heavy metals, according to the effect on investigated parameter, nickel was the most toxic in both treatments. The lowest toxicity achieved lead and that is in accordance with results of Bojarczuk (2004) who found significant decrease of poplar clones grown *in vitro* in concentration of 2 mM Pb^{2+} .

Table 1. Influence of different concentrations of investigated heavy metals on height of shoots (mm) of poplar clones

Nickel					
Clone	K	0.1 mM		0.5 mM	
L-12	37.440 cd	60.000 a	(60.26 %)[1)]	22.720 ef	(-39.32 %)
L-80	51.960 ab	37.160 cd	(-28.48 %)	19.823 f	(-61.85 %)
L-111/81	33.760 de	47.480 bc	(40.64 %)	18.960 f	(-43.84 %)
LBM	26.360 def	26.140 def	(-0.83 %)	18.160 f	(-31.11 %)
Average	37.380 a	42.695 a	(14.22 %)	19.916 b	(-46.72%)

Cadmium					
Clone	K	0.1		0.5	
L-12	32.320 abc	36.280 ab	(12.25 %)	24.520 cd	(-24.13 %)
L-80	41.480 a	38.200 a	(-7.91 %)	22.840 cd	(-44.94 %)
L-111/81	32.480 abc	37.760 ab	(16.26 %)	23.240 cd	(-28.45 %)
LBM	26.280 bcd	32.160 abc	(22.37 %)	20.440 d	(-22.22 %)
Average	33.140 a	36.100 a	(8.93 %)	22.760 b	(-31.32 %)

Lead						
Clone	K	0.1 mM		K	0.5 mM	
L-12	67.68 a	57.27 b	(-15.38 %)	32.12a	22.36 bc	(-30.39 %)
L-80	44.6 c	33.7 d	(-24.44 %)	27.04 ab	15.44 de	(-42.90 %)
L-111/81	25.88 de	21.96 e	(-15.15 %)	18.76 cd	14.40 de	(-23.24 %)
LBM	32.32 de	27.6 de	(-14.60 %)	26.00 ab	16.84 cde	(-35.23 %)
Average	42.62 a	35.13 b	(-17.57 %)	25.98 a	17.26 b	(-33.56 %)

1) – values in brackets represent relative difference from control (((treatment value-controle value)/controle value)*100%)

Table 2. Influence of different concentrations of investigated heavy metals on biomass of shoots (g) of poplar clones

Nickel					
Clone	K	0.1 mM		0.5 mM	
L-12	0.381 b	0.235 cd	(-38.32 %)1)	0.073 e	(-80,84%)
L-80	0.490 a	0.203 d	(-58.57 %)	0.089 e	(-81.84 %)
L-111/81	0.294 bc	0.323 bc	(9.86 %)	0.065 e	(-77.89 %)
LBM	0.201 d	0.077 e	(-61.69 %)	0.047 e	(-76.61 %)
Average	0.341 a	0.210 b	(-38.41 %)	0.069 c	(-79.76 %)

Cadmium					
Clone	K	0.1		0.5	
L-12	0.217 cde	0.329 abc	(51.52 %)	0.098 ef	(-54.83 %)
L-80	0.238 bcd	0.341 ab	(43.28 %)	0.103 ef	(-56.72 %)
L-111/81	0.205 de	0.385 a	(87.80 %)	0.075 f	(-63.41 %)
LBM	0.147 def	0.226 bcd	(53.74 %)	0.052 f	(-64.62 %)
Average	0.201 b	0.320 a	(59.2 %)	0.082 c	(-59.2 %)

Lead						
Clone	K	0.1 mM		K	0.5 mM	
L-12	0.628 a	0.498 ab	(-20.70 %)	0.198 ab	0.130 bc	(-34.34 %)
L-80	0.476 b	0.312 cde	(-34.45 %)	0.273 a	0.206 ab	(-24.54 %)
L-111/81	0.088 g	0.118 fg	(34.09 %)	0.054 cd	0.029 d	(-46.30 %)
LBM	0.176 efg	0.146 fg	(-17.04 %)	0.101 cd	0.083 cd	(-17.82 %)
Average	0.342 a	0.268 ab	(-21.64 %)	0.157 a	0.112 ab	(-28.66 %)

1) – values in brackets represent relative difference from control (((treatment value-controle value)/controle value)*100%)

Positive effect of heavy metals lower concentration recorded on poplar shoots' growth is in the accordance with results of researches conducted on wheat, cotton, tomato, pepper and potato that were treated with nickel (Mishra & Kar, 1974). Similar results were achieved by Zornoza *et al.* (1999) who treated sunflower in water culture with nickel. Also, Kališova-Špirochova *et al.* (2003) recorded biomass increase in poplar grown *in vitro* with addition of lead in low concentration. It was proved, as well, that in low concentration lead has stimulative effect on root growth (Seregin & Ivanov, 2001). In low doses, also cadmium can stimulate root growth in *Allium sativum* (Liu and Kottke, 2003).

Inhibitory effect of heavy metals on plant growth was proved by numerous researches. Pilipović *et al.* (2006) cultivated three poplar clones in water cultures and treated them with 0, 10 and 100 ppm of nickel, cadmium or lead. Presence of every particular heavy metal in medium caused stagnation of biomass accumulation in all investigated clones.

Table 3. Influence of different concentrations of investigated heavy metals on multiplication of shoots of poplar clones

Nickel					
Clone	K	0.1 mM		0.5 mM	
L-12	5.720 a	2.280 c	(-60.14 %)[1)]	0.04 d	(-99.30 %)
L-80	4.320 b	0.200 d	(-95.37 %)	0 d	(-100.00 %)
L-111/81	5.200 ab	1.560 c	(-70.00 %)	0.04 d	(-99.23 %)
LBM	4.360 b	0.520 d	(-88.07 %)	0 d	(-100.00 %)
Average	4.900a	1.140 b	(-78.39 %)	0.02 c	(-99.63 %)
Cadmium					
Clone	K	0.1		0.5	
L-12	4.04 ab	3.400 abc	(-15.84 %)	0.840 de	(-79.21 %)
L-80	3.200 abc	2.760 bc	(-13.75 %)	0.280 e	(-91.25 %)
L-111/81	3.880 ab	4.640 a	(19.59 %)	0.840 de	(-78.35 %)
LBM	2.040 cd	2.640 bc	(29.41 %)	0.120 e	(-94.11 %)
Average	3.290 a	3.360 a	(2.12 %)	0.520 b	(-84.19 %)

Lead						
Clone	K	0.1 mM		K	0.5 mM	
L-12	5.52 ab	5.8 ab	(5.07 %)	3.49 a	0.64 cd	(-81.66 %)
L-80	3.65 c	3.3 c	(-9.59 %)	2.92 ab	2.6 ab	(-10.96 %)
L-111/81	3.6 c	4.12 bc	(14.44 %)	2.95 ab	0.92 cd	(-68.81 %)
LBM	4.52 bc	3.12 c	(-30.97 %)	2.6 ab	1.29 bcd	(-50.38 %)
Average	4.32 a	4.09 a	(-5.32%)	2.99 a	1.36 b	(-54.43 %)

1) – values in brackets represent relative difference from control (((treatment value-controle value)/controle value) *100%)

Investigated clones differed in their response to heavy metals. In comparison to other clones, in clone L-111/81 were recorded the best growth parameters: height, biomass and multiplication in the treatment with 0, 1 mM of all investigated heavy metals. In this clone, not only that measured parameters were not significantly decreased compared to control, but stimulative effects were measured. Clone LBM showed significant level of tolerance to investigated heavy metals in concentration of 0, 5 mM.

CONCLUSIONS

All three heavy metals, in the concentration of 0.5 mM, mainly decreased height, biomass and multiplication of all investigated clones compared to control.

Applied in concentration of 0, 1 mM, heavy metals had different effects on shoots of investigated clones of white poplars: cadmium generally increased shoots' biomass, nickel decreased, and effect of lead on shoots biomass was not statistically significant.

In concentration of 0, 1 mM nickel had inhibitory effect on multiplication of shoots, but effects of cadmium and lead in the same concentration were not statistically significant.

Investigated clones differed among themselves in their responses to heavy metals presence. Clone L-111/81 had the best growth parameters: height, biomass and multiplication in the treatment with lower concentration of all investigated heavy metals compared to other clones.

Clone L-111/81 could be a good candidate for phytoremediation of soils contaminated with lower concentration of nickel, cadmium or lead, while clone LBM could be used on sites with higher concentration of these heavy metals.

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DAMAGE CAUSED BY RED DEER (*Cervus elaphus*) ON YOUNG POPLAR PLANTATIONS

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Summary: *The aim of this study was to determine the main causes and incidence of damage caused by red deer on young poplar plantations within fenced part of the hunting ground „Podunavsko lovište Plavna“. Within 46 rows on three young poplar plantations (sites A, B, C) 2294 stems were examined in autumn 2007. Besides, 2773 newly planted stems have been measured in spring 2008, whereof 477 stems were planted in the place of damaged ones within the site A. The extent of damage among three young poplar plantations ranged between 10.5% (site C) and 73.1% (site A) of damaged stems. The most frequent types of damage were stem breaking (18.4%, 421 stems), browsing (8.8%, 201 stems), bark stripping (1.6%, 36 stems) and other types of damage - insects or fungi (6.5%, 149 stems). Undamaged stems at all young poplar plantations had a significantly larger median DBH than the damaged stems. Our results suggest that the main cause of damage to young poplar plantations lies in the high population density of red deer (29.3–32.3 individuals / 100 ha, 2005 and 2006, respectively) and that larger stems are far less damaged by red deer.*
Key words: *poplar, Cervus elaphus, damage, Vojvodina*

INTRODUCTION

Poplars (*Populus sp.*) are widely used in North America and Europe (eg France, Italy, Hungary, Croatia, Serbia). Poplar cultivation practices vary worldwide according to different needs, tradition, climate, plant material, available sites, and current economy and legislation in a particular country or region (Karačić, 2005). Interest in growing poplars has fluctuated, and objectives have shifted between producing saw logs, pulpwood, or more densely spaced „woodgrass“ or biofuels (Stanturf et *al.*, 2001). In recent years, most poplar plantations are established for biofuels (heat, ethanol, hydrogen gas, dimethylether, electricity) and pulpwood production in short-rotation forestry, but interest in sawlog production and environmental services is increasing (Mitchell et *al.*, 1999; Stanturf et *al.*, 2001; Kauter et *al.*, 2003; Weih et *al.*, 2003; Christersson, 2008; Danilović, 2008).

The growth and productivity of poplar plantations can be influenced by many factors: species or clone, site quality, climate, nutrient and water availability, plant material, type and intensity of silvicultural system, and the efficiency of pest and disease control (Stanturf et *al.*, 2001; Karačić, 2005). However, many studies have reported that large herbivores (eg red deer, white-tailed deer and moose) may have a profound effect on forest and forest plantations by grazing, browsing, trampling, rooting and bark stripping (Netzer, 1984; Putman & Moore, 1998; Bergqvist et *al.*, 2001; Heroldová et *al.*, 2003; Gačić et *al.*, 2006; Gill, 2006). Damage by red deer may consist of pulling-out newly planted trees, browsing the leading shoots of young trees and stripping the bark from older trees, while fraying, rubbing and trashing with antlers seldom causes significant damage (Ratcliffe, 1987). Therefore, the aim of this study was to determine the main causes and incidence of damage caused by red deer on young poplar plantations within fenced part of the hunting ground „Podunavsko lovište Plavna“.

MATERIALS AND METHODS

The study was conducted within fenced part (ca. 630 ha) of the hunting ground „Podunavsko lovište Plavna“ (ca. 2619 ha), situated in the western part of Vojvodina (region Bačka) along the left bank of the Danube River (45°17’–45°30’N, 16°40’–16°52’E). The relief of the study area is lowland (80–87 m a.s.l.), the climate is moderately continental with clearly distinguished cold and warm seasons. Mean annual temperature is 10.9°C (varying from 21.4°C in July to −1.4°C in January), mean annual precipitation is 601 mm, of which 55% occurs during the vegetation period.

The studied poplar plants were set up by means of the technology of shallow planting in the holes having depth of 80 cm, with the previous ground preparation which comprised the removal of forest waste and looping after the cutting down and lumbering, but without splintering of tree stumps and surface land cultivation. At two sites (A and B) the planting was carried out in December and January, and the clone M1 was used. The age of the planting material was 1/2, while the planting interval equaled 6×6 m. In 2007, considering the care and protection measures, the chemical protection from defoliates and the treating weed by means of herbicides based on glyphosate had been implemented. However, the care and protection measures for the planting were not being implemented during 2008.

Within 46 rows on three young poplar plantations (sites A , B, C) 2294 stems were examined in autumn 2007. The data were collected for the following indexes: diameter at breast height (DBH) of the accuracy up to 0.1 mm, the description of damage (type, time of appearing and distance from the surface of the ground) and the description of individual protection (sort of wire and its condition). Many stems were broken in two localities (A and B) so their DBH could not be measured, and due to this, the sample for the statistical analyses includes data for 1971 stems (See: Table 1). Besides, 2773 newly planted stems have been measured (both height and DBH of a stem were included), in spring 2008, whereof 477 stems were planted in the place of damaged ones within the site A.

Prior to statistical analyses, all DBH data were tested for normality. Stem DBH data were not normally distributed, due to the appropriate non-parametric statistical test was used. Therefore, we compared the medians of the DBH of damaged and undamaged stems using a Kruskal-Wallis one-way ANOVA. The results were considered significant at $p \leq 0.05$ level. The relationship between height and the DBH was derived by the methods of regression and correlation analysis. All calculations were performed by means of the program STATGRAPHICS Plus 5.1 for Windows.

RESULTS

The extent of damage among three young poplar plantations ranged between 10.5% (site C) and 73.1% (site A) of damaged stems (Table 1). The most frequent types of damage were stem breaking (18.4%, 421 stems), stem browsing (8.8%, 201 stems), bark stripping (1.6%, 36 stems) and other types of damage – insects or fungi (6.5%, 149 stems). The medians of the DBH for all damaged and undamaged poplar stems were significantly different (Kruskal-Wallis statistic = 378.8; $p < 0.05$). Undamaged poplar stems at all young poplar plantations had a significantly larger median DBH than damaged poplar stems (site A: Kruskal-Wallis statistic = 16.0; site B: Kruskal-Wallis statistic = 48.7; site C: Kruskal-Wallis statistic = 77.7; $p < 0.05$). The average DBH of damaged poplar stems had small variation on site A and B (16.0 mm and 17.3 mm, respectively), while on site C it was 44.9 mm (Fig. 1) which may be explained by longer age of the plantation (4 years). There were obvious differences in type of damage between sites - stem breaking and stem browsing were dominant on site A and B, while bark stripping was dominant on site C.

The unbroken stems on both sites had a larger mean DBH than broken stems: site A (17.4 and 16.0 mm, respectively) and site B (19.4 and 17.2 mm, respectively). The stem height at the point of breakage was between 0 and 230 cm (site A: mean = 83.8; n = 105), and between 0 and 250 cm (site B: mean = 142.0; n = 113). The mean DBH of the browsing stems was 15.8 mm on site A (range = 9.0 to 21.0), and 17.1 mm on site B (range = 12.0 to 21.0). The stem height at the point of browsing was between 125 and 220 cm (site A: mean = 191.1; n = 52), and between 130 and 240 cm (site B: mean = 191.5; n = 55). Bark stripping damage by deer is concentrated at the base of the poplar. Out of 29 stems for which this measure was recorded (site C), 75.9% had bark stripping occurring between 10 and 150 cm. The mean DBH of bark stripped stems was 67.0 mm (range = 26.0 to 118.0) and stems which had no bark stripping damage had a mean DBH of 63.0 mm (range = 22.0 to 121.0). Bark stripping could reach up to 1.9 meters but this was not common.

Additionally, 2773 newly planted stems on two locations were measured in spring 2008, whereof 477 stems were planted in the place of damaged ones within the site A. There was a statistically significant positive relationship between height and DBH in young poplar plantations ($F_{1,2668}$ = 11436.2; $p < 0.01$; r = 0.90) (Fig. 2).

Table 1. Damage caused by red deer on young poplar plantations in fenced part of the hunting ground „Podunavsko lovište Plavna“ (2007)

Diameter - DBH (*mm*)	Total stem (*n*)	Incidence and types of damage (*n*)			
		Stem breaking	Stem browsing	Bark stripping	Other type of damage
site A					
≤ 10	20	5	2	-	3
11-15	96	38	19	-	12
16-20	189	49	30	2	16
21-25	44	13	1	-	4
≥ 26	1	-	-	-	-
without DBH	229	124	86	-	19
Total	*579*	*229*	*138*	*2*	*54*
site B					
≤ 10	4	2	-	-	1
11-15	110	30	14	2	5
16-20	348	68	39	2	8
21-25	154	12	2	1	4
≥ 26	18	1	-	-	1
without DBH	88	72	8	-	8
Total	*722*	*185*	*63*	*5*	*27*
site C					
≤ 20	10	-	-	-	10
21-30	27	2	-	1	14
31-40	90	1	-	4	21
41-50	150	-	-	3	10
51-60	196	-	-	4	9
61-70	222	-	-	5	1
71-80	167	-	-	3	-
81-90	80	-	-	5	-
≥ 90	45	-	-	4	1
without DBH	6	4	-	-	2
Total	*993*	*7*	*-*	*29*	*68*
Total	**2294**	**421**	**201**	**36**	**149**

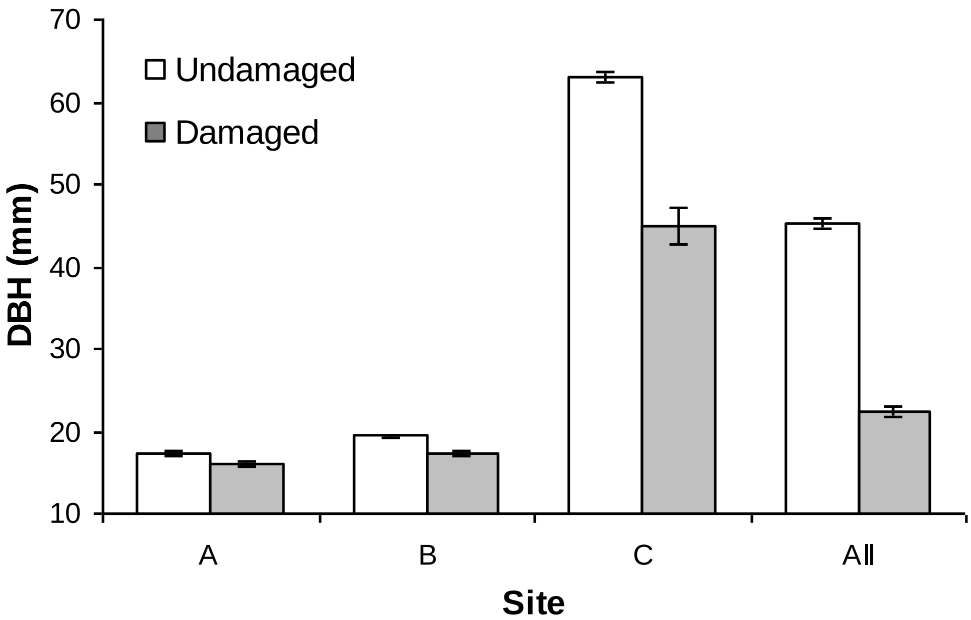

Figure 1. Average DBH of damaged and undamaged poplar stems (with standard error bars) for all poplar stems on all sites in fenced part of the hunting ground „Podunavsko lovište Plavna“

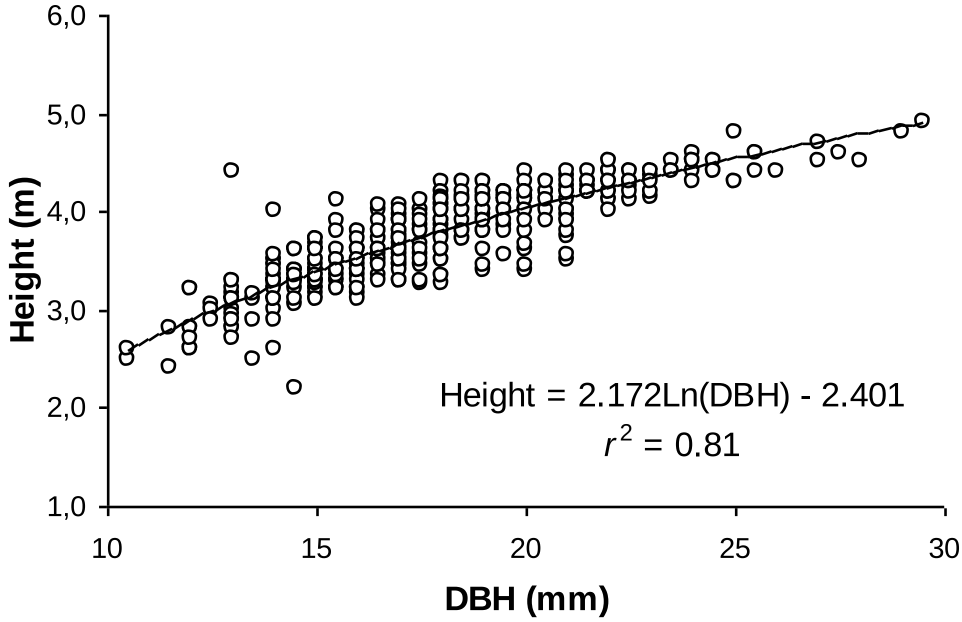

Figure 2. The relationship between height and DBH in young poplar plantations (n = 2670)

The survey of the spring deer stocks in fenced part of the hunting ground „Podunavsko lovište Plavna" is summarized in Figure 3. Red deer numbers have increased considerably over the last six years (from 88 individuals in 2001 to 194 individuals in 2006), mainly due to insufficient harvest size, especially due to the low proportion of females in the adult harvest. However, the present stock values of the red deer and the values of its population density are significantly lower (137 individuals in 2007 and 125 individuals in 2008), but still they do not correspond to the actual carrying capacity (80 individuals).

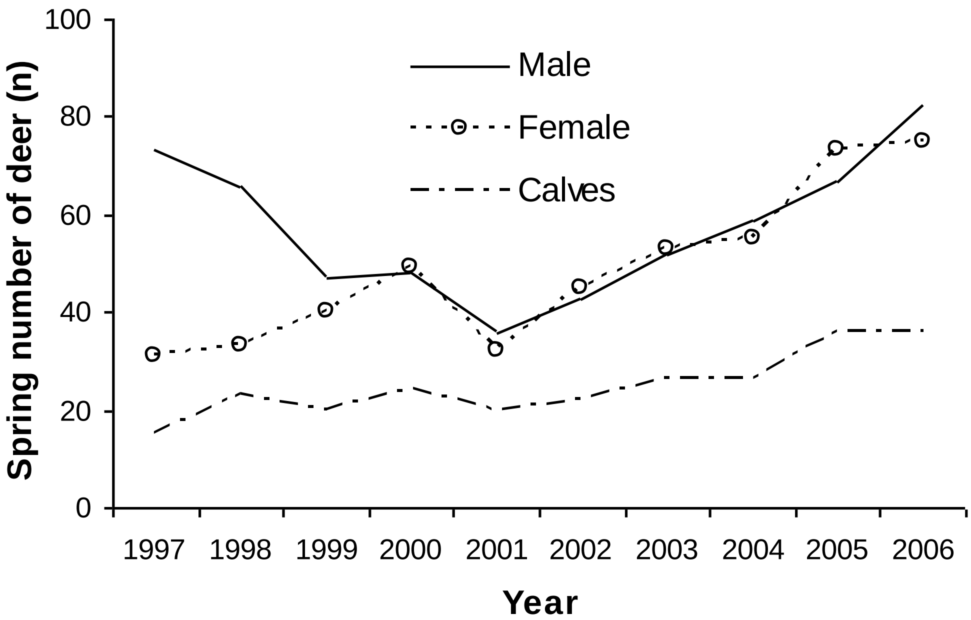

Figure 3. Red deer population dynamics - changes in proportion of calves, females and males

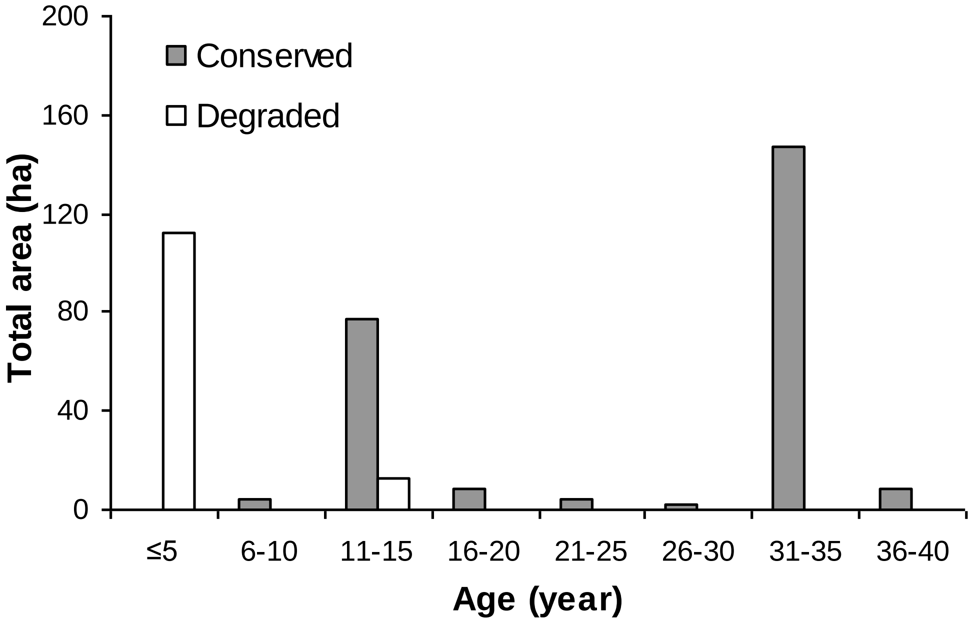

Figure 4. Age class distribution of all poplar plantations in fenced part of the hunting ground „Podunavsko lovište Plavna“ (age class I = ≤5 year; age class II = 6-10 year; and so forth)

Poplar plantations in lowland Serbia are usually managed with rotations of 20–25 years except where they are subject to high risk from large mammals (eg fenced hunting grounds), in which case longer rotations are used. In fenced part of the hunting ground „Podunavsko lovište Plavna“ the proportion of poplar plantations is important (451 ha or 73% of the total area), and their age class distribution (mature plantations occupied 160 ha) are greatly unfavourable in relation to the availability of food and shelter for red deer population (Fig. 4).

DISCUSSION

It is well known that large herbivores can damage poplar plantations by removing shoots and leaves („browsing“), stripping bark, and breaking or fraying stems. Poplars are a preferred browse for most cervid species (eg white-tailed deer) and may cause establishment failure, especially of smaller plantations subject to high browsing pressure (Verch, 1979; Stanturf et *al.*, 2001). Large mammal browsing can be so serious that the land user is left with only two options: to put fence or to forget growing poplar. In general, Hodge & Pepper (1998) reported that tree protection in woodland may be obtained by barriers (erection of fencing, tree guards or tree-shelters; use of chemical repellents), control of animal numbers (eg shooting, trapping), and habitat management (regrettably, this is a highly theoretical option at present). Netzer (1984) suggests that good plantation establishment techniques along with fast growing clones result in minimal deer damage to hybrid poplar plantations. With proper establishment, trees grow to reach or beyond reach of browsing deer in the first year and well beyond in the second, while poorly established plantations result in slower growing trees that are likely to be browsed for several years. Further, Karačić (2005) reported that fencing of poplar plantations is probably the most effective measure against browsing damages of large mammals. However, results of our study suggest that individual protection of poplar stems by wire mesh guards was ineffective measure in fenced part of the hunting ground „Podunavsko lovište Plavna“ beacuse of too many red deer.

Typical poplar cultivation issues are selection and production of clone material, site selection, soil preparation, spacing and planting technique, weed and pest control, irrigation, fertilization, thinning, rotation length and harvest techniques. Most of these issues are closely related to end use of crop (Karačić, 2005). Poplar plantation culture depends on three things: planting the best quality stock on high-quality sites and providing timely and appropriate cultural treatments (Stanturf et *al.*, 2001). According to Jović (1968) artificial restitution of composition contents together with the entire land cultivation is very unfavorable from the viewpoint of conditions for nutrition and habitat of deer. The lower levels of vegetation are destroyed by land tillage and the agrotechnical measures afterwards, and thus the later formation of vegetation is being prevented from occurring. The lack of natural food, the absence of the possibility of having shelter, frequent presence of humans and mechanization due to the necessary measures for care and protection of plantations contribute to the fact that these land areas practically disappear for deer. Thus, this author suggests that the planting as the composition contents should be omitted in conditions of high population of deer and intensifying hunting camps. Also, the number of authors (eg Reimoser & Gossow, 1996) reported that forestry must rehabilitate monocultural forests to be more natural, and natural regeneration strategies must be the preferred ones. Additionally, clear cutting with a need for artificial reafforestation should be avoided, thereby further reducing the predisposition of a forest to deer damage.

The total deer population size in fenced part of the hunting ground „Podunavsko lovište Plavna“ was significantly higher during last eight years (Fig. 3) than the optimal deer population size determined by the actual hunting management plan (80 individuals in the spring stock what means an average density of 13 individuals per 100 ha of the hunting area). Novaković (1999) suggests that in the most free hunting grounds of Serbia the density of red deer population needs to be in the range of 2–7 individuals / 100 ha of the hunting area. Similarly, Gill (2000) reported that deer populations need to be in the range of 4–7 individuals / 100 ha in the uplands to ensure adequate regeneration and maintain plant diversity.

Deer are usually managed by attempts to control their numbers to achieve a reduction in damage and natural mortality, and by the exploitation of surpluses from venison and from trophy hunting (Ratcliffe, 1987). Deer management should aim to maintain healthy deer populations in balance with their environment and prevent serious damage to woodlands, trees, crops gardens and other wildlife. The goal should be the prevention of deer problems, and management of deer problems requires a combination of three approaches: (1) design and management of the habitat, especially woodlands; (2) physical protection of vulnerable areas or individual trees; and (3) humane culling of deer over reasonably large areas to reduce and then maintain numbers at an acceptable level (Mayle, 1999).

In conclusion, our results suggest that the main cause of damage on young poplar plantations within fenced part of the hunting ground „Podunavsko lovište Plavna“ lies in the high population density of red deer (19.8 individuals / 100 ha of the hunting area in spring 2008), and that larger poplar stems are far less damaged by red deer. The reduction of mature poplar plantations at recent and future years will result in a reduction of the capacity of this fenced part to support red deer, and this may in turn lead to increased damage to poplar plantations. Therefore, the hunting management objective should aim to reduce the deer population to a level at which the establishment of poplar plantations is possible (13 individuals / 100 ha), but the forest management should aim to promote natural processes of regeneration, indigenous species, multi-species forest stands, and individual poplar stem protection by strengthened upgraded wire mesh guards.

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REGENERATION DYNAMICS OF UNEVEN-AGED FOREST STAND AFTER THE ESTABLISHMENT OF NATIONAL PARK 'RISNJAK' AND THE ABANDONMENT OF THE MANAGEMENT ACTIVITIES

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Summary: *Monitoring of the stand structure development and regeneration processes on 1 ha large permanent plot in the national park 'Risnjak' started in 1998. Main objective was to gain an insight in the processes underway in previously managed uneven-aged forest stand in the national park 'Risnjak' in which no management activities were performed since 1953. Following problems were detected: beech dominated left side of the diameter distribution indicates a halt in the recruitment of fir seedlings some decades ago; share of fir seedlings in the regeneration decreased from 23.4% in 1998 to 8.5% in 2007 while the share of beech seedlings increased from 16.1% up to 58.3%; overall picture of beech domination is completed by large proportion of beech canopy cover over the plot. It is well known that recruitment of the fir seedlings could be improved by some form of well known silvicultural intervention, like creating a gap by removing old fir trees. Problem lies in strict form of protection forbidding any kind of intervention. Therefore we explored possibilities of two software packages for the presentation of research results to stakeholders in question. Properly executed steps in the chain: research – simulation and analysis – visualization and presentation, should, at least, initiate comprehensive silvicultural project to test different management options, and draw scientifically based conclusions on the future of the forests in national park 'Risnjak'.*
Key words: *silver fir and beech forest, uneven-aged forest; regeneration, national park, stand visualization*

INTRODUCTION

The main requirement for an area under consideration for the stricter categories of nature protection, like national park, is its naturalness, i.e. the area should contain one or more ecosystems unchanged by human activities (IUCN, 1994; Anon., 2005). This is a difficult task in the case of forest ecosystems, especially in southern and central parts of Europe where forests experienced long, extensive and constant pressure of human activities (Parviainen et *al.*, 2000). Thus, the national parks are usually established in forested areas previously exposed to forestry management of different intensity, and such activities are abandoned following the establishment of protected areas. In 1953 'Risnjak' national park was established in Croatia for the protection of silver fir (*Abies alba* Mill.) and beech (*Fagus sylvatica* L.) forest ecosystem. These forests were not 'virgin forests' at the time of the park establishment, although the natural species composition has been preserved. Forest management in the form of selection system based on the regeneration processes driven by the gap dynamics ('Plenterwald') resulted in stable forest ecosystems of exceptional scenic beauty. With the rise of the national park, forest management was forbidden and forests were given back to the nature. Since 1953 no management activities were performed, except for the removal of dead trees along the roads, paths and trails that were clear danger to park visitors.

Shade-tolerant silver fir is a tree species well suited to perform as a major building block of an uneven-aged multilayered forest stands (Schütz, 2001), like those in NP Risnjak. However, the decline of silver fir vitality in Central Europe during the recent decades is well documented (Brinkmann, 1997; Bončina et *al.*, 2003; Bigler et *al.*, 2004). Probable causes for fir decline are: climate change; industrial pollution of water, air and soil; changes in the populations of large herbivores (Bončina, 2000; Nagel et *al.*, 2006). The most pronounced symptoms of fir decline are absence of transition of fir regeneration to canopy layer

(Dobrowolska, 1998; Motta, 1996) and process of species alternation with beech substituting silver fir (Bončina et *al.*, 2003).

Silvicultural measures aimed at improving stand structural attributes to enhance regeneration in previously production forests, and now protected forest areas, may require felling of old trees and creation of different-sized gaps (Toivonen, 2000). In traditional selection silvicultural system, that was applied to these forests prior to the establishment of the national park, gaps were created by removal of overmature dominant trees to spark the regeneration flushes. Such interventions are destined to provoke vigorous response from a number of organizations and individuals concerned with nature protection. Hence, the request for the silvicultural interventions in protected areas should be very carefully presented to the legislative bodies and nature protection organizations with reasoning behind the request clearly formulated.

In the past, artists' renditions were used in depicting visual impacts of different managerial interventions or the lack of thereof, but ever-increasing variety of management options, and the broadening of the time-horizons for which the impact on landscape should be visualized, make the application of artistic renditions impossible (McGaughey, 2004). Today, foresters and administrators in protected areas have a number of tools at their disposal to transfer the reasons behind their actions to the concerned stakeholders in a visually appealing way. These tools are computer programs for the visualization of forest development over time, on stand and landscape level. For example, outputs of stand-level growth and development model for different scenarios can be utilized in a holistic (landscape-level) land-use model trough which various conflicts between stakeholders in managing forests or other natural resources can be addressed and hopefully resolved (Hjortsø et *al.*, 2006; Wollenberg et *al.*, 2000). Models and simulation methods are useful as a scientific base for whole array of administrative operations, e. g. zoning of protected areas (Sabatini et *al.*, 2007). Nevertheless, these models require extensive data from long-term monitoring and silvicultural experiments as a basis for simulations and scenario analysis.

Aim of this paper is to present results of decade-long monitoring of regeneration processes, with special emphasis on fir regeneration, in formerly managed uneven-aged forest stand of beech and fir in which the management was abandoned after the establishment of the national park Risnjak in 1953. Additionally, two software packages for the visualization of the permanent experimental plot were tested to asses the possibilities for their future use in the presentation of the results to the stakeholders.

MATERIALS AND METHODS

National park 'Risnjak' is situated in the north-west part of Croatia (Fig. 1). The highest mountain peak is 'Veliki Risnjak', 1528 m above sea level. Total area of NP 'Risnjak' amounts to 6400 ha of which 95% is under forest cover, while meadows and rocky outcrop make up the remaining 5% of the park's area. The major part of forest cover is comprised of uneven-aged mixed stands of silver fir and beech (Vukelic, 1985). The mean annual temperature and mean annual precipitation for the 30-year period (1961-1990) are 7.4 °C and 2280 mm, respectively.

In 1998 a permanent experimental plot 100 x 100 m in size was set up in the typical uneven-aged stand of fir and beech at 680 m a.s.l. The plot is part of the network of permanent experimental plots established in forested Croatian national parks for the monitoring of forest dynamics in protected areas.

All trees over 7.5 cm in diameter at breast height (DBH) were stem-mapped, numbered and measured for DBH with calipers by taking two perpendicular measurements on 1.3 m above ground. Definite height and direction of the first DBH measurement was point-marked on the stem with permanent forest paint. Stand structure was measured in detail on a subplot 60 x 60 m in size (Fig. 2a) in 1998 and remeasured in 2007. All marked trees on the subplot were measured for total height and height to crown base, and detailed map of crown projections was made. Terrain relief was surveyed at the position of each mapped tree and several additional characteristic terrain points on the subplot with handheld laser range finder LaserAce 300 (Measurements Devices Ltd., UK). Obtained terrain heights were used to create three-dimensional digital model of terrain in ESRI's ArcMap (Hayakawa et *al.*, 2007) (Fig. 2b). Maps of gaps, canopy cover by tree species and by canopy layers were produced from the map of crown projections. Abundance and structure of regeneration was recorded in 1998, 2001, 2003 and 2007 on three strips within the subplot, each 120 m^2 in size (2 x 60 m). All plants on these strips were tallied according to their form (tree or shrub), species, age (one-year old or older) and height. In 2007 each of these three strips was further subdivided into six 2 x 10 m sub-strips. Average slope was calculated for each of these 18 sub-strips.

Figure 1. Location of national park Risnjak shown on Landsat ETM satellite image draped over digital elevation model

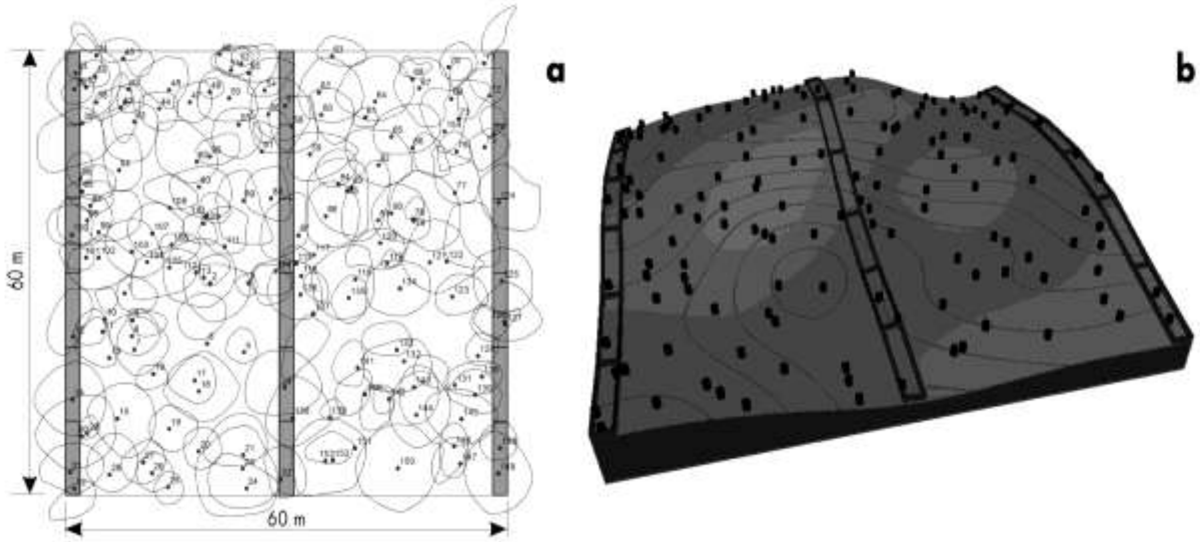

Figure 2. (a) Subplot layout with tree positions, strips on which regeneration was measured and crown projections; (b) three-dimensional terrain model with superimposed tree positions and strips for regeneration monitoring

Crown damage status (i.e. crown transparency) of each tree on the subplot was assessed four times during the monitoring period (1998, 2001, 2003 and 2005) according to ICP Forest methodology (UN-ECE, 1998).

Two software packages were used for the visualization of experimental plot: 3ds Max (Autodesk, USA) and EnVision (USDA Forest Service, USA). The first one is commercial software with wide application in developing three-dimensional computer projects, ranging from architecture to Hollywood movies. The EnVision software was developed in USDA Forest Service especially for forestry, for the purpose of stand and landscape level visualization of forest ecosystems.

RESULTS

Stand structure

Basic structural relationships between canopy tree species remained basically the same ten years after the establishment of the experimental plot (Table 1). Stand basal area is dominated by silver fir (32.60 m^2/ha, or 73% in 2007), and most abundant tree species by the number of trees per hectare is beech (230/ha, or 67% in 2007). Two other tree species are marginally represented by few stems per hectare. Total stand basal area increment over nine years (4.53 m^2/ha) is unevenly distributed between tree species with 60% being generated by large fir trees. Share of overmature firs in stand structure is also evident from the diameter distributions (Fig. 3). Absence of fir from the lower diameter classes suggests that fir recruitment came to a halt few decades ago. Instead of fir, beech is filling in the lower part of diameter distribution.

Table 1. Basic structural features of plot's canopy trees between two measurements (1998 and 2007)

Tree species	Year				Change (2007-1998)	
	1998		2007			
	Density	Basal area	Density	Basal area	Density	Basal area
	trees/ha	m^2/ha	trees/ha	m^2/ha	trees/ha	m^2/ha
Silver fir	114	29.88	111	32.60	-3	+2.72
Beech	231	9.47	230	11.27	-1	+1.80
Other tree species	6	0.52	5	0.52	-1	+0.01
Total	351	39.87	346	44.40	-5	+4.53

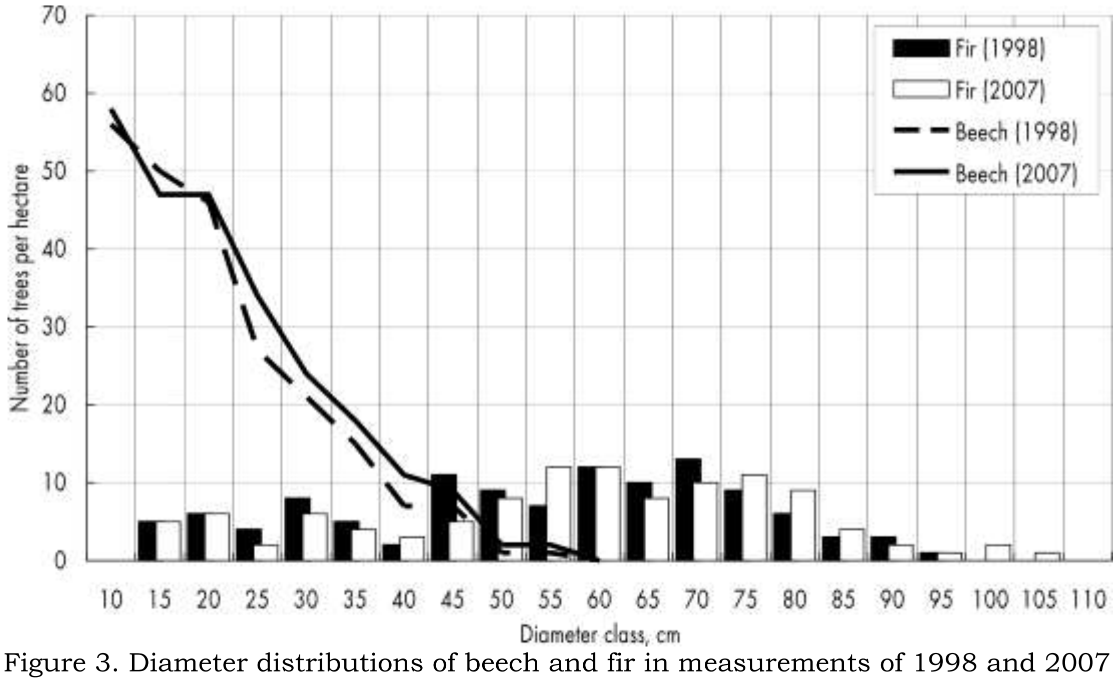

Figure 3. Diameter distributions of beech and fir in measurements of 1998 and 2007

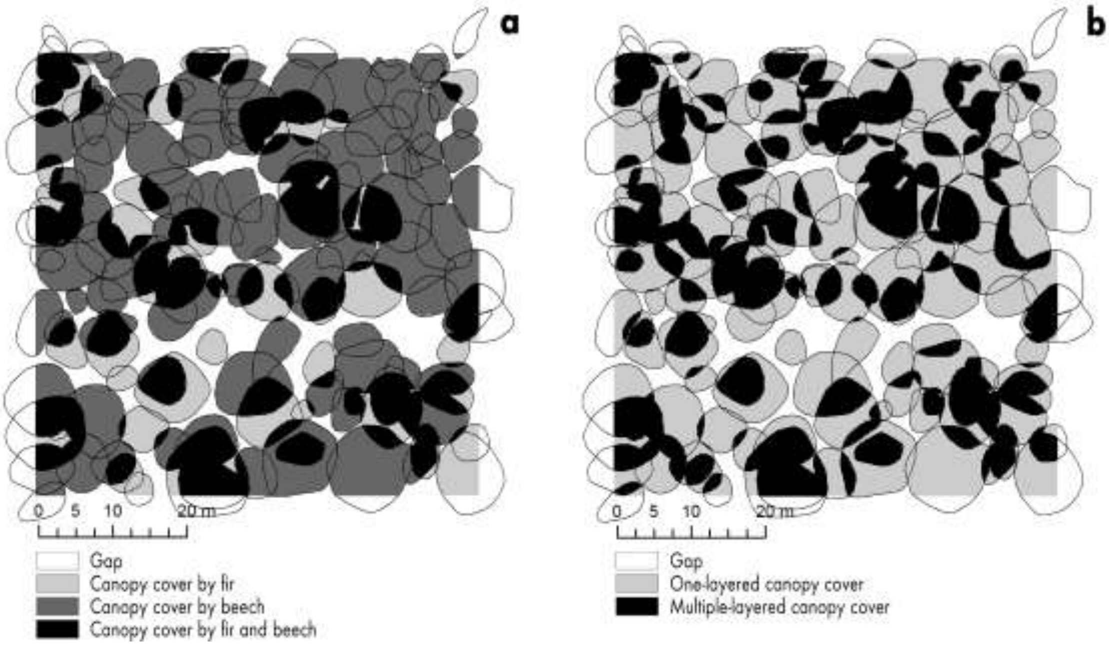

Figure 4. Horizontal texture (a) and vertical structure (b) of canopy cover of the stand on experimental plot

Analysis of horizontal texture and vertical structure of canopy cover on experimental plot was based on map of crown projections (Fig. 2a). Proportion of gaps in canopy layer amounts to 11.5% of plot's horizontal surface. Boncina (2000) found that in managed Slovenian beech and fir forests average proportion of gaps amounts to 15%, while in virgin forest remnants gaps cover only 1.2% of the forest area. Analysis of canopy cover by tree species revealed predominance of canopy cover by beech. Proportion of plot's horizontal area covered by beech crowns (either solely or in combination with fir crowns) is as high as 73.9% (Fig. 4a), in spite of larger average canopy diameter and crown projection area of fir trees. In contrast, fir crowns (solely or overlapping with beech crowns) cover only 38.2% of the plot's area.

Forests of beech and fir that managed by selective cutting are rich in vertical structure with multiple canopy layers (Dubravac et *al.*, 2005, Dubravac et *al.*, 2007). In our case, highly structured canopy with multiple layers covers only 34.3% of the plot's area (Fig. 4b). Remainder of the plot's area is covered by unstructured, one-layered canopy.

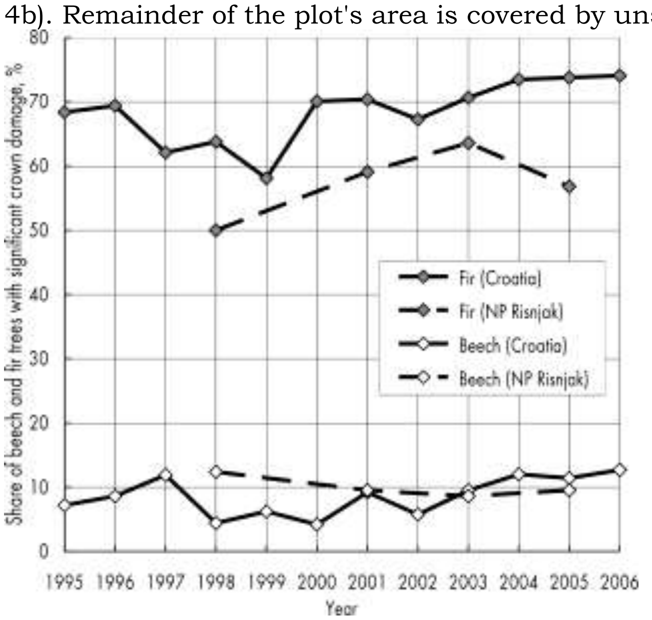

Figure 5. Share of beech and fir trees with significant crown damage (crown transparency over 25%) on experimental plot and an average for Croatia (1995-2006)

The crown damage of individual trees on the plot (Fig. 5) was assessed according to the ICP Forests methodology (UN-ECE, 1998), in which trees with crown transparency greater than 25% are considered significantly damaged. Fir and beech trees on the plot follow general trend observed for these tree species in Croatia during the last decade (Potočić & Seletković, 2006). Share of fir trees on the plot with significant crown damage was five to six times greater than the share of damaged beech trees. This confirms the status of fir as the most endangered tree species in Croatia, and also in the national park 'Risnjak'.

Regeneration dynamics

Regeneration was surveyed four times between 1998 and 2007 on three strips with total area of 360 m^2. Regeneration dynamics was assessed with respect to the occurrence of one-year-old and older fir seedlings, occurrence of beech plants and occurrence of other tree species and shrubs. All plants were also measured for height and assorted into height classes (Table 2).

The most striking feature regarding height of the fir plants is that none of the plants measured over 30 cm in height over the whole of the monitoring period. Beech plants, on the other hand, ranged up to 3 m in height. We have counted 11111 and 18805 one-year-old fir seedlings per hectare in 1998 and 2007, respectively. There seems to be no problem with the establishment of one-year-old fir seedlings, but rather in the transition of fir seedlings to upper height classes. Roženbergar et *al.* (2007) obtained similar results in the old-growth forest of beech and fir in Slovenia, where fir seedlings fail to recruit into height classes over 50 cm. Dobrowolska (1998) came across the same problem in Jata Reserve in Poland, and Mazur (1989) states that large number of seedlings that appear after seed years does not have large impact where high mortality during the first years of life occurs.

Table 2. Regeneration dynamics by species and height (plants per hectare)

Height class, cm	Measurement year											
	1998			2001			2003			2007		
	Fir	Beech	Other	Fir	Beech	Other	Fir	Beech	Other	Fir	Beech	Other
< 30	8945	3916	19778	7221	15971	22471	7611	41389	20888	4695	28659	12944
31-60		1194	2500		1749	3584		1083	3889		1694	3861
61-130		417	1361		333	1250		589	1306		1056	1556
131-150		28			167	167		56	222		222	222
151-200		83			56			167			83	139
201-250		139			139			55			167	
251>		361	28		361	28		500	28		333	28
Total	8945	6138	23667	7221	18776	27500	7611	43839	26333	4695	32214	18750

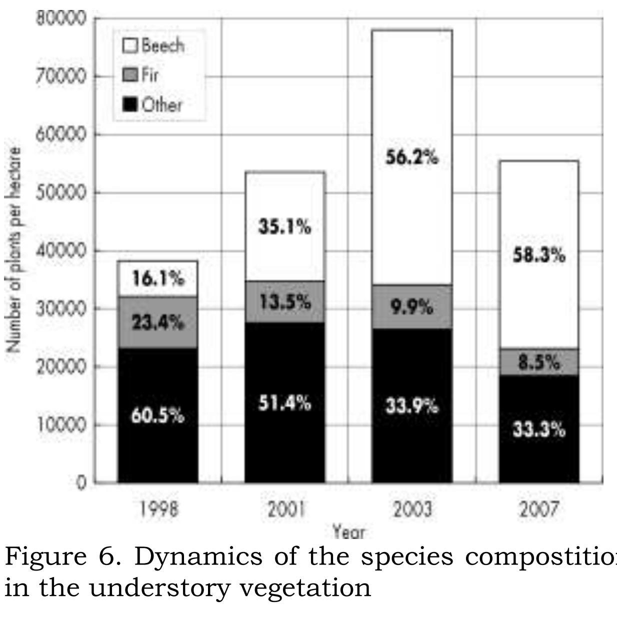

Figure 6. Dynamics of the species compostition in the understory vegetation

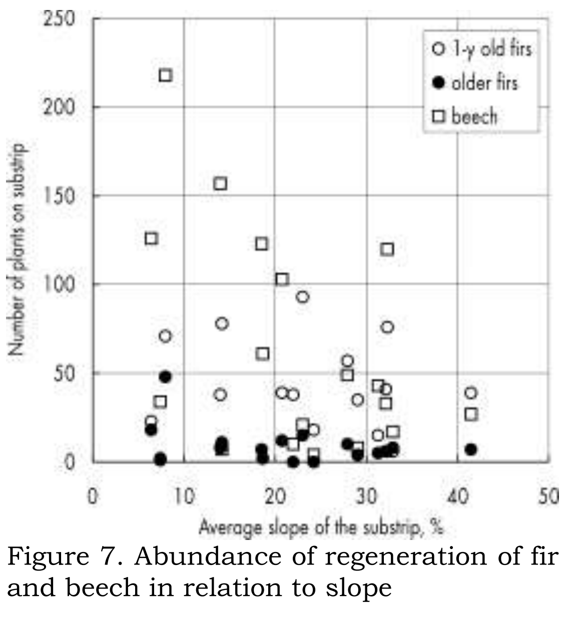

Figure 7. Abundance of regeneration of fir and beech in relation to slope

Other important finding regarding the regeneration dynamics in the investigated stand is fluctuation in the composition of species (Fig. 6). At the beginning of the monitoring period, beech comprised only 16.1% of the understory layer, and nine years later share of beech amounts to 58.3%. This flush of beech regeneration was induced by the rich seed-rain in 2001. During the same period share of fir decreased from 23.4 to only 8.5% in 2007. Increase in the number of beech regenerants and simultaneous decrease of fir together with the inability of fir to transgress above 30 cm in height may have important negative implications for the future of fir in this stand. Moreover, under over-excessive canopy cover and levels of above canopy light below 20%, probability of stem forking in beech increases (Stancioiu & O'Hara, 2006). We have also found that, thanks to its morphological plasticity in growth, beech was able to occupy empty spaces left by fir, but the stem quality of these plants is very low, frequently with forking.

Occurrence of fir and beech regeneration was also analyzed in relation to slope as the main determinant of the microtopography. Average slope for each of the 18 substrips was calculated from the three-dimensional relief model, and number of beech and fir plants on substrip was plotted against the average slope (Fig. 7). Total number of plants decreases with the increase in slope. This is mainly due to the large proportion of beech plants and the fact that only beech shows statistically significant, although weak, negative correlation with slope (Pearson's $r = -0.483$; $p < 0.05$), because of the influence of gravitation on heavy beech seeds during the phase of seed dispersal.

Stand visualization

To asses the ability of visualization tools in presenting the results of this research, we have utilized two software packages. As a representative of the commercial product intended for broad spectrum of users with need for three-dimensional computer graphics, 3ds Max (Autodesk, USA) software package was selected. On the other hand, EnVision (USDA Forest Service, USA) was selected as a specialized computer product developed by the foresters and for the use in forestry research and practice. Examples of visualizations of the experimental plot in the selected software packages are presented in Fig. 8.

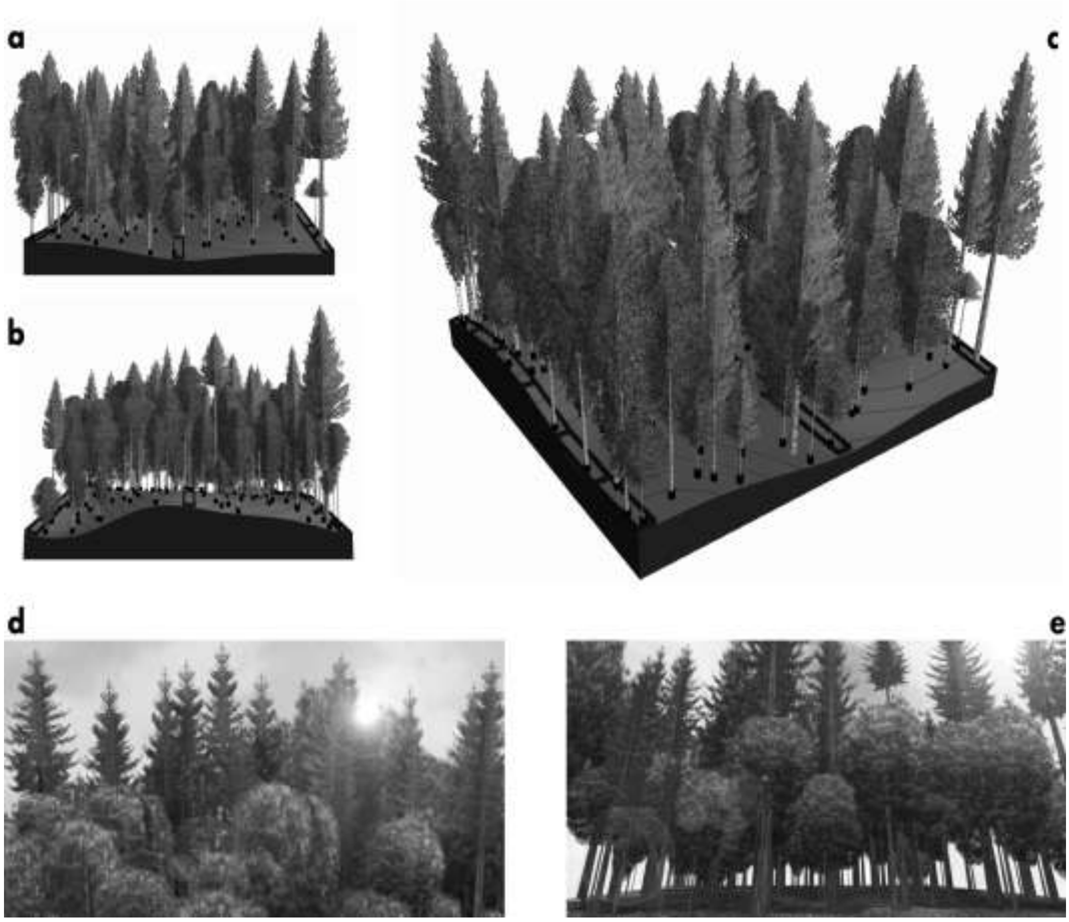

Figure 8. Visualization examples of the experimental plot in two software packages: EnVision (a, b and c) and 3ds Max (d and e)

EnVision was finally selected as a more suitable for our research needs among two tested software packages. The major advantage of the EnVision software is its ability to use already available forestry datasets collected trough every-day forestry activities, like tree lists, stand tables, and most of all, GIS layers such as forest stand boundaries and digital elevation models. Although the overall quality of the stand visualization in 3ds Max exceeds that of the EnVision, the amount of work-hours and software-specific knowledge required to achieve such quality level is significantly higher than to achieve standard quality of the

EnVision visualization. Furthermore, the final visualization product of the EnVision can be easily updated by changing the input tables of the datasets used for the visualization.

DISCUSSION

Stand structure remained unchanged during the monitoring period, with mature over-sized fir trees dominating stand basal area and stand basal area increment, and beech dominating the left side of diameter distribution indicating a halt in the recruitment of the fir seedlings some decades ago.

During the nine years of monitoring a decrease in the number of young fir plants was recorded and a lack of transition of firs from seedling to sapling stage. None of the young fir plants grew over 30 cm in height during the last nine years. The share of beech in the total number of understory plants has increased from 16.1% in 1998, up to 58.3% in 2007.

As old fir trees die, the source of fir seed will diminish, and the few emerged seedlings will have to strive in beech dominated environment. This could ultimately lead to species substitution as already noticed in nearby Slovenian beech and fir forests. Process of species substitution is even more concerning since it is underway in the forests of the national park 'Risnjak' where uneven-aged fir and beech forests are the main feature. The following question emerges: Should we protect the natural processes such as species substitution and prohibit any kind of human intervention, or should we try to protect the forests that are a product of these processes and ultimately the very reason for the establishment of the NP 'Risnjak'? Similar question could be easily raised in a number of protected areas with strict protection regimes, especially in the light of climate change processes.

To answer this complex question, in the case of Risnjak national park, further research is needed. Monitoring of regeneration and stand structure development should be continued and further developed, and the data obtained should be utilized trough stand simulators to produce several scenarios as a basis for the decision-making process.

Results of simulations for different scenarios could be easily presented to key players in decision making process trough EnVision software package on landscape level. Properly executed steps in the chain: research-simulation and analysis-visualization and presentation, should, at least, initiate comprehensive silvicultural project to test different management options, and draw scientifically based conclusions on the future of the forests in national park 'Risnjak'.

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MIGRANT NOCTUIDAE SPECIES OF NATIONAL PARK FRUŠKA GORA *(Noctuidae, Lepidoptera)*

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Abstract: *This is a brief report about migratory species found in National Park Fruška Gora. Altogether, 26 migratory species have been collected since 2002. Until now, migratory species have not been explored in National Park Fruška Gora. Founded species are clasified according to adopted classification into eumigrators, paramigrators, emmigrants, dismigratory species and potential migrators. Presented list of migratory Noctuids is a part of a wider list of migratory species of order Lepidoptera of National Park Fruška Gora, which will be also published soon.*
Key Words: *Migratory species, Noctuidae, Fruška gora*

INTRODUCTION

This is a brief report about migratory species found in National Park Fruška Gora. The classiffication of migratory species is given in (Eitschberger et all, 1991) and also adopted in (Beshkov, 1994a, 1994b, 1996a, 1996b, Vajgand, 2000, Zečević, 1995, Zečević, 2002).

Eumigrators are those ones which leave their breeding grounds and travel to specific areas (seasonal areas) at specific times during the year, due to reproduction. Here young are produced, and they or their offspring migrate back to the original breeding areas to reproduce. These offspring which migrate further than the specific seasonal areas are not capable of migrating back and perish. They are also known as the seasonal migrants 1st order.

Paramigrators, or temporary migrants, are those species which leave the native land to survive the particular season (i.e. during the winter, or summer). The same specimen come back to the native land to continue the reproduction.

Emmigrant species occasionally move within the areal and never come back to the domicile.

Dismigratory species (or evasional species) are those ones which are moving, extend their areals and leave the domiciles without particular goals (i.e. reproduction or surviving). They tend to expand or change the population.

Potential migrators are species for which is supposed that they would move and expand their areals.

MATERIALS AND METHODS

Several standard methods, which are usually used for collecting of specimen and monitoring of Noctuidae population has been also used in this research. Two kinds of light traps have been used - 400W mercury bulbs and petromax lamp, also with power 400W.

Some more valuable specimen are prepared and deposited in the standard entomological boxes. Furthermore, the permanent preparations of genital armatures has been made, due to confirm the determination in particular ambiguous cases.

RESULTS AND DISCUSSION

Altogether, 26 migratory species of the Noctuidae family have been collected in National Park Fruška Gora since 2002. They are recognized and classified as follows: two eumigrators, three paramigrators, twelve emmigrants, five species, which belong to the dismigratory ones and four species identified as potential migrators. In the rest of the section, the list of recorded migrant species is given. There are two numbers attached to the each species. The first number is identification number of species in the private collection of author. The second number is the number of species according to Karsholt & Razowski (1996) classification.

1. The Seasonal migrants 1st order, recorded on Fruška Gora are:

46. 9056 ***Autographa gamma*** (Linnaeus, 1758) (Figure 1)
191. 10346 ***Agrotis ipsilon*** (Hufnagel, 1766) (Figure 2)

2a. Emmigrant species - local migrants 1st order recorded on Fruška Gora are:

94. 9505 ***Phlogophora meticulosa*** (Linnaeus, 1758) (Figure 3)
176.10096 ***Noctua pronuba*** (Linnaeus, 1758) (Figure 4)
179.10100 ***Noctua fimbriata*** (Schreber, 1759) (Figure 5)

2b. Emmigrant species - local migrants 2st order recorded on Fruška Gora are:

31. 8965 ***Tyta luctuosa*** (Denis & Schiffermüller, 1775) (Figure 6)
44. 9051 ***Macdunnoughia confusa*** (Stephens, 1850) (Figure 7)
48. 9081 ***Trichoplusia ni*** (Hübner, 1803) (Figure 8)
53. 9100 ***Acontia lucida*** (Hufnagel, 1766)
73. 9358 ***Schinia scutosa*** ((Denis & Schiffermüller, 1775)
74. 9364 ***Heliothis viriplaca*** (Hufnagel, 1766)
75. 9367 ***Heliothis peltigera*** (Denis & Schiffermüller, 1775)
76. 9370 ***Helicoverpa armigera*** (Hübner, 1808) (Figure 9)
86. 9460 ***Spodoptera exigua*** (Hübner, 1808)
158.10003 ***Mythimna vitellina*** (Hübner, 1808)
188.10238 ***Peridroma saucia*** (Hübner, 1803)
201.10445 ***Nycteola asiatica*** (Krulikovsky, 1904)

3. Occasional species recorded on Fruška Gora are:

157.10002 ***Mythimna albipuncta*** (Denis & Schiffermüller, 1775)
162.10022 ***Mythimna l-album*** (Linnaeus, 1767) (Figure 10)
182.10199 ***Xestia c-nigrum*** (Linnaeus, 1758)
192.10348 ***Agrotis exclamationis*** (Linnaeus, 1758)
193.10351 ***Agrotis segetum*** (Denis & Schiffermüller, 1775)

4. Possible migrants recorded on Fruška Gora are:

64.9307 ***Amphipyra pyramidea*** (Linnaeus, 1758)
65.9308 ***Amphipyra berbera*** Rungs, 1949
82.9450 ***Hoplodrina blanda*** (Denis & Schiffermüller, 1775)
153.9987 ***Mamestra brassicae*** (Linnaeus, 1758)

Figure 1. Autographa gamma (Linnaeus, 1758)

Figure 2. Agrotis ipsilon (Hufnagel, 1766)

Figure 3. Phlogophora meticulosa (Linnaeus, 1758)

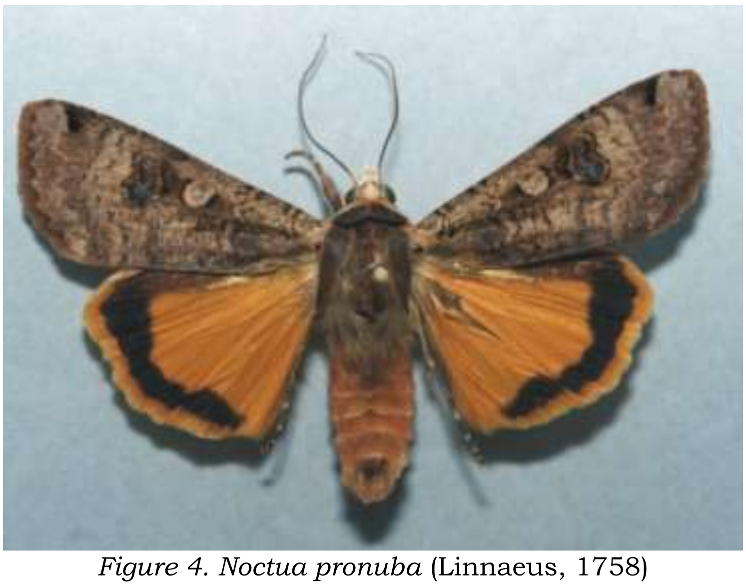

Figure 4. Noctua pronuba (Linnaeus, 1758)

Figure 5. Noctua fimbriata (Schreber, 1759)

Figure 6: Tyta luctuosa (Denis & Schiffermüller, 1775)

Figure 7. Macdunnoughia confusa (Stephens, 1850)

Figure 8. Trichoplusia ni (Hübner, 1803)

Figure 9. Helicoverpa armigera (Hübner, 1808)

Figure 10. Mythimna l-album (Linnaeus, 1767)

CONCLUSION

Until now, migratory species have not been explored in National Park Fruška Gora. Founded species are clasified according to adopted classification into eumigrators, paramigrators, emmigrants, dismigratory species and potential migrators. Presented list of migratory Noctuids is a part of a wider list of migratory species of order Lepidoptera of National Park Fruška Gora, which will be also published soon.

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MICROSATELLITE GENETIC RELATIONSHIPS OF POPULUS CLONES, CULTIVARS AND HYBRIDS FROM TWO SECTIONS OF GENUS POPULUS

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Abstract: *The objective of this paper was to examine the functionality and informational value of 12 microsatellite DNA markers in order to establish a fingerprinting profiles starting from germplasm (different genetic backround) of the core collection of the Institute of Lowland Forestry and Environment, Novi Sad, Serbia. The markers polymorphisms and its levels were determined within 13 genotypes of two sections (Aigeiros and Tacamahaca) of genus Populus. Twelve sets of microsatellite primers, were chosen for this study. Genomic DNA was isolated from the leaf tissue using a modification of the Permigeant et al. (1998) method. PCR amplifications of the desired fragments were carried out in a volume of 25 µL according to Gomez et al. (2003). All SSR markers were successfully amplified and sized. Among 12 loci, PTR1, PTR3, PTR6, and PTR14 were the most polymorphic whereas PTR11, PTR15 and PTR2 showed a low level of polymorphisms. Monomorphic loci were PTR4, PTR12 while PTR7 and PTR8 loci haven't shown any amplification for all genotypes examined. Revealing polymorphisms within genotypes of interest, this microsatellite DNA marker could be successfully used in distinguishing tree genotypes, in revealing the fingerprinting pattern so could be of value in poplar breeding programs.*
Key words: *poplar, microsatellites, polymorphisms*

INTRODUCTION

Genus Populus L. (Salicaceae) includes about 30 species classified in six sections, distributed widely, mainly in the forests of temperate and cold regions of the Northern Hemisphere.

The economically most important species are in the section Aigeiros Duby, Tacamahaca Spach. and Leuce Duby. Many poplar species and their interspecific hybrids are suitable for poplar culture and intensive forest plantations. *P. deltoides* Marsh. (cottonwood) and *P. nigra* L. (black poplar) of section Aigeiros and *P. trichocarpa* Torr. Ex Gray (black cottonwood) of the section Tacamahaca are one of the most important species for interspecific poplar breeding programs worldwide. These species and their interspecific hybrids are suitable for short-rotation intensive poplar culture and agroforestry.

Indentification of populus clones and cultivars and knowledge of their genetic interrelationship are essential for effective selection, breeding and genetic resource management programs, varietal control, protection and registration and handling of planting and breeding stocks.

P. deltoides and *P. trichocarpa* are one of the most important poplar timber species and plays a central role in poplar breeding programs and has contributed to many successful interspecific hybrids (Frison *et al.*, 1995, and Weisgerber and Han, 2001). Thus hybrids *P.x euramericana* have found its utility as a source of timber whereas *P. nigra* 'Italica' is used as a windbreak or for landscaping purposes. Hybrid Pannonia (*P.x euramericana)* was successfully raised for a high growth vigor (Orlović *et al*, 2006).

A main concern confronting poplar breeders is to improve knowledge of genetic constitution, relationships and indentification of clones, cultivars and varieties.

Highly polymorphic, consistent and codominant markers, microsatellites or simple sequence repeats (SSRs), are a reliable marker for clone and cultivar identification in poplars. Molecular markers gives new dimension to genetic and population studies, as well as to breeding practice, protection of breeder's rights (while the methods of gene manipulation enabled fast introduction of desired genes in interesting genotypes, which is of special importance in breeding of perennial wood species (Kovačević *et al.*, 2002; Galović *et al.*, 2007).

The aim of this study was to use microsatellite DNA markers for evaluating DNA profiles and make differentiation of selected clones, cultivars and their hybrids from two sections of genus Populus, and determine their molecular genetic interrelationships. Also, it is tested the informativeness of the SSR DNA markers.

MATERIAL AND METHODS

Thirteen genotypes with different genetic background (clones, cultivars, hybrids) and from two sections of genus Populus (Aigeiros and Tacamahaca) from Novi Sad core collection, were sampled (Tab 1). All genotypes originated from Vojvodina province and they are representatives of Institute of Lowland Forestry and Environment, Novi Sad, Serbia. Genomic DNA was isolated from the leaf tissue of individual plants using a modification of the Permigeant et al. (1998) method. Twelve SSR DNA markers (PTR1, PTR2, PTR3 PTR4, PTR5, PTR6, PTR7, PTR8, PTR11, PTR12, PTR14, PTR15), (Tab. 1) developed for *P. tremuloides* (Rahman et al., 2000) were used to evaluate it's functionality and informational value on thirteen populus genotypes. Also to determine the identity and genetic relationship of different genotypes of Populus species. The SSR markers were amplified individually using 12 SSR primer pairs according to different PCR protocols (Rahman et al. 2000). The PCR reactions were carried out in a volume of 25µL (Gomez et al., 2003).The amplified products were separated on 6% denaturing PAA and visualized by silver staining procedure.Genetic similarities and relationships among the clones were determined by calculating Jaccard's similarities based on their SSR genotypes by using NTSYS version 2.1 software. A UPGMA cluster analyses was performed based on interclonal genotypic Jaccard's similarities and a cluster plot of the 13 genotypes was constructed. Jaccard distance (J') for individual genotypes was determined as following

$J' = \frac{M_{01} + M_{10}}{M_{01} + M_{10} + M_{11}}$, M11 - number of alleles that bought genotypes posses; (M01 + M10) - number of alleles that one genotype posses and the other not. Discrimination index (D) for individual microsatellite DNA loci was determined as follows (Kloosterman et al., 1993): $PD = 1 - \sum P_i^2$, where P_i stands for the frequency of the genotype with the i^{th} type.

RESULTS AND DISCUSSION

The SSR analysis was performed for 13 *Populus* genotypes belonging to two sections: Aigeiros Duby and Tacamahaca Spach, as clones, cultivars and hybrids (Tab1).

Table 1. Species of populus used to test *Populus tremuloides* SSR primer pair utility

Genotypes under observation:	**Section**
1.B81 (*Populus deltoides*)	Aigeiros Duby
2.B229 (*Populus deltoides*)	Aigeiros Duby
3.Pannonia - M1 (*Px euramericana*)	Aigeiros Duby
4.182/81 (*Populus deltoides*)	Aigeiros Duby
5.PD100 (*Populus deltoides*)	Aigeiros Duby
6.NS001 (*Populus nigra L.*)	Aigeiros Duby
7.NS002 (*Populus nigra L.*)	Aigeiros Duby
8.I-214 (*P x euramericana*)	Aigeiros Duby
9.102/81 (*P. nigra x P. maximowiczii*)	
10.9111/93 (*P. nigra x P. maximowiczii*) x *P. italica*	
11.*P. italica*	Aigeiros Duby
12.1007(*P. trichocarpa*)	Tacamahaca Spach
13.1004 (*P. trichocarpa*)	Tacamahaca Spach

Genetic variation was detected among them by investigating 12 nuclear SSR marker loci. Ten out of twelve loci analyzed were polymorphic among the *Populus species.* A total of 6 allels were detected among the amplification products. The numbers of alleles detected per locus ranged from 1 at PTR4 and PTR12 to 6 at PTR1, PTR3, PTR6 and PTR14 with an average of 2 alleles per locus. The power of discrimination of each nuclear SSR locus varied widely, from 0,132 to 0,811 (Table 2).

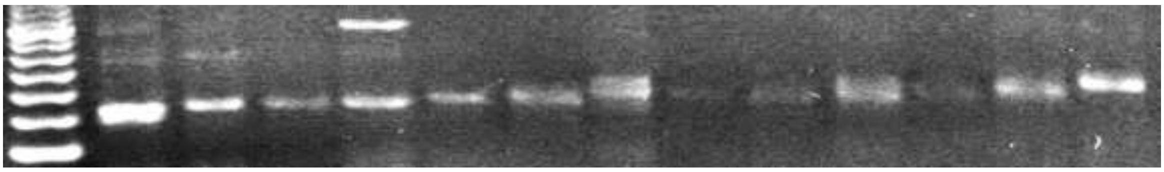
Table 2. Power of discrimination (PD)

Loci	PD
PTR1	0.769231
PTR2	0.528926
PTR3	0.793388
PTR4	0
PTR5	0.686391
PTR6	0.769231
PTR11	0.131944
PTR12	0
PTR14	0.810651
PTR15	0.641975

Loci, PTR3 and PTR14, were found to be the most informative one for species differentiation, whereas PTR11 was the least informative locus. Regarding that there was no amplification record on loci PTR4 and PTR12, power of discrimination designated as „0”. Figure 1 showed PCR products amplified using PTR1, PTR4, PTR11, PTR14, PTR15 SSR loci with: 1. B81 (*Populus deltoides*), 2. B229 (*Populus deltoides*), 3. Pannonia – M1 (*Px euramericana*, 4. 182/81 (*Populus deltoides*), 5. PE19/66 Neoplanta (*Populus deltoides*), 6. NS001 (*Populus nigra* L.), 7. NS002 (*Populus nigra* L.), 8. I-2/4 (*Px euramericana*), 9. 102/81 (*P. nigra x P. maximowiczii*), 10. 9111/93 (*P. nigra x P. maximowiczii*) x *P. italica*, 11. *P. italica*, 12. 1007(*P. trichocarpa*), 13. 1004 (*P. trichocarpa*) DNA. Lane M is a 50bp ladder DNA size marker (Fermentas). The figures were selected upon their representative polymorphic patterns where was undoubtedly detected the polymorphism of the particular locus due to presence or absence of the DNA fragment.

PTR1

kb M 1 2 3 4 5 6 7 8 9 1 11 12 13



0,2

PTR4

kb M 1 2 3 4 5 6 7 8 9 1 11 12 13

0,2

PTR11

kb M 1 2 3 4 5 6 7 8 9 1 11 12 13

0,2

PTR14

kb M 1 2 3 4 5 6 7 8 9 1 11 12 13

0,2

PTR15

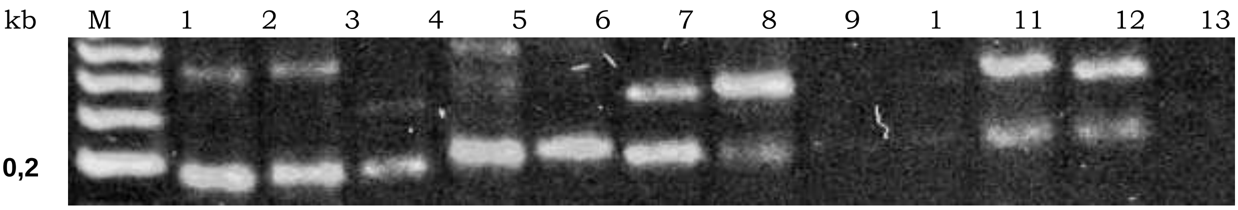


Figure 1. PCR products amplified using PTR1, PTR4, PTR11, PTR14, PTR15 SSR loci with 1. B81 (Populus deltoides), 2. B229 (Populus deltoides), 3. Pannonia - M1 (Px euramericana, 4. 182/81 (Populus deltoides), 5. PE19/66 Neoplanta (Populus deltoides), 6. NS001 (Populus nigra L.), 7. NS002 (Populus nigra L.), 8. I-2/4 (Px euramericana), 9. 102/81 (P. nigra x P. maximowiczii), 10. 9111/93 (P. nigra x P. maximowiczii) x P. italica, 11. P. italica, 12. 1007(P. trichocarpa), 13. 1004 (P. trichocarpa) DNA. Lane M is a 50bp ladder DNA size marker (Fermentas).

Based on the SSR data obtained from ten polymorphic loci, according to Jaccard's coefficient of similarities (Tab. 3), *Populus* species were discriminated into four different clusters, as shown in the UPGMA tree (Fig. 2)

Table 3. Jaccards' distances for the examined poplar genotypes

Genotypes	**B-81**	**B229**	**Pannonia**	**182/81**	**PD100**	**NS001**	**NS002**	**I-214**	**102/81**	**9111/93**	**P.italica**	**1007**	**1004**
B-81	0.000	0.583	0.938	0.786	0.786	1.000	0.846	0.929	0.818	0.938	0.917	0.846	0.846
B-229	0.583	0.000	0.875	0.364	0.615	0.933	0.857	0.857	0.727	0.875	0.833	0.769	0.769
Pannonia	0.938	0.875	0.000	0.800	0.800	0.875	0.714	0.769	0.833	0.824	0.769	0.857	0.769
182/81	0.786	0.364	0.800	0.000	0.714	0.933	0.857	0.769	0.833	0.875	0.833	0.857	0.857
PD100	0.786	0.615	0.800	0.714	0.000	0.769	0.769	0.769	0.444	0.714	0.727	0.667	0.545
NS001	1.000	0.933	0.875	0.933	0.769	0.000	0.400	0.727	0.800	0.615	0.444	0.833	0.833
NS002	0.846	0.857	0.714	0.857	0.769	0.400	0.000	0.600	0.800	0.714	0.545	0.727	0.727
I-214	0.929	0.857	0.769	0.769	0.769	0.727	0.600	0.000	0.833	0.857	0.667	0.769	0.769
102/81	0.818	0.727	0.833	0.833	0.444	0.800	0.800	0.833	0.000	0.833	0.667	0.444	0.444
9111/93	0.938	0.875	0.824	0.875	0.714	0.615	0.714	0.857	0.833	0.000	0.545	0.857	0.667
P. italica	0.917	0.833	0.769	0.833	0.727	0.444	0.545	0.667	0.667	0.545	0.000	0.800	0.667
1007	0.846	0.769	0.857	0.857	0.667	0.833	0.727	0.769	0.444	0.857	0.800	0.000	0.545
1004	0.846	0.769	0.769	0.857	0.545	0.833	0.727	0.769	0.444	0.667	0.667	0.545	0.000

First group shared all *P. deltoides* species (B81, B229, 182/81) from Aigeiros section. The second group represents cultivar Pannonija (*Px euramericana*), Aigeiros section with the highest Jaccards coefficient. The third group obtained clone PD100 which belongs to *Populus deltoides*, hybrid 102/81 (*P. nigra x P. maximowiczii*), clone 1007 (*P. trichocarpa*) and clone 1004 (*P. trichocarpa*). Here is interesting to point out that there is a close relations of PD100 *P. deltoides* clone (which belongs to Aigeiros section) with clones 1007 and 1004 of *P. trichocarpa* species that belongs to Tacamahaca section. All others belongs to fourth group of *P. nigra* species, Aigeiros section.

This data could be in accordance with the observations of Hamzeh, 2004 and Cervera, 2005 who suggest that the genomic segments containing the SSR loci developed for *P. nigra* are homologous in the *Populus* species examined in the present study. They stated that the section Aigeiros has a close affinity to the lineages of section Tacamahaca. Eckenwalder, 1996 was also suggested a close association of two sections and mentioned that this close affinity gives the high informativeness of the *P. nigra* SSR markers and therefore the similarities between two sections is not unexpected.

The results in this paper shown that SSR markers developed for P*opulus tremuloides* are also informative for *Populus nigra*. This study proved that the microsatellite DNA markers used for evaluating DNA profiles could make differentiation of selected clones, cultivars and their hybrids from two sections of genus Populus, and determine their molecular genetic interrelationships.

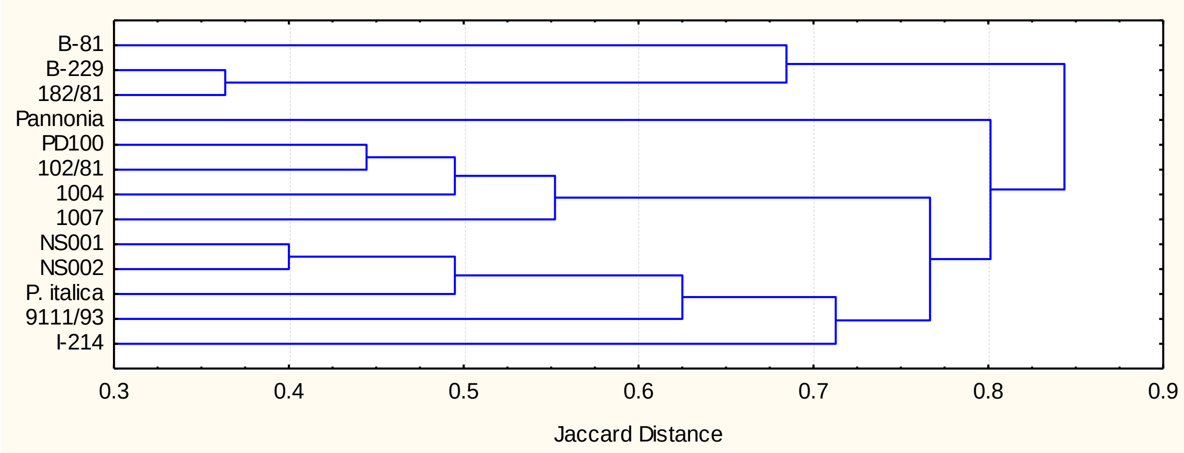


Figure 2. Cluster analysis, UPMGA – linkage method, for examined poplar genotypes

CONCLUSIONS

All SSR markers, except PTR7 and PTR8, from genus *Populus* have been successfully amplified. Out of 12, ten loci were polymorphic, two monomorphic for all genotypes analysed. Number of alleles per locus varied from 1-6. In average number of alleles per locus was 2. Jaccard's coefficient of similarities discriminated all genotypes under observation in four distinct groups. First group shared all *P. deltoides* species (B81, B229, 182/81) from Aigeiros section. The second group represents cultivar Pannonija (*Px euramericana*), Aigeiros section with the highest Jaccards coefficient. The third group obtained clone PD100 which belongs to *Populus deltoides*, hybrid 102/81 (*P. nigra x P. maximowiczii*), clone 1007 (*P. trichocarpa*) and clone 1004 (*P. trichocarpa*). Here is interesting to point out that there is a close relations of clone PD100 *P. deltoides* (which belongs to Aigeiros section) with clones 1007 and 1004 of *P. trichocarpa* species that belongs to Tacamahaca section. All others belongs to fourth group in *P. nigra* species Aigeiros section.

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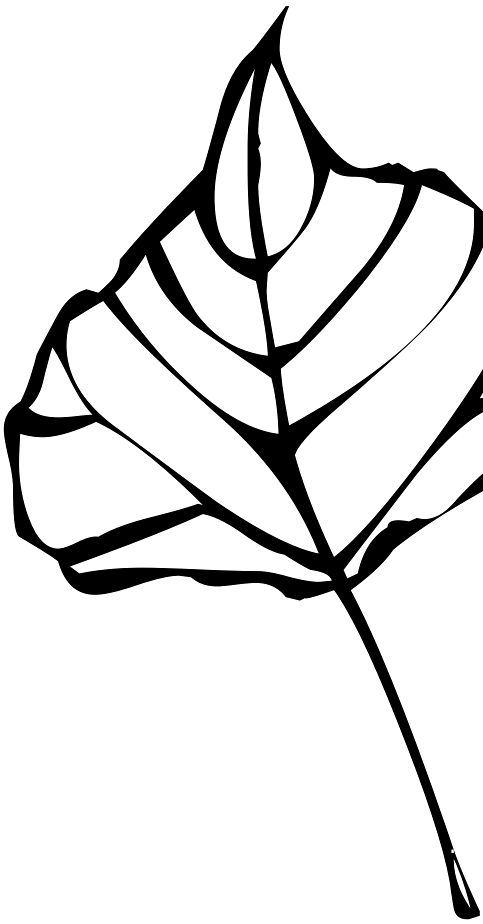

POSSIBILITIES OF HARDWOODS AND CONIFEROUS SEEDLINGS MYCORRHIZATION IN THE FIELD CONDITIONS IN PODGORICA

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Summary: *In natural soils, all forest trees form symbiotic, mutually beneficial associations between their roots and specialized soil fungi, called mycorrhiza. Mycorrhiza provides many benefits to the seedlings and adult tree, especially in enhancing water and nutrient uptake.*
Soil and climate conditions of Montenegro demand paying special attention to plant material production, used both for reforestation, forest regeneration and recultivation of anthropogenically degraded areas. There should also keep in mind forthcoming climate changes, global warming with higher air temperatures and droughts.
Seedling mycorrhization experiments have started in 2005. and 2006., in the open field in Podgorica.
Mycorrhization enclosed:

- *eight coniferous tree species: Pinus nigra Aiton, Pinus sylvestris L., Pinus heldreichii Christ., Pinus halepensis Mill, Picea abies (L.)H. Karst., Picea omorika (Pancic.) Purk., Abies alba Mill., Pseudotsuga mensiesii (Mirb.)Franko.,*
- *five hardwood tree species: Acer dasycarpum Erth., Celtis australis L., Liriodendron tulipifera L, Platanus x acerifolia hort., Ulmus x hollandica hort characterised by vesicular-arbuscular mycorrhiza, and also hardwood Quercus ilex L., characterised by ectomycorrhiza.*

Mycorrhized seedlings have faster growth and better developed root system. Differences between treated and control seedlings depend on examined species and, generally, become more noticeable on hardwoods ones. They are more evident with seedlings age, especially on conifers where they become clearer only in second year after sowing.
Cultivation of coniferous in open field in Podgorica is troublous, faced with lot of problems, and hardly sustainable. Mycorrization of coniferous were not equal on all plants, but could be treated as successful. The best results were obtained in mycorrhization of Picea omorika and Pinus halepensis. For achieving good results of mycorrization on open field, it is recommendable to use higher dosage of fungal inoculum.
Chosen hardwood species tolerate much better cultivating conditions in Podgorica's climate in open, and have very good reaction on mycorrhization- with faster growth and much more developed root system.

INTRODUCTION

Seedling production is one of the most important areas concerning forestry techniques in whole, since both successful forestation and quick development highly depend on the seedling quality. The seedlings quality is being assessed by the evaluation of both morphological and physiological parameters which allows an anticipation of the success of the field installation. This also depends on the seed genetic information, production conditions, handling of the seedlings and the plantation site (Claro et al, 1998).

Foresters and nursery managers are well aware of the critical stress period that seedlings experience during transplanting. Thus, it is of outmost priority that nursery grows and sends to the reforestation site properly prepared plants. Soil and climate conditions of Montenegro demand paying special attention on plant material production, used both for reforestation, forest regeneration and recultivation of anthropogenically degraded areas.

In natural soils, all forest trees form symbiotic, mutually beneficial associations between their roots and specialized soil fungi, called mycorrhiza. Mycorrhiza provides many benefits to the seedlings and adult tree. Mycorrhiza ameliorate the physiological status on seedlings mainly by improving water and nutrient uptake from the soil (Smith & Read, 1977, cit. Rincon et al., 2001) and can play an important role in the protection of plants against environmental stress factors such as drought, pathogenic agents or heavy metal pollutions (Marx, 1973; Boyd & Hellebrand, 1991; Leyval et al., 1997, cit Rincon et al., 2001). Seedlings without mycorrhiza will have to form it before the seedlings begin to actively take up water and nutrients from the soil. Seedlings with mycorrhiza are better

prepared to immediately begin the soil exploration and so stand a better chance for survival than nonmycorrhizal seedlings. (Castellano & Molina, 1989) Controlled inoculation techniques are useful as an additional nursery culture method to increase field performance of out-planting seedlings (Cordell et al, 1987, Mousian et al., 1987., cit. Rincon et al, 2001)
Two major mycorrhizal types, that prevail among forest trees, are: ectomycorrhiza, formed with the important coniferous species of the *Pinaceae* and hardwoods in the *Fagaceae* and *Betulaceae*; and vesicular-arbuscular (VA) mycorrhiza, common on the other hardwoods.

Controlled inoculation is usually conducted in sheltered areas for nursery production, such as greenhouses. In that case, control and management of environmental parameters, like temperature and moisture, are possible. That ensures equal germination of seed and seedling development, but also affect on development and survival of fungal symbiont, process of mycorrhization in whole, and later mycorrhizal development.

Due to lack of such areas for nursery production, we tried to make seedling mycorrhization of few coniferous and deciduous tree species in the open field condition. We relied upon submediterranean climate of Podgorica, primarily on relatively high and uniform air temperature in spring period.

MATERIAL AND METHODS

Seedling mycorrhization experiments have started in 2005. and 2006. in the open fild in Podgorica. Mycorrhization enclosed eight coniferous tree species: *Pinus nigra* Aiton., *Pinus sylvestris* L., *Pinus heldreichii* Christ., *Pinus halepensis* Mill., *Picea abies* (L.)H. Karst, *Picea omorika* (Pancic)Purk, *Abies alba* Mill., *Pseudotsuga mensiesii* (Mirb.)Franko, and also hardwood *Quercus ilex* L., characterised by ectomycorrhiza.and five hardwood tree species: *Acer dasycarpum* Ehrh., *Celtis australis* L., *Liriodendron tulipifera* L., *Platanus x acerifolia* hort., *Ulmus x hollandica* hort. characterised by vesicular-arbuscular mycorrhiza.
Plant material:

For sowing were used *P. abies* and *P omorika* seeds, which were collected in NP Tara (Srbija) (2005), *P. nigra* in Prijepolje (Srbija) (2005), *A. alba*, *P. menziesii* and *P. heldreichii* in Kolašin (Montenegro) (2005), and also certified seed *P. sylvestris* from Slovenija («Mengeš»). *A. dasycarpum* and *U. hollandica* seeds were collected in Belgrade (IV 2006), and *C. australis* (2005), *L. tulipifera* (X 2004), *P. acerifolia* (II 2006) and *Q. ilex*(X 2005) in Podgorica.
Sowings were done in mixture of peat (Floragard, TKS 1 or Gramaflor, Gramaflor Vertriebs, Germany): Sand : Perlite in 3:2:1 proportion. Basely the same substrate was used for replanting.

«Bosna plast» containers (with 53 planting sites) were used for conifers. Sowing were done from 10.-12. 04. 2006. in Podgorica. Before germination seeds of *P. nigra*, *P. sylvestris*, *P. abies* and *P. omorika* were rinsed in Perfit solution (1:8 - 3% H_2O_2), in 30 min duration. After that seeds were washed and sowed. *A. alba* and *P. menziesii* seeds were rinsed in tap water over 24 h, and after that treated with Perfit, as described for previous seeds. Preventive treatment with Befungin WP-50 in conc. 0.05% was done after sowing. *P. nigra* seedlings were replanted in 2007. in 0.8 l pots. Most of *P. nigra* seedlings dried during the summer of 2008.

«Bosna plast» containers (with 53 planting sites) were used for *Q. ilex* and *U. holandica*, also. Sowing was done in April 2006. in Podgorica. *A. dasycarpum* (16. V. 2006), *L. tulipifera* (IV. 2005.) were sowed in woody boxes, and *P. acerifolia* (26.III 2006.) was sowed in boxes under the glass. Before germination seeds of hardwoods were rinsed in Perfit solution (1:8 - 3% H_2O_2), in 30 min duration. Preventive treatment with Mankogal-80 in conc. 0.25% was done after sowing. In 3 week period (*A. dasycarpum* - 2.VI) to 3 months (*P.acerifolia* 10-18. VI) after sowing seedlings were replanted in 0.3-0.5 l pots. During the next year, *A. dasycarpum*, *C. australis* and *L. tulipifera* were replanted in 1-2 l plastic bags, and *P. acerifolia* in 0.8 l pots. Most of *L. tulipifera* and *C. australis* plants dried during the summer of 2008.

Fungal inocula and inoculation:

Coniferous and *Q. ilex* were inoculated with Aegis-Ecto, Sygenta (Spain), composed of *Rhizopogon* sp., *Pisolithus* sp, *Scleroderma* sp., with more then 10^7 spore/ plant (1 g of inoculums). Inoculation of substrate had been done (measurement of substrate mixture enough for filling of one container was done, and 53 g of inoculums were added), and seed sowed in inoculated substrate.

Hardwoods were inoculated with vesicular-arbuscular mycorrhizal inokulum Aegis, Sygenta (Spain), composed of *Glomes intraradices* and *Glomes mosseae* propagula, (100 infective propagule of fungus per ml). *C. australis, L. tulipifera, P. acerifolia* and *U. hollandica* was inoculated with 5 ml of inoculums - 500 infective propagule per plant. *A. dasycarpum* was inoculated with 3 different doses of inoculums per plant: 5 ml, 7.5 ml, 10 ml. Inoculation of plants was done during the replanting of plants in plastic bags or pots except in *U. hollandica*, where inoculation of substrate was done and seeds sowed in inoculated substrate.

Plants were shaded, watered when needed: daily - 3 times per week during the summer, late spring and early autumn, and fertilized 3- 4 times during the season with water soluble fertilizer Polyfeed 17:10:27+me.

Culture conditions:

Seedlings were cultivated in the field conditions in Podgorica (Stari aerodrom 2005, 2006, 2007, and Ljeskopolje 2008). But, because of very hard climatic conditions in Podgorica, *P. abies, P. omorika, A. alba, P. mensiesii* were cultivated in Medun locality, nearby Podgorica, on cca 400m altitude.

According the HMI of Montenegro data, average yearly air temperature in Podgorica is 15,4˚ C (1961-2000), i.e.15,8 ˚ C in 2006th, i 17˚ C in 2007 th. (Average seasonal temperatures, as indicator of thermal characteristic of place, are for summer (very hot) 25,1˚ C, winter (mild) 6,2˚ C, autumn 16,0˚ C and spring14,3˚ C.)

Av. month air temperatures in Podgorica are pretty equal, and for first half of vegetation season are- for March: av.10,0; av.. max 15,1; av. min 5,1˚ C; April: av. 13,9; av. max 19,1; av. min 9,0˚ C; May: av. 19,1;av. max 24,5; av. min 13,7˚ C, June: av. 23,2; av.max 28,8; av. min 17,6˚ C.

Average year precipitation in Podgorica is 1637,4 mm (1961-2000), but summer precipitation is only 10 % of all, and only 2 % in July (37, 8 mm av., and only 0.2 mm in 2007.). Average yearly humidity is 64.7%, minimally in July-51.2 %, and maximally in November-74.3%. Average year duration of sun light is 2477,1 h, what means 55.6% of possible. During the summer number of sunny hours is very big 10,1 hour daily, i.e.69,1 % of possible. The most frequent is north wind (13.8 % of total number of frequent directions during the year, with 3.3 m/s), then south-southeast wind (11.1%, with 2.1 m/s).

Measured parameters:

Coniferous plants were measured for stem height and root collar diameter (mm), total dry weight, shoot and root dry weight at the end of each growing season (except *A. alba*, *P. mensiesii and P. heldreichii*, which were measured at the end of 3rd , because of small number of inoculated plants. Sample for measurement of coniferous were 10 plants per treatment. Stem height and root collar diameter of hardwoods were measured on 15-30 plants at the end of each growing season, and dry weight on 5 plants per treatment, at the end of third growing season. (except *A. dasycarpum*, at the end of first and third growing season) There were calculated average values of measured parameters.

Samples were prepared for measuring; root was rinsed in water and carefully cleaned from substrate. Dry weight (g) of one year old plants were measured after oven dried on 65 °C, 18 h, two and three years old coniferous and three years old hardwoods after oven dried on 80˚ C, 48 h, and three years old *A. dasycarpum* after 72 h on 80˚ C. Dry weights of coniferous in 1st and 2nd growing season was measured on 0.0001 g accurency, and on 0.001g accurency in 3rd. Rounded on 0.01g, which were shown in tables, were done after calculation of average values. Shoot vs. root ratio was calculated.

Quantitative assessment of ectomycorrhiza was done for coniferous. Entire root systems were examined at 5 X magnification and the percentage of all short roots with ectomycorrhiza is visually estimated.It was graded as excellent ++++ (75-100%), good +++ (50-74%), moderate ++ (24-49 %), poor + (1-24 %) (Marx et all, 1994)

RESULTS

Seedling growth characteristics and ectomycorrhiza development of coniferous species during three years after sowing and inoculation were shown in Table 1. and Table 2. Differences between treated and control seedlings, as expected, depend on examined species and they are more evident with seedling age. They become clearer only in second year after inoculation. Percentage of root mycorization also rises with seedling age.

But, mycorryization was not equal on all treated seedlings, what was evidenced on *P. halepensis* and *P. omorika*, but also on *P. sylvestris*, *P. abies P. menziesii.* Among treated plants of mentioned species there were two groups of plants, one (group I) where plants were bigger (shoot high, root collar diameter, needle length, but also branching, and branch length in *P. omorika*). Root analysis of those plants shoved much more developed mycorrhiza on them, with dense and obvious mycelium of fungal symbiont on plant root and substrate. Differences in size and mycorrization degree among the treated plants of *P. omorika* and *P. halepensis* three years after sowing, are shown in table 3.

Influence of mycorrhization on growth of hardwood seedlings was obvious after one growing season. Differences in size between treated and controlled plants are more evident with seedling age. In all examined hardwood species inoculated with VAM, except obviously better growth in stem height and depth, bigger leaf mass (which we were not measured, but evident), much more developed root was reported.

Table 1. Seedling growth characteristics and ectomycorrhiza development of some coniferous species during three years after sowing and inoculation

Plant species	year	treat	shoot high	diam	dry weight	shoot dry w	root dry w	s/r	myco-rrhizaton
			mm		g				
P. nigra	2006	T	85.82	1.27	0.27	0.19	0.10	1.90	++
		0	82.11	1.16	0.22	0.17	0.07	2.43	
	2007	T	123.3	2.70	2,04	1.44	0.60	2.40	++
		0	115.0	2.57	1.78	1.10	0.68	1.62	
P. sylvestris	2006	T	99.12	1.14	0.17	0.13	0.04	3.25	++
		0	87.44	1.04	0.13	0.10	0.03	3.33	
	2007	T	155.0	1.92	1.96	1,15	0.81	1.42	++
		0	122.0	1.58	0.66	0.45	0.21	2.14	
	2008	T	219.38	0.29	2.13	1.58	0.55	2.93	+++
		0	182.00	0.20	1.18	0.82	0.36	2.34	
P. halepensis	2006	T	183.75	1.70	0.40	0.32	0.08	4.00	++
		0	137.50	1.35	0.26	0.22	0.04	5.50	
	2007	T	255.1	2	1.87	1.25	0.62	2.02	+++
		0	159.25	1.65	1.26	0.88	0.38	2.32	
	2008	T	213.63	2.9	2.57	1.83	0.66	2.77	+++
		0	230.00	2.2	1.28	0.84	0.36	2.33	
P.abies	2006	T	77.55	1.09	0.20	0.12	0.15	0.80	+
		0	64.08	1.02	0.15	0.09	0.06	1.50	
	2007	T	96.25	1.39	0.55	0.29	0.25	1.16	++
		0	83.33	1.38	0.46	0.24	0.21	1.14	
	2008	T	127.00	2.2	1.29	0.75	0.54	1.39	++
		0	89.64	1.40	0.54	0.30	0.24	1.25	
P.omorika	2006	T	63.00	0.82	0.16	0.08	0.08	1.00	+
		0	60.62	0.92	0.11	0.07	0.04	1.75	
	2007	T	87.27	1.44	0.50	0.28	0.22	1.27	++
		0	75.00	1.07	0.34	0.17	0.18	0.94	
	2008	T	152.14	2.1	2.03	1.22	0.81	1.51	+++
		0	97.00	1.3	0.72	0.40	0.32	1.25	

Effects of mycorrhization were not so evident on *Q. ilex* (ECM). There are some differences in size of plants, treated ones are little bigger on shoot and also on plant root

L. tulipifera and *P. acerifolia* grow unequally from the start, probably because of plants genetics. Because of that, sizes in treated and also in controlled plants *L. tulipifera* are very variable, and shoot high differences are about 30 cm (in 2nd year). In 2nd year we examined only well grown *P. acerifolia.*

Seedling growth characteristics of hardwoods during three years after sowing and inoculation were shown in Table 4 and Table 5.

Table 2. Seedling growth characteristics and ectomycorrhiza development of some coniferous species three years after sowing and inoculation

Plant species	treat	shoot high	diam.	dry weight	shoot dry w	root dry w	s/r	myco-rrhizaton
		mm		g				
P. heldreichii	T	108.75	0.21	1.27	0.75	0.52	1.44	++++
	0	103.33	0.17	0.80	0.46	0.34	1.31	+
A. alba	T	125.00	0.20	1.07	0.57	0.50	1.14	+++
	0	101.25	0.17	0.68	0.40	0.28	1.43	
P. mensiessi	T	137.50	0.18	1.00	0.49	0.50	0.98	++
	0	83.00	0.16	0.60	0.31	0.29	1.07	

Table 3. Differences in size and mycorrhization among the treated seedlings of *P. omorika* and *P. halepensis* three years after sowing and inoculation

Plant sp.	group	shoot high	diam.	dry weight	shoot dry w.	root dry w	s/r	myco-rrhizaton
		mm		g				
P. omorika	T-I	185	2.9	3,4	2,1	1,3	1.62	++++
	T-II	145	2.0	1,5	0,8	0,7	1.14	+++
	0	105	1.4	0,95	0,55	0,4	1.38	
P. halepensis	T-I	310	3.5	3.40	2.60	0.80	3.25	++++
	T-II	275	2.0	1.70	1.25	0.45	2.78	++
	0	210	1.9	0.97	0.74	0.23	3.22	

Table 4. Seedling growth characteristics (stem height, root collar diam,) of hardwoods during three years after sowing and inoculation (all measured values are in mm)

Plant sp.	tr	2006		2007	
		height	diam	height	diam
*L. tulipifera**	T	320.5	6.3	802.0	15
	0	205.0	4.7	686.5	15
*C. australis**	T	276.0	3	740	20.5
	0	150	1,4	660	20

Plant sp.	tr	2006		2007		2008	
		height	diam	height	diam	height	diam
P. acerifolia	T	108.2	3	204.6	4.1	48.33	6.2
	0	75.2	3	170	3.5	39.90	3.5
U. holandica	T	185	2	248.5	2.4	65.84	6.8
	0	156	1.5	198	2.2	48.7	4.9
Q. ilex	T	135	2.4	268	4.8	50.12	7.5
	0	107.9	2	216.5	4.8	45.2	6.5

* sowing in 2005.
all measures (values) in mm
Each value represents a mean of 15 seedlings.

Results of *A. dasycarpum* mycorrhization were interesting, raising the question of optimal dose of myccorhizal inoculums in different plant species. A. dasycarpum seedlings inoculated with 5 ml of VAM inoculums shoved just a little better growth then controlled plants. Growth of plants which were treated with 7.5 and 10 ml of VAM inoculums were much better and differences between those two treatments were small. In first year plants treated with 7.5 ml of VAM inoculum grew better, so in that case we could recommend this, 7.5 treatment.

Table 5. Seedling growth characteristics of U. *hollandica*, *P. acerifolia*, and *Q. ilex* 3 years after sowing and inoculation. (measured values are in mm)

Plant species	treat	shoot height	diam.	dry weight	shoot dry w	root dry w	s/r
U. hollandica	T	640	6.6	17.15	5.33	11.82	0.45
	0	430	4.3	6.21	1.59	4.62	0.34
P. acerifolia	T	400	6.4	9.55	3.88	5.67	0.68
	0	358	3.6	2.36	0.82	1.54	0.53
Plant species	treat	shoot height	diam.	shoot dry weight	steam dry w.	root dry weight	leaf dry weight
Q. ilex	T	455	6.8	9.06	4.72	5.80	4.36
	0	460	6.4	7.88	4.28	4.89	3.59

Each value represents a mean of 5 chosen seedlings.

Table 6. Seedling growth characteristics of A. *dasycarpum* during 3 years after sowing and inoculation with different inoculation dose

year	treat	shoot height	diam.	shoot dry weight	root dry weight	s/r
		mm		g		
2006	10 ml	386.7	5.5	1.60	1.75	0.91
	7.5 ml	388.3	4.5	1.37	1.11	1.23
	5ml	313.3	4.17	0.77	0.55	1.4
	0	268	4.17	0.75	0.48	1.56
2007	10 ml	607	7.5	-	-	-
	7.5 ml	665	7.5	-	-	-
	5ml	477.5	5	-	-	-
	0	490.7	4.5	-	-	-
2008	10 ml	852.2	9.2	16.03	17.12	0.94
	7.5 ml	838.7	8.7	10.15	14.00	0.73
	5ml	688.7	7.3	5.38	8.30	0.65
	0	661.6	8.6	5.30	8.50	0.62

DISCUSSION

This investigation was primarily aimed to the possibilities of seedlings mycorrhization of tree species in open field conditions, and also mycorrhization in general. So, *A. alba*, *P.abies*, *P. omorika* and *P. mensiesii* are the three species which hardly could be developed and cultured in Podgorica, or in conditions of submediterranean climate at all. They were used only in experimental purposes. Because of not enough known effects of fertilizing, and also concrete fertilizer on mycorrhiza development, their application during this test were small, what had a negative consequences mostly on development of conifers.

For culturing the trees in open field conditions in Podgorica, shading (late May-October) is necessary and also generous watering during the summer. Because of small volume of planting pots (and containers) during the warmest July and August days those daily watering could also be not sufficient.

P. nigra seedlings in 1st and 2nd year after sowing reach sizes adequate for I qualitative class (JUS). *P. sylvestris*, *P. abies* and *P. halepensis* seedlings in 1st and 2nd year have good growth in height, belonging to I qualitative class, but root collar diameter to II qualitative class. In 3rd year seedlings slow down in growth and should be replanted. *P. omorika* in 2nd year in treatment and control belonged to II qualitative class, but in 3rd reached sizes proposed for I qualitative class. *A. alba* in 3rd year have height adequate for I qualitative class, but collar root diameter for II. Douglas fir seedlings were below the standard.

Mycorrhization of coniferous and hardwoods seedlings in the field conditions was successful.

It was known that mycorrhization depend on environmental conditions: temperature, humidity, mechanical features and aeration of substrata. It could be assumed that relatively high and uniform spring and summer temperatures of air influenced on substrate temperature. They could be important in first few months after inoculation and could positively influence growth of fungal symbiont and mycorrhizal establishment, and later development in whole. Some mycorrhizal fungi will tolerate wide temperature fluctuation during seedling production, others will not. Some mycorrhizal fungi grew well and formed abundant ectomycorrhizae at 20-25˚C, but *Pisollithus tinctorius* also at 40˚ C. (Castellano, Molina, 1989).

Mycorrhization did not have same success, and mycorrhiza was not equally developed in all treated coniferous. It is obvious that some plants have better established symbiosis with inoculated fungi. In that case plants were bigger, and mycorrhiza on their roots was obvious and abundant (mantle, mycelium, rhizomorphae). It is especially in case of *P. halepensis* and *P. omorika*. It also could be said that mycorrhization have greater success in case of this two species. Differences in mycorization among treated plants could be consequence of not equal environmental conditions (bigger plants are mostly grouped in the middle of containers, where the fluctuations in substrate temperature and moisture are smaller), but also in inoculation technique - way on which inoculation was done (mix of inoculums with substrate measured for entire container). In case that the mycorrhization could be carried in open field we suppose that quantity of inoculums per plant could be increased.

Effects in VAM inoculation was pretty good. Mycorrhizal inoculums in case of those plants were applied in higer doses, but also in each plant alone. Investigation of effective dosage of mycorrhizal inoculums for certain tree species started with *A. dasycarpum*, and could be continued with the others. Treated hardwood plants, which we planted on terrain during 2008, showed good reception_and excellent and fast growth, which could be also considered as good mycorrhization consequence. Chosen hardwood species much better tolerate cultivating conditions in Podgorica in open, so their production seems to be more certain.

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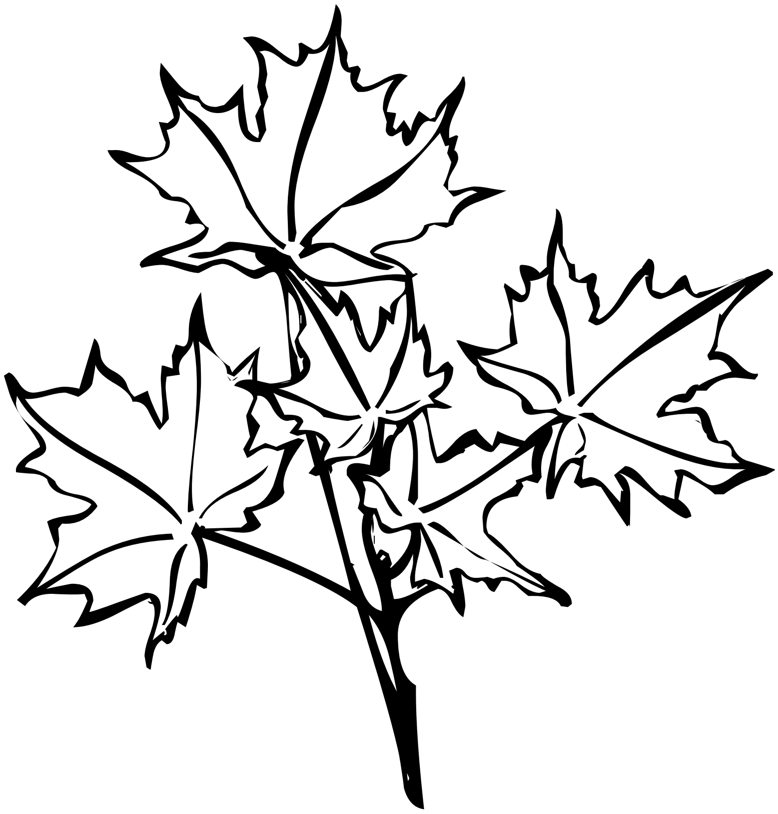

PRODUCTION CHARACTERISTICS OF BLACK POPLAR CLONES ON THE RIVER SAVA INUNDATION

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Abstract: *Results from studies associated with production and structural stand characteristics of registered black poplar clones (I-214, 618, 457, 55/65 i S_{6-36}) after 20 and 30 years of stand development on Sava river alluvium soil are published in this paper. Experimental stand was established in three replicates at planting distance of 4,25 × 4,25 m, and nine years later the mainly shematic thinning out was conveyed, so the planting distance was brought down to 6 x 6 m.*
Twenty years later no significant differences in elements of stand growth ($d_{g20\%}$, d_g, g_s, N, G) were found. Determined mean basal area diameters ranging from 31.7-33.3 cm, mean heights from 30-32 m, basal areas from 21.1-23.8 $m^2 \cdot ha^{-1}$, and volumes from 278.7-314.1 $m^3 \cdot ha^{-1}$ with high survival rate after 20 years pointed out to significant production potential of all studied clones.
Thirty years after development of the stand, the studied clones had similar mean elements of the growth ($d_{g20\%}$, d_g, g_s), while difference found in elements of stand growth (N, G, V) was due to decreased number of trees in the period between 20 and 30 years. Achieved values of mean basal area diameters ranging from 38.1-40.4 cm, mean heights from 37.0-37.9 m, basal areas from 28.5-30.5 $m^2 \cdot ha^{-1}$ and volumes from 495-585 $m^3 \cdot ha^{-1}$ after 30 years of investigation confirmed the high production potential of studied clones under the conditions of Sava river alluvium soil.
Numerical indicators of diameter structure showed smaller variability (9.7-13.7%) and left asimmetry in all clones. In clones 457 and S_{6-36} similar variability of diameters at breasts height was determined. There was a significant difference in flat thickness structure 30 years upon stand establishment.
Difference between studied clones in the structure of tree number, basal area and volume per diameter classes was determined using Kolmogorov-Smirnov test. The same test was used to confirm significant retardation of clone 618 structure in comparison to clones structure I-214, 55/65 and 457.
Results of investigation confirmed high production potential of registered poplar tree varieties (I-214, 618, 457, 55/65, S_{6-36}). Clones achieved similar mean elements of stand growth, while difference was found in stand structure, so the significance of knowing structural characteristics of stands of black poplar tree clones was pronounced.
Key words: *poplar, clones, wood volume production, stand structure*

INTRODUCTION

The basic parameters controlling poplar tree production are the following: choice of variety (clone), choice of habitat and appropriate technology for establishment, care and protection of poplar stand. All the above mentioned parameters are mutually depended, and the proper poplar variety choice is the basic prerequisite for optimal usage of potential habitats. Chosen variety should have a high genetic potential in regard to production and adaptibility to habitat conditions in inundations, tolerance to economically significant pathogens and pests, as well as the ability for successful rooting of reproductive and planting material.

Relatively wide range of potential habitats for growing poplars demands knowledge about their basic characteristics as the starting elements for choice of variety (clone), and the choice of appropriate technology for establishment and proper care of stand, intention, i.e. the purpose of production.

In our region poplars are the types of trees characteristic of the highest growing potential of wood volume. Significant usage of poplar trees was initiated by artificial afforestation with first cultivars after the I World War. With growing demand for poplar wood after the II World War, first of all the wood for chemical industry (Guzina, et al, 1991/b), a significant swing was achieved by introduction and mass expanssion of new poplar cultivars (Serotina, Marilandica, Regenerata, Robusta). Introduction of new poplar varieties back then contributed to significant increase of product assortment, as well as the increase of poplar stand value in relation to natural poplar forests.

Mass establishment of poplar stands with one cultivar (clone) caused development of ecosystems with monoclonal stands on large areas which never existed in natural ecosystems until then. Very soon, after some 10-15 years from introduction of new cultivars first signs of biological instability of poplar stands, even dying off of the whole stands, especially if established on inappropriate soils started to appear.

The only solution was to introduce new, Italian clones during the sixties of the last century. Among them the clone I-214 (*Populus* × *euramericana* (Dode) Guinier), which had very high production potential, and was very resistant to pathogen was the most widely spread. Mass expansion of clone I-214 on large areas caused gaining gradual susceptibility to pathogens of the cortical tissue (*Dothichiza populea* Sacc. et Br.), and leaf (*Melampsora sp.* and *Marssonina brunnea* Ell. et Ev.). Culmination took place in 1977-78 when epiphytotia of *Dothichiza populea* Sacc. et Br. pathogen appeared on large areas (Marinković, 1980).

During that period a significant contribution to solving of the above mentioned problems was given by the Poplar Research Institute which developed and introduced new clones of Eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.). First official registration of domesticated varieties (I-214, I-45/54, Ostia, Robusta), and new varieties of Eastern cottonwood (varieties 618, 457, 450, 55/65 and 725) started in 1980. After that registration of new poplar varieties in 1987 (varieties S_{1-8}, S_{6-36}, S_{1-20}), in 1998 (varieties NS_{11-8}, NS_{1-3}, Pannonia (M-1)), and in 2004 (varieties Novi Sad 1, Novi Sad 2) followed.

By introduction of new varieties into production significant improvements in regard to usage of poplar genetic potential were achieved. However, it was also revealed that genotypes used in production over long period were greatly endangered by the harmful effects of micoflora and entomofauna, and their genetic potential was lessened as the time passed. It was especially pronounced in the cases where small number of genotypes was used in production.

Long-term solutions of the above mentioned problems could be achieved by introducing into production new, experimentally tested varieties in combination with several varieties in stands. Such decisions were based on the view that no production of poplar clones which would mean a permanent solution was expected in the near future in regard to resistance to relatively large number of potential provokers of diseases and damages, i.e. which would mean the permanent solution in regard to biological stability of the stand. Permanent security of the production in poplar stands could thus be achieved only by using larger number of resistant varieties (clones) having great genetic production potential. For that reason the forest practice should be provided by reasonably greater number of resistant varieties (clones) with desirable production traits (some of them would have diminished production potential compared to the clones used up to now due to greater resistance to diseases and pests) which should be introduced into production as soon as possible.

The basic purpose of the poplar stand is, at least for now, the production of woods, i.e. the production function of the forests is pronounced, although other functions of poplar stands are not negligible. The most realistic parameter which enables production forest function estimation is the wood volume obtained at the end of rotation period with accompanying structure of tree assortment, i.e. its value.

The aim of this paper was to reveal production characteristics of the stand of registered poplar clones in Serbia at the age close to the length of rotation period in production of the most valuable poplar selections. By pointing out to individual elements of stand structure detail characteristics of stand of studied poplar clones are obtained, and also the possibility of their usage at differentiating stands of different poplar clones according to Andrašev, et al, (2004); Andrašev, (2008).

MATERIAL AND METHOD

The trial was set in three replicates according to block design system at the distance between planting of 4,25 × 4,25 m, i.e. 555 trees per hectare, so called "normal planting" using nursary plants type 1/1. Nine years upon stand development thinning cutting, with prevailing shematic character (distance between planting was reduced to 6 × 6 m, i.e. 278 trees per hectare) was undertaken. The following clones were included in the trial: I-214 (*Populus* × *eurmaricana* Dode Guinier, domesticated clone), 618, 457, 55/65 (*Populus deltoides* Bartr. ex Marsh., clones registered in Serbia in 1980), and S_{6-36} (*Populus deltoides* Bartr. ex Marsh., clone registered in Serbia in 1987).

A pedology profile up to the depth of the underground water was opened in the stand. The samples for laboratory analysis were collected, and systematic belonging of the soil was defined. Studied soil belonged to fluvisol type, double layer variety with fossil soil where old soil of clay texture composition (A_b) was covered by recently loamy deposition 68 cm in depth (Table 1).

Table 1. Physico-chemical soil properties

Hori-zon	*Depth*	*Granulometric composition [%]*					*Chemical properties*		
		Coarse sand	*Fine sand*	*Silt*	*Clay*	*Texture class*	*pH in H_2O*	*Humus*	*C_aCO_3*
	[cm]	*[%]*						*[%]*	*[%]*
A_a	0-15	6.32	17.28	46.44	29.96	Clayly loam	7.40	4.40	1.67
G_{so}	16-68	4.32	15.00	46.16	34.52	Silty clay loam	7.56	1.97	2.92
A_b	68-140	1.26	28.34	28.36	41.76	Clay	7.56	1.11	0.84
G_r	140-150	2.85	16.79	32.60	47.76	Clay	7.53	0.80	3.34

Twenty years after stand development diameters at breast height were measured in the sample composed of approx. 20 trees (two central rows) for each replicate. In each studied clone falling of each tree and detail analysis of one basal area diameter mean tree (d_g) were done.

Thirty years upon stand development all diameters at breast height were measured, and in each replication basal area diameter mean tree was cut for detail analysis. With the aim of more realistic determination of stand volume data for construction of so called "volume line" for each clone were collected. For that purpose at least one tree was cut and measured. Degrees of thickness was 5 cm in width.

Data processing was based on application of standard indicators of numerical structure, statistical tests of variance analysis, and test of the least significant difference at the level of 5%, regression analysis, and Kolmogorov-Smirnov non-parameter test. Programs EXCELL and STATISTICA 6.0. were used for data processing.

RESULTS OF INVESTIGATION

Surveying of trial stand after 20 years from stand establishment revealed that there were no significant differences between studied clones in regard to obtained mean values for elements of stand growth ($d_{g20\%}$, d_g, g_s, N, G) (Table 2).

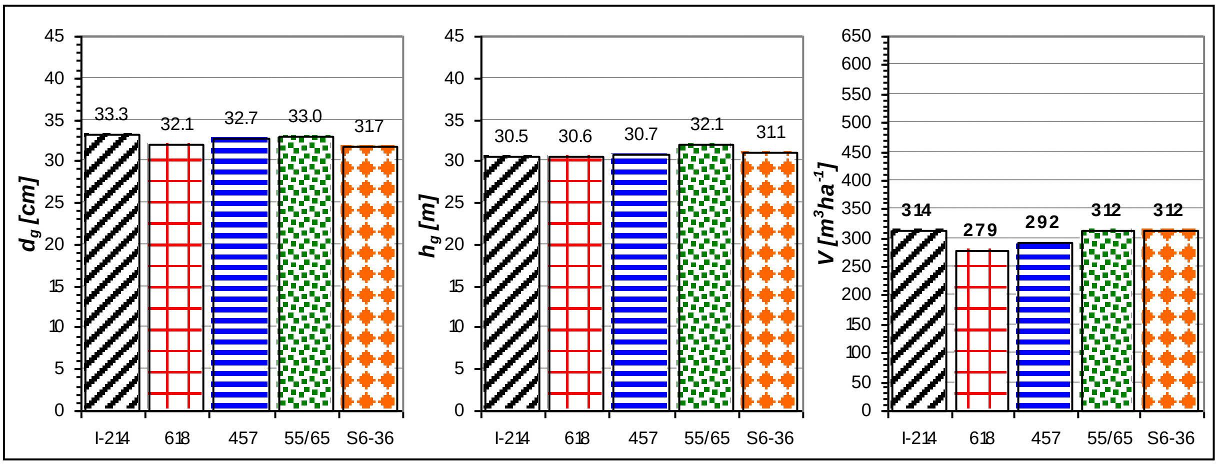
Obtained mean basal area diameters after 20 years ranging from 31,7-33,3 *cm* (d_g) and from 33,8-36,2 *cm* ($d_{g20\%}$), and height of mean trees from 30,5-32,1 *m* (Graph 1) pointed out to average favorable habitat conditions for black poplar cultivation.

Determined number of trees per hectare ranging from 250-278 (90-100% from initial number of trees for planting distance of 6 × 6 *m*) was the consequence of high establishment and survival of trees, but also of the fact that after nine years of stand development the thinning cutting, with prevailing shematic character, was done (eliminated from 52,65 $m^3 \cdot ha^{-1}$ in clone 618, to 72,82 $m^3 \cdot ha^{-1}$ in clone 457) reducing the number of trees to total overgrowth. Such high survival rate caused significant amount of basal areas per hectare from 21,1-23,8 $m^2 \cdot ha^{-1}$.

Table 2. Mean value of growth elements of stands of studied poplar clones 20 years after establishment

Clone	$d_{g20\%}$	d_g	g_s	*N*	*G*
	[cm]	*[cm]*	*[m²]*	*[trees·ha⁻¹]*	*[m²·ha⁻¹]*
I-214	36.2 a	33.3 a	0.087 a	249 a	21.72 a
618	35.7 a	32.1 a	0.081 a	259 a	21.06 a
457	35.8 a	32.7 a	0.084 a	278 a	23.35 a
55/65	35.2 a	33.0 a	0.086 a	278 a	23.85 a
S6-36	33.8 a	31.7 a	0.079 a	269 a	21.19 a
F	1.14^{ns}	0.86^{ns}	0.86^{ns}	1.80^{ns}	1.28^{ns}
p	0.3919	0.5187	0.5223	0.2065	0.3413

$d_{g20\%}$ - mean basal area diameter 20% of the thickest trees in a stand; d_g – mean basal area diameter; g_s – basal area of mean tree; N – number of trees per hectare; G – basal areas per hectare



Graph 1. Mean values of diameters at breast height, height and volumes of stands per hectare of studied poplar clones 20 years upon establishment

Twenty years upon establishment a wood volume from 278,7 $m^3 \cdot ha^{-1}$ (clone 618) to 314,1 $m^3 \cdot ha^{-1}$ (clone I-214) (graph. 1) was obtained. An average increase in volume ranged from 13,94-15,70 $m^3 \cdot ha^{-1} \cdot god^{-1}$. Clone 618 had the lowest volume, which in comparison to clone I-214 (clone with the greatest wood volume), accounted for 88.7%.

Thirty years upon stand establishment the elements of studied clones were close to those of average trees ($d_{g20\%}$, d_g, h_g) (Table 3, graph 2). Obtained mean diameters at breast height from 38,1 *cm* (clone 618) to 40,4 *cm* (clone I-214), mean diamteres 20% of thickest trees in stand from 42,6 *cm* (clone 618) to 45,8 *cm* (clone 457), as mean heights from 37,0-37,9 *m* pointed out to significant production possibilities of studied clones under conditions of a given habitat.

During period from 20-30 years of stand development number of trees of all clones was reduced. Greater reduction in number of trees was determined for clones with smaller number of trees per hectare (clones I-214 and 618), which caused significant difference in number of trees after 30 years of stand development (Table 3).

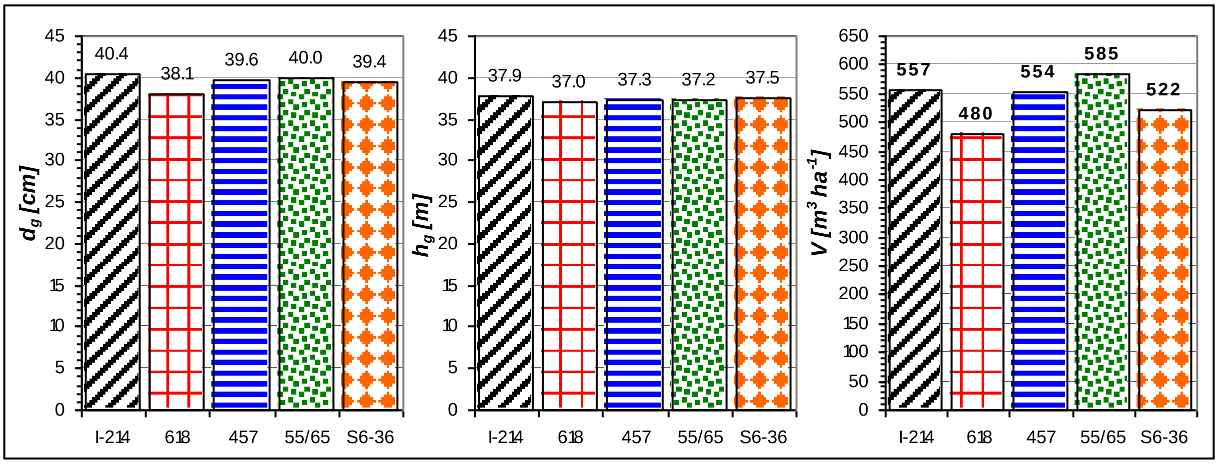
Difference in number of trees in stands of studied clones caused significant difference in basal area per hectare. Clones 618 and I-214, with 28.5-30.5 $m^2 \cdot ha^{-1}$ fell behind clone 55/65 significantly – the clone 55/65 had the bigest basal areas of 34.5 $m^2 \cdot ha^{-1}$.

In the base of volume line a curved line dependence of total tree volume on its diameters at breast height exists (Graph 3, Table 4).

Table 3. Mean values for elements of stand growth of studied poplar clones 30 years upon establishment

Clone	$d_{g20\%}$	d_g	h_g	g_s	N	G	V_{mt}	V_{vl}	$V_{d>40cm}$
	[cm]	[cm]	[m]	[m²]	[trees·ha⁻¹]	[m²·ha⁻¹]	[m³·ha⁻¹]	[m³·ha⁻¹]	[%]
I-214	45.4 a	40.4 a	37.90 a	0.1284 a	238 b	30.53 b	550.83 ab	557.35 ab	71.9 a
618	42.6 a	38.1 b	36.99 a	0.1139 b	250 b	28.45 c	494.61 b	479.71 c	41.8 b
457	45.8 a	39.6 ab	37.25 a	0.1233 ab	272 a	33.52 ab	536.85 ab	553.80 ab	61.0 ab
55/65	45.1 a	40.0 ab	37.23 a	0.1258 ab	274 a	34.55 a	587.32 a	585.39 a	49.3 ab
S_{6-36}	45.1 a	39.4 a	37.51 a	0.1221 ab	256 ab	31.16 bc	510.71 b	521.98 bc	46.4 b
F	1.04^{ns}	1.53^{ns}	0.23^{ns}	1.56^{ns}	6.27^{**}	6.73^{**}	2.47^{ns}	4.10^{*}	2.40^{ns}
p	0.4324	0.2672	0.9130	0.2582	0.0086	0.0068	0.1120	0.0321	0.1191

$d_{g20\%}$ - mean basal area diameter 20% of the thickest trees in a stand; d_g – mean basal area diameter; h_g –tree height with diameter d_g; g_s – basal area of mean tree; N – number of trees per hectare; G – basal areas per hectare; V_{mt} – volume per hectare obtained by mean tree method; V_{vl} – volume per hectare obtained by using constructed volume line; $V_{d>40cm}$ – participation of tree volume with diameter at breast height larger than 40 cm



Graph 2. Mean values of diameters at breast height, height and volume of stand per hectare of studied poplar clones 30 years after establishment

Chosen models of volume lines had high determination coefficients (R^2>98.7%). All studied clones had similar tree volumes for smaller diameters at breast height ($d_{1.30}$<40*cm*), while there was a difference among studied clones for greater diameters at breast height (Graph 3).

Volume per hectare determined according to method of mean tree stand volume (V_{mt}) revealed no significant differences among clones, although LSD test at the level of 5% confirmed that the clone 618 with 495 $m^3 \cdot ha^{-1}$ was significantly smaller than the clone 55/65 with more than 585 $m^3 \cdot ha^{-1}$ (Table 3).

Stand volume obtained using constructed volume lines (V_{vl}) revealed significant differences among studied clones. Clones 618 (480 $m^3 \cdot ha^{-1}$) and S_{6-36} (520 $m^3 \cdot ha^{-1}$) produced significantly smaller wood volumes per hectare in relation to clone 55/65, which produced more than 585 $m^3 \cdot ha^{-1}$ (Table 3, graph 2).

Graph 3. Treeline volume (v_{st}) of studied poplar clones

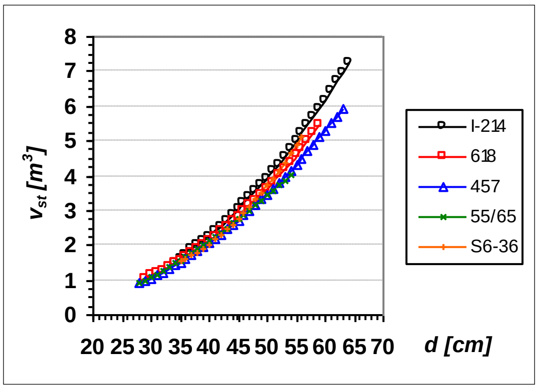


Table 4. Model, parameters and estimation of models of volume lines of studied poplar clones

Clone	*Function*	*Function parameters*			*Estimation of models*		
		a	*b*	*c*	R^2	s_e	*n*
I-214	$y=a\cdot x^b$	$2.477\cdot10^{-4}$	2.473		0.9938	$1.47\cdot10^{-1}$	9
618	$y=a\cdot x^2+b\cdot x+c$	$2.56\cdot10^{-3}$	$-7.916\cdot10^{-2}$	1.205	0.9986	$6.50\cdot10^{-2}$	8
457	$y=a\cdot x^b$	$4.014\cdot10^{-4}$	2.317		0.9897	$1.56\cdot10^{-1}$	11
55/65	$y=a\cdot x^2+b\cdot x+c$	$1.224\cdot10^{-3}$	$1.979\cdot10^{-2}$	$-6.168\cdot10^{-1}$	0.9987	$4.97\cdot10^{-2}$	6
$S_{6\text{-}36}$	$y=a\cdot x^2+b\cdot x+c$	$4.346\cdot10^{-3}$	$-2.229\cdot10^{-1}$	4.004	0.9878	$1.41\cdot10^{-1}$	11

Table 5. Numerical parameters of structure of thickness after 30 years of stand development

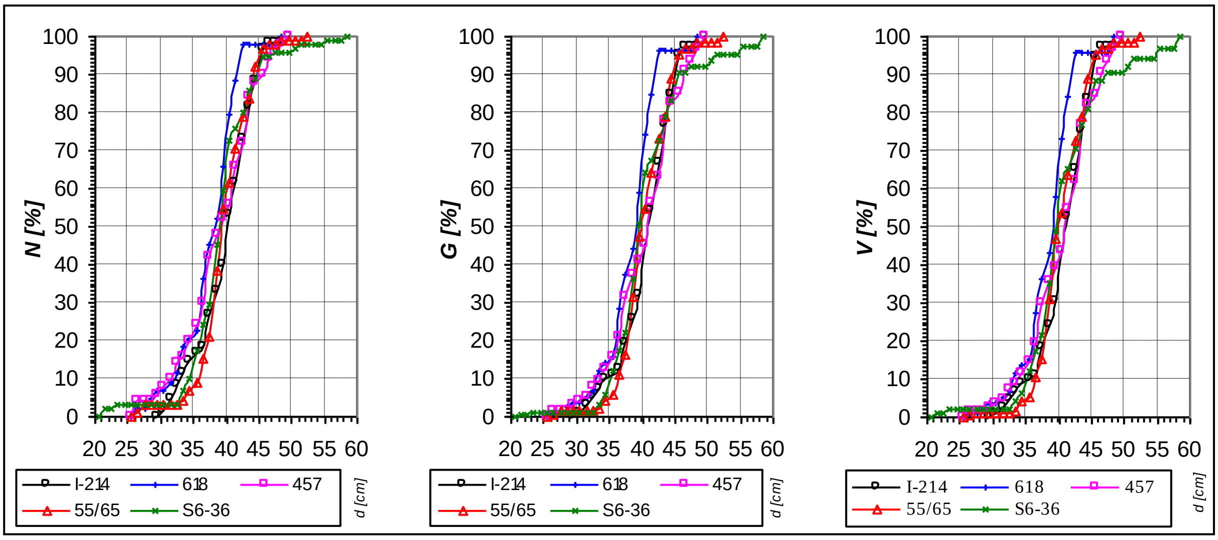
Clone	*n*	d_a	s_d	c_v	d_{min}	d_{max}	v_w	α_3	α_4
	[trees]	*[cm]*	*[cm]*	*[%]*	*[cm]*	*[cm]*	*[cm]*		
I-214	60	40.2	4.17	10.4	30.9	49.4	18.5	-0.449	2.691
618	44	37.9	4.22	11.1	26.3	48.3	22.0	-0.595	3.698
457	50	39.2	5.36	13.7	26.2	49.6	23.4	-0.392	2.727
55/65	99	40.0	3.89	9.7	26.1	52.4	26.3	-0.594	5.761
$S_{6\text{-}36}$	91	39.5	5.38	13.6	21.2	58.5	37.4	-0.163	7.113

n – number of measured trees; d_a – arithmetical mean diameter at breast height; s_d – standard deviation of diameters; c_v – variation coefficient; d_{min} – minimal diameter; d_{max} – maximal diameter; v_w – variation width; α_3 – coefficient assimetry; α_4 – coefficient of external

Numerical indicators of thickness structure of studied clones 30 years after establishment showed close arithmetic mean diameters at breast height (37,9-40,2 *cm*), small variability (9.7-13.7 %), as well as weak to medium expressed left asymmetry (Table 5). Greater difference was found for variation width (18.5-37.4 *cm*), and flatness of thickness structure of studied clones (α_4 = 2.73-7.11). Clones 457 and $S_{6\text{-}36}$ had very similar arithmetic mean diameters at breast height (39.2-39.5 *cm*), sizes of standard deviations (5.36-5.38 *cm*), and variation coefficients (13.6-13.7 %). However, numerical parameters describing asymmetry (α_3), and exterior of thickness structure (α_4) were different.

Table 6. Results of Kolmogorov-Smirnov test for comparison of diameter structures, basal area and volume structures per diameter classes of studied clones after 30 years of stand establishment

		Diameter structure (N%)					*Wood volume structure (V%)*			
	Clone	I-214	618	457	55/65	$S_{6\text{-}36}$	618	457	55/65	$S_{6\text{-}36}$
Basal area structure (G%)	I-214	-	0.2789^{***}	0.16^{ns}	0.1455^{ns}	0.2096^{*}	0.3163^{***}	0.1251^{ns}	0.1676^{ns}	0.1870^{ns}
	618	0.3194^{***}	-	0.2598^{**}	0.2323^{**}	0.1673^{ns}	-	0.3366^{**}	0.2349^{**}	0.2604^{**}
	457	0.1263^{ns}	0.33^{***}	-	0.2079^{*}	0.1796^{ns}	-	-	0.1501^{ns}	0.1744^{ns}
	55/65	0.1739^{ns}	0.22^{*}	0.15^{ns}	-	0.1234^{ns}	-	-	-	0.0808^{ns}
	$S_{6\text{-}36}$	0.1949^{*}	0.2372^{**}	0.1837^{ns}	0.0837^{ns}	-	-	-	-	-



Graph 4. Summary curve for thickness structure, basal area structure, and volume per degrees of thickness 30 years after stand establishment

Non-parametric Kolmogorov-Smirnov test revealed significant differences between structure of thickness (Table 6, graph 4). Clone 618 achieved the smallest diameters at breast height, and its diameter structure significantly differed from that of clones I-214, 55/65 and 457. Basal area and volume structure per diameter degrees significantly differed between clone 618 from one, and the rest of the clones from another side.

Participation of tree volume with diameters at breast height greater than 40 *cm* ($V_{d>40cm}$ *[%]*) per hectare, served as an appropriate expression of valorization of the value of achieved volume of studied clones. Although no significant difference among studied clones for the survey as a whole was not found (Table 3), the result the least significant difference test at the *5*% level of significance confirmed that clone I-214 with participation of volume of trees thicker than 40 cm of 71.9%, significantly differed from clones 618 (41.8%) and S_{6-36} (46.4%).

DISCUSSION

Obtained mean values of stand growth elements ($d_{g20\%}$, d_g, g_s, G) pointed out to mean favourable habitat conditions for growth of studied poplar clones. Achieved mean height in 20 years ranging from 30-32 *m* was smaller than mean values achieved on habitats for optimal black poplar growth, (Marković i Pudar, 1990; Marković, et al, 2001), but higher than those achieved on habitats less favourable for their growth, such as brown soil (Živanov, et al, 1985), humogley (Živanov and Jovin, 1983), sandy forms of fluvisol (Rončević, et al., 1999; Andrašev, et al., 2004; Andrašev, 2008). Since a reliable production differentiation in poplar stands can be done in 20 years old stands (Andrašev, 2008), according to bonitet in regard to poplar height (Marković, et al, 1987) the studied stand belonged to bonitet class II.

Diameters at breast height, and stand volume of studied clones in the 20th year significantly depended, besides the influence of stand bonitet class and poplar clone (cultivar), on stand density as well. Obtained mean diameters at breast height ranging from 31,7-33,3 *cm*, as well as the stand volume ranging from 280-315 $m^3 \cdot ha^{-1}$ in addition to 53-73 $m^3 \cdot ha^{-1}$ of wood volume obtained by thinning after 9 years, confirmed that the stand was fairly favorable for poplar growth and was in accordance with achieved heights. This was confirmed by the results of previous investigations in stands where wood volume ranging from 350 to 400 $m^3 \cdot ha^{-1}$ in the optimal habitats was achieved in the 13 to 15 year age range (Herpka i Marković, 1975; Lazarević, 1977; Marković i Pudar, 1990; Marković, et al, 1997).

In previous investigation connected to stand productivity under our conditions there were fewer results from trials aged over 25 years. With the aim of comparison of achieved production potential, an average stand volume increase of the age close to the length of rotation period can be used as a suitable element. Although there were no data on developing tree characteristics in the studied trial, it was assumed on the basis of previous investigations that the stand aged 30 years was close to the optimal length of production cycle for production of the most valuable poplar selections.

Achieved average volume increment per hectare after 30 years ranging from 16 $m^3 \cdot ha^{-1} \cdot god^{-1}$ (clone 618) to 19.5 $m^3 \cdot ha^{-1} \cdot god^{-1}$ (clone 55/65) can be classified as bonitet class II (Marković et al, 1987; Herpka, et al, 1987). Modern investigations revealed that limits of bonitet classes from 2 $m^3 \cdot ha^{-1} \cdot god^{-1}$ in old age close to the rotation period can be accepted (ANDRAŠEV, 2008), so the studied clones 618 and S_{6-36} would belong to one (from 16-18 $m^3 \cdot ha^{-1} \cdot god^{-1}$), and clones 55/65, 457, I-214 to another bonitet class (18-20 $m^3 \cdot ha^{-1} \cdot god^{-1}$).

Previous results relating to young poplar stands revealed that almost all registered clones of Eastern cottonwood (618, 457, 450, 55/65, 725, S_{1-8}, S_{6-36}) have greater growth elements in stand aged from 5-10 years in relation to clone I-214. Clones registered in 1987 (S_{1-8}, S_{6-36}) in stands 5-10 years old were more productive in relation to clones registered in 1980 (618, 457, 450, 55/65, 725), while between clones registered in 1980 there was no significant difference (Herpka, 1985; Marković, et al., 1986; Herpka i Guzina, 1987; Rončević andi Belčić, 1987; Guzina, et al., 1991/a; Marković, et al., 1991).

Results of investigation (Table 2, 3, graphs 1, 2) revealed that studied poplar clones had similar productivity in stands aged 20 years. Similar growth elements of clones 618 and 457 aged 10 years were quoted by Marković, et al., (2001), while at age 22 years clone 457 achieved greater dimensions on soil optimal for poplar cultivation. On soil of lower production potential (sandy form of fluvisol) aged 24 years similar growth elements of clone 618 and 457 were mentioned by Andrašev, et al., (2004). Achieved gross wood volume of clones 618 and 457 with the usage of constructed model of volume lines (V_{vl}) 30 years after stand establishment (table 3, graph 2) revealed that clone 457 was more productive, which was in accordance with quotation of Marković, et al (2001). As studied habitat belonged to height bonitet of class II it could be concluded that smaller differences in productivity between clones appeared later, which was in accordance with quotation of ANDRAŠEV (2008), as well as that differences were expressed later on habitats with lower bonitet class.

On soils with lower production potential (sandy form of fluvisol) aged 24 years Andrašev, et al., (2004) mentioned similar growth elements for clones 618 and 457, while clone S_{6-36} achieved significantly greater growth elements at densities between 400 and 625 trees per hectare. Results of investigation (graph 2, table 3) revealed that clone S_{6-36} achieved similar (clone 618) or smaller (clones 457, 55/65) productivity than the clones registered in 1980, which was in contrast to quotation of Guzina, et al., (1991/a), Andrašev, et al, (2004), Andrašev, (2008). Since clones have determined specific reaction to habitat conditions, and having in mind the results of Andrašev, (2008) that optimal habitats for clone S_{6-36} was loamier texture, it could be concluded that clone S_{6-36} under condition of studied habitat was not inside range of its ecological optimum.

Guzina, et al., (1991/*a*) quoted that lagging of clone I-214 (*Populus* × *euramericana* (Dode) Guinier) behind clones of Eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.) in young stands was partialy the results of successful selection of new genotypes with greater genetic potential, but also in great deal the consequence of situation that that clone, under changed environmental conditions with extended populations of disease provokers, can not express its genetic potential in regards to increase of wood volume. As a consequence of minor appearance of disease of corticular tissue (*Dothichiza populea* Sacc. et Br.) and leaf (*Melampsora sp.* and *Marssonina brunnea* Ell. et Ev.), it was mentioned by Marković, et al., (1997) that at the age of 15 years clone I-214 achieved growth elements similar to those for Eastern cottonwood clones. Results of investigation (Table 2, 3) showed that clone I-214 achieved significant production yields similar to those of the most productive Eastern cottonwood clones (55/65, 457), with the most favorable participation of tree volume more than 40 *cm* in diameter ($V_{d>40cm}$, *[%]*). It pointed out that under conditions of diminished infection of disease provoker this clone still presents the basis for achieving high production effects.

Studied poplar clones have achieved different level of productivities at the age of 30 years, which was confirmed by the size of amount of gross wood volume per hectare obtained by the use of constructed model of volume lines (V_{vl}). Mean values of diameters (d_g, $d_{g20\%}$), heights (h_g), and volumes (V_{mt}) of stand did not point to significant differences between clones. Minor priviledge of using heights while conveying differentiation of stand productivity of different poplar clones was also confirmed by Andrašev (2008). However, some differences in structure construction of the stand of studied clones, which in addition to the significance of obtained differences in thickness structures, basal area and volume structures per diameter classes pointed out to possibility of their usage in differentiation of poplar stands.

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DECAY FUNGI OF MEDITERRANEAN AND SUBMEDITERRANEAN PART OF MONTENEGRO

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Summary: *Decay fungi inhabit woody plants, being parasites of weakness on standing trees or saprobes on dead wood and woody materials. Decay fungi treated in this paper belong to Basidiomycetes, Aphyllophorales.*
During the last couple of years, in the maritime cities green areas, Virpazar and Podgorica, on mostly decidious trees as the hosts, there have been recorded numerous decay fungi fruit bodies (carpophores). Ganoderma adspersum Bound was noted on 5 different host plants, on 32 standing trees in total, Inonotus hispidus (Bolton) Karst on 6 different host plants, on 15 standing trees, a Phellinus. torulosus (Pers.)Bourd.&Galz. on 15 different host plants, on 41 standing tree, and 5 stamps. Besides, in urban green areas, singularly or with few records Fomes fomentarius (L.) Kickx, Phellinus igniarius (L.) Quel, and Phellinus punctatus (Fr.) Pilat have been noted. These fungi develop as parasites of weakness, and their appearance in such a number could be dangerous. In Castanea sativa forest stands in Boka Kotorska bay and Lake Skadarsko surroundings, Fistulina hepatica (Sch.)Fr. has been frequently noted.
On the other hand, on forest stands and recreational areas in Adriatic coast and Podgorica surroundings (submediterranean following interesting and rare species have been noted: ***Bjerkandera adusta*** *(Willd)Karst,* ***Coriolopsis gallica*** *(Fr.) Ryvarden,* ***Cylindrobasidium evolvensb*** *(Fr.) Julich,* ***Hapalopilus rutilans*** *(Pers.)Karst,* ***Hymenochaete rubiginosa*** *(Schrad.)Lev.,* ***Irpex violaceus*** *(Pers.) Quél,* ***Grandinia crustosa*** *(Pers.) Fr,* ***Lenzites warnieri*** *Dur. Month,* ***Lopharia spadicea*** *(Pers.) Boidin,* ***Oligoporus subcaesius*** *(David) Ryvarden & Gilb.,* ***Perenniporia ochrolruca*** *(Berk.) Ryvarden,* ***Pulchericum caeruleum*** *(Lam.) Parmasto,* ***Steccherinum basibadium*** *Banker.*
Morphological characteristics of ***Lopharia spadicea*** *and* ***Steccherinum basibadium*** *have also been described, with macroscopic and microscopic descriptions, photography and micrographs.*
Key words: *decay fungi, Mediterranean and submediterranean part of Montenegro, diversity, host plants, Ganoderma resinaceum, Inonotus hispidus, Phellinus torulosus; Lopharia spadicea, Steccheruinum basibadium.*

INTRODUCTION

Decay fungi inhabit woody plants, being parasites of weakness on standing trees or saprobes on dead wood and woody materials. As a consequence of decay fungi presence, physical features of woods are changing trough reduction of their mechanical stability. It may lead to early tree mortality from a variety of factors, such a breakage, blow down or predisposition to the other forest health factors. In the urban environment those trees could create hazardous situation, where property damage or serious body injury could have resulted from falling limbs or the toppling of the entire tree. But, decay fungi also play an important role in creating gaps in forest canopies and play a lead role in nutrient cycling, species succession, biodiversity and creation a wildlife habitat.

During the last few years, basidiomes of decay fungi have been frequently noted on forest stands on Adriatic coast and in Podgorica surroundings, as well as in maritime cities green areas and parks or gardens in Podgorica and Virpazar.

Decay fungi treated in this paper belong to *Basidiomycetes*, *Aphyllophorales.*
On researched area, forest vegetation is composed of natural or artificial mediterranean forests of Aleppo pine (*P. halepensis*), dalmatic pine (*P. nigra dalmatica*), pinea (*P. pinea*) with *Cupressus sempervirens* and *P. pinaster*; which appear at different degradation stage of climatogenic forests of *Quercetalia ilicis* on Adriatic coast, and also termophilous submediterranean broadleaves forests with *Quercus pubescens*, *Q. trojana*, *Q. cerris* as dominant plant species (Alliance *Ostryio – Carpinion orientalis,* Ass. *Querco – Carpinetum orientalis*, Ass. *Quercetum trojanae*) and, fragmentary, chestnut forests on acid soils from Ass. *Querco- Castanetum submmediterraneum.* in submediterranean region.

Besides, in the maritime cities green areas, there are many alochthonous and exotic plants typically cultivated in Mediterranean region. In combination with autochthonous vegetation, they create specific and obvious mediterranean landscapes, mostly trough combinations of evergreen deciduous and coniferous. So, there are *Magnolia grandiflora*, *Ligustrum ovalifolium*, *Laurus nobilis*, *Pittosporum tobira*, *Eucaliptus* sp, and also deciduous species usual in continental regions, also: *Platanus acerifolia*, *Sophora japonica*, ashes and limes.

MATERIALS AND METHODS

During the last couple of years (2004-2008), basidiomes of decay fungi have been noted on forest stands and urban green areas. Researches have included locations on Adriatc coastal area, maritime cities and their surroundings (Tivat, Kotor, Stari Stoliv, Budva, Petrovac, Lucice, Bar), as well as submediterranean region, with some locations near Scadar Lake (Virpazar, Livari, Brčeli) and Podgorica (Podgorica within city limits and Bolesestre). This wasn't some systematic research of decay fungi, but part of one wider examination of fungi of this area, so during the field visits with other goals in mind, interesting material have been collected.

Samples of basidiomes were noted, collected and used for analyses. Determination of fungi has been done according to basidiomes, spore bearing organs and structures, and spores of fungi. Microscopic analyses are done on fresh material. Melzer reagent, Kongo red and 5 % KOH were used for staining of slides.

For determination of collected material, following keys have been used: Bernicchia (2005), Breitenbach & Kranzlin (1986), Bondartseva (1998), Cetto (1990), Jülich (1989), Ryvarden & Gilbertson (1993).

The fungi founds are evidenced, and most of species have been followed by photo documentation of macroscopic appearance and micro details, later ilustrated with drawings. The most of the exicata are collected in mycological collection of Montenegrin Micological Center in Podgorica. Naming of fungi was done according Index fungorum, (www. Index fungorum.org), and their systematic position were done according Bondartseva (1998), Jülich (1989), Breitenbach & Kranzlin (1986).

RESULTS

During the last few years in Mediterranean and submediterranean part of Montenegro basidiomes of following decay fungi **(***Basidiomycetes*, *Aphyllophorales*) have been noted on forest stands and green areas.

Bjerkandera adusta (Willd) Karst (Fam. *Poriaceae*, Subfam *Tyromycetoideae*)
hornbeam stump, Bolesestre, 31 III 2005., leg & det B. Perić.

Coriolopsis gallica (Fr.) Ryvarden (Fam. *Poriaceae*, Subfam *Trametoidae*)
Punica granatum branch in Perićs family garden, Masline, Podgorica, 22. X 2007., leg & det B. Perić

Cylindrobasidium evolvens (Fr.) Julich (Fam. Corticiaceae)
deciduous twig (hornbeam), Bolesestre, 29. III 2005. leg & det B. Perić.

Fistulina hepatica (Schaeff) Fr. *(Fam. Fistulinaceae)*
Castanea sativa forest stand, (Livari **frequent** X 2004-2008; Stari Stoliv **frequent** X 2006), leg & det J.Lazarević

Fomes fomentarius (L.) Kickx (Fam. *Poriaceae*, Subfam *Fomitoideae*)
Liriodendron tulipifera (Tivat **1**, IX 2004.), *Platanus acerifolia* (Tivat **1**, IX 2006), *Populus* sp (Podgorica **2**, X 2004, 2005), leg & det J.Lazarević

Ganoderma resinaceum Bound (Fam *Ganodermataceae*)
Acer negundo (Budva **2**, VI 2006) *Celtis australis* (Budva **1**, VI 2006), *Laurus nobilis* (Tivat **6**, IX 2004-X 2008, Kotor **6,** IX 2006-III 2008), *Ligustrum ovalifolium* (Kotor **4,** IX 2006, Tivat **4**, IX 2004-X 2008), *Platanus acerifolia* (Tivat **8,** IX 2004- IX 2006, Virpazar **1**, X 2004-2005), leg & det J.Lazarević

Grandinia crustosa (Pers.) Fr (Fam. *Corticiaceae*)
decayed *Pinus halepensis* brench, Gorica, Podgorica, 14. IV 2005., leg & det B. Perić

Hapalopilus rutilans (Pers.) Karst (Fam. *Poriaceae*, Subfam *Tyromycetoideae*)
deciduous twig (hornbeam), Bolesestre, 29. III 2005., leg & det B. Perić

Hymenochaete rubiginosa (Schr.)Lev. (Fam. *Hymenochaetaceae,*Subfam. *Hymenochaetales*)
oak brench, Brčeli, Crmnica, 03. IV 2005, leg & det B. Perić

Inonotus hispidus (Bolton) Karst (Fam. *Hymenochaetaceae*)

Acer dasycarpum (Podgorica **3,** X 2004-06), *Acer negundo* (Podgorica X 2004-06), *Celtis australis* (Budva **1**, VI 2006), *Platanus acerifolia* (Podgorica **1**, X 2004-07, Tivat **2**, X 2006), *Salix* sp (Bar **1**, I 2004-IV 2008), *Sophora japonica* (Tivat **6**, IX 2004-X 2006), leg & det J.Lazarević

Irpex violaceus (Pers.) Quel (Fam. *Poriaceae*, Subfam *Steccherinoideae*)

Pinus halepensis stump, Podgorica, 14. II 2007., leg D. Raspopović, det B. Perić.

Lenzites warnieri Dur. Month (Fam. *Poriaceae*, Subfam *Trametoideae*)

oak brench, Brčeli, 07. IV 2005., leg & det B. Perić

Lopharia spadicea (Pers.) Boidin (Fam. *Corticiaceae*)

dead twig of *Vitis vinifera* in Perićs family garden, Masline, Podgorica, 17. III 2008., leg & det B. Perić.

Oligoporus subcaesius (David)Ryvarden & Gbb. (Fam.*Poriaceae*, Subfam *Tyromycetoideae*)

decayed hornbeam branch, Bolesestre, 31. III 2005., leg & det. B. Perić

Peniophora incarnata (Pers.) Karst (Fam *Corticiaceae*)

deciduous twig (lime, honeborn, hazel), Bolesestre 19., 29. III 2005., leg & det B. Perić.

Perenniporia ochrolruca (Berk.) Ryvarden (Fam. *Poriaceae*, Subfam *Fomitoideae*)

Erica arborea brench, Petrovac, 16. III 2005., leg O. Perić, det. B Perić

Phellinus igniarius (L.) Quel (Fam. *Hymenochaetaceae*)

Platanus acerifolia (Tivat **1**, X 2004-X 2008), leg & det J.Lazarević

Phellinus punctatus (Fr.) Pilat (Fam. *Hymenochaetaceae*)

Laurus nobilis (Tivat **5,** X 2004-2008), *Robinia pseudoacacia* (Gorica **2**, X 2005-2007; Tivat **2**, X 2008), leg & det J.Lazarević

Phellinus torulosus (Pers.) Bourdot & Galzin (Fam. *Hymenochaetaceae*)

Castanea sativa (Livari **1**, X 2004; Stari stoliv **2**, X 2006), *Cupressus sempervrens* (Tivat **2**, IX 2006), *Laurocerassus officinaslis* (Virpazar **1**, X 2006), *Laurus nobilis* (Tivat **1**, IX 2004-X 2006), *Ligustrum ovalifolium* (Tivat **1**, IX 2004-X 2006), *Ligustrum vulgare* (Podgorica **1**, XI 2004), *Olea europea* (Tivat **1**, IX 2004),*Padus racemosa* (Tivat **1**, X 2006), *Pittosporum tobira* (Tivat **6**, IX 2004-X 2006, Kotor **1**, IX 2006, Virpazar **1**, X 2006), *Platanus acerifolia* (Tivat **1**, X 2006-X 2008), *Prunus spinosa* (Tivat **3**, IX 2004-X 2008), *Pyrocantha coccinea* (Podgorica **1**, II 2005), *Quercus pubescens* (Tivat **2**, IX 2002, Perazića do **1**, VII 2005), *Robnia pseudoaccacia* (Tivat **8**, IX 2004-2008), *Viburnum tinus* (Pogorica **1**, II 2005, Tivat **1**, X 2008), stump of broadleav (Podgorica **2**, II 2004-2006, Tivat **3**, IX 2004-2006), leg & det J.Lazarević, oak stump, Brčeli, 03. IV 2005., leg O. Perić, det B. Perić.

Pulchericum caeruleum (Lam.) Parmasto (Fam. *Corticiaceae*)

dead hornbeam brench, in Perićs family garden, Masline, Podgorica, 29. III 05, on dead twig *Vitis vinfera*, in Perićs family garden, Masline, Podgorica, I-III 2005, old decayed prop of *Castanea sativa*, in Perićs family garden, Masline, Podgorica, I-III , 2005., leg det. B. Perić

Steccherinum basibadium Banker (Fam. *Poriaceae,* Subfam *Steccherinoideae*)

Punica granatum barkless branch in Perićs family garden, Masline, Podgorica, 17. III 2008., leg & det B. Perić.

1- 6 (bolded number)–number of trees on which the basidiomes of fungi have been noted

L. spadicea and ***S.basibadium*** are species new for Montenegro, rare and interesting, so we decided to present them in detail.

Lopharia spadicea (Pers.) Boidin (Fam. *Corticiaceae*)

Makro characteristics

Basidiocarps: annual, resupinanate, embedded with *Vitis* twig, ellipsoid, only partly rises above substrate in irregular shell alike or fan – shaped form. Sterile surface irregular, spotty concave in the middle; around margins are irregularly stick out pilous, lighter then basic color. Margin is distinct, slightly woven, light cream in color. Pore surface is slightly irregular, somewhere with cloddy protuberances, light gray-brown to dark gray-brown. Thu fungus consists of three parts, laterally bonded. Radius of the central, the bigest part is 25-40 mm, 15-18 mm of width, while the radius of lateral, young basidiocarps is only 5-7 mm.

Habitat**:** On twig of *Vitis vinifera* remain after winter pruning in Perićs family garden, Masline, Podgorica, 17. III 2008. *Leg & det.* B. Perić.

Mikro characteristics

A – basidiospores ellipsoid to cylindrical, hyaline in water, nonamyloid, 6,80 - 9,40 x 3,40 - 4,00 μm.

B – basidia tetrasporic, lengthy clavate, 24,50- 28,30 x 4,40-5,80 μm, with sterigma 0-2,40 μm.

C – pseudocistide in form of pleurocystida, very lengthy, 87,30 -108,60 x 4,60-8,40 μm curved in hymenial zone, brownie, very inkrustrated in the ends, ends are 39,30 - 49,30 μm. Generative hyphae hyaline or slightly brown, 4-5 μm wide, septated with clamps. Sceletal hyphae brownie, 5-6 μm wide, without clamps.

Steccheruinum basibadium Banker (Fam. *Poriaceae*, Subfam *Steccherinoideae*)
Makro characteristics
Basidiocarps annual, 65 x 25 mm, developed as small shell, mostly embedded over substrata, resupinate, irregular shaped, with distinct margin, lighter and without needles. Hymenophore is composed of small, short (1-1.5 mm) and dense, thick needles cream with little yellow orange.
Habitat**:** On dead twig of Punica granatum remain after winter pruning in Perićs family garden, Masline, Podgorica, 17. III 2008. *Leg & det.* B. Perić.

Mikro features
A – basidiospores rounded or oval, hyaline, guttulate, 3,40 - 4,60 x 2,50- 3,70 μm, nonamiloid.
B – basidia tetrasporic clavate, 17,80 -24,90 x 24,90-5,80 μm, with sterigma -2 μm.
C – pseudocistide in form of pleurocystida, lengty 39,60 -96,30 x -9,50-14,00 μm, curved in hymenial zone, browny, inkrustrated in the ends.
Generative hyphae hyaline or slightly brown, 4-4,5 μm wide, septated with clamps. Sceletal hyphae brownie, 5-8 μm wide, ended as pseudocistide 30-50 μm.

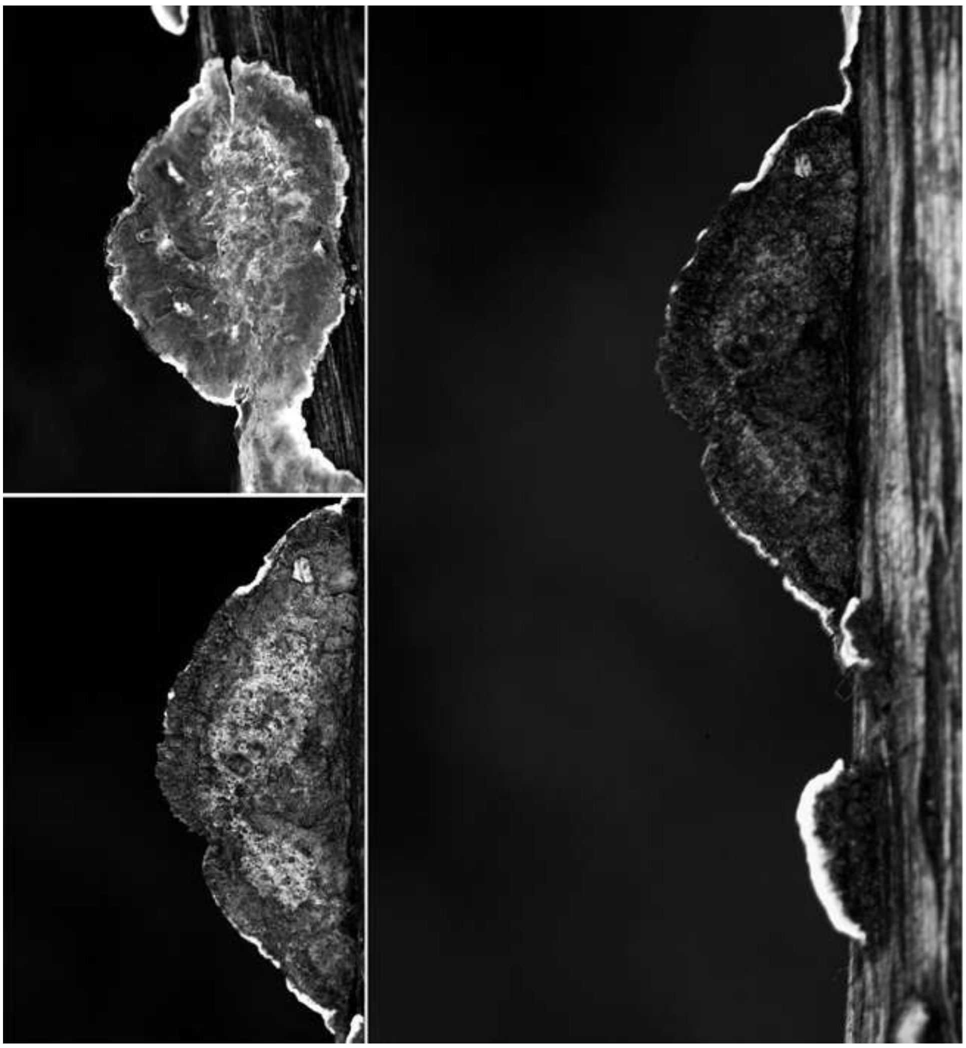
DISCUSSION

20 species of fungi that cause decay of trees have been noted in mediterranean and submediterranean part of Montenegro in last few years (2004-2008). They were recorded on 28 different host plants.

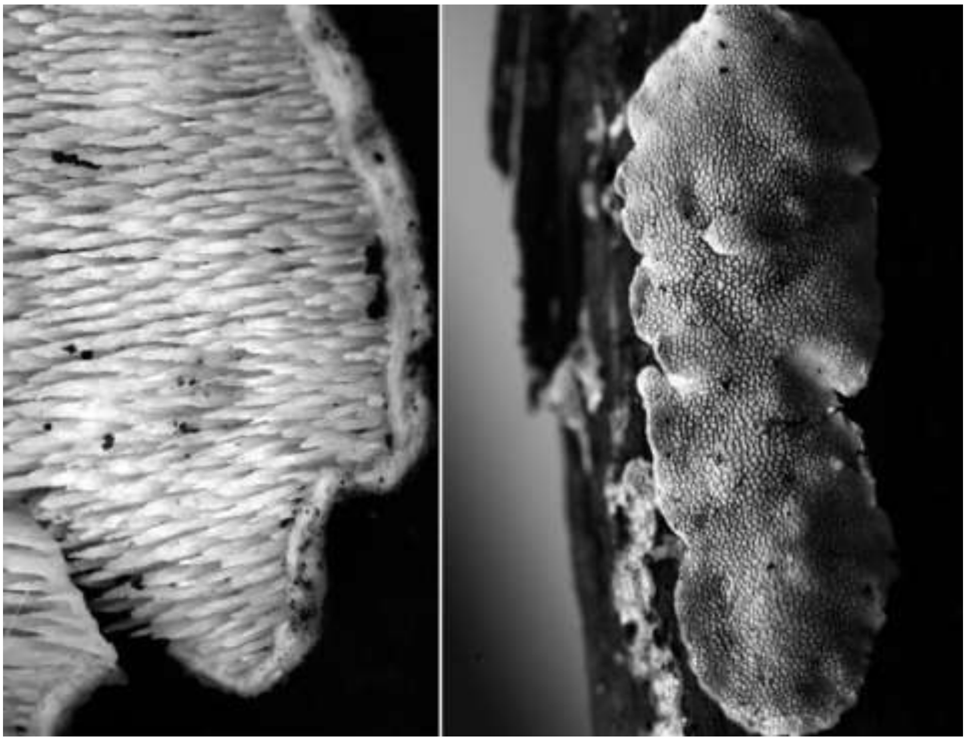
Fomes fomentarius, *Ganoderma resinaceum, Inonotus hispidus*, *Phellinus igniarius*, *Phellinus punctatus* and *Phellinus torulosus* have been developed on standing trees as parasites of weakness, but also could occur as saprobe. Those are white rot fungi that also decay hardwood in roots and lower stems or stem and branches, and invade and kill cortical tissue. As the majority of decay fungi, they have been infecting trees by basidiospores trough mechanical injuries on stems and branches. Intensity of decay varies of fungal species, host and also of environmental conditions, so the time required for tree death varies.(Bernard, 1981; Campaniele et al, 2004; Karadžić et al, 2002.) Total number of standing trees on which the basidioms of these fungi were developed is 101, all in urban or recreational areas.

G. resinaceum, I. hispidus and *P. torulosus* could be treated as frequent and widespread decay fungi in urban green areas in Mediterranean and submediterranean part of Montenegro. During the last couple of years *G. adspersum* was noted on 5 different host plants, on 32 standing trees in total, *I. hispidus* on 6 different host plants, on 15 standing trees, a *P. torulosus* on 15 different host plants, on 41 standing tree, and 5 stamps.. Basidioms of *G. resinaceum* and *P. torulosus* developes in stam base and root and those of *I. hispidus* on trunk or main branch of crown. All three species are fungi typical for Mediterranean region, i.e. south of Europe (Ryvarden et al, 1993; Bundartseva, 1998). It is thought that *I. hispidus* is aggressive parasite of deciduous species (Toole, 1955; Bernard, 1981), also as G. *resinaceum* which belongs to group of aggressive ganodermas. *P. torulosus* was frequent in forest ecosystems in Mediterranean area where it took important role in oak decline. Investigations conducted in Italy showed that it should be kept under close watch since it could become a serious risk to broadleaf trees in the Mediterranean- type ecosystem (Panconesi et al., 1994 cit. Campaniele et al, 2004).

Fistulina hepatica have been evidenced in *Castanea sativa* stands in Boka Kotorska bay and in the surrounding of Lake Skadarsko, were it was frequent. It causes brown rot on standing trees. It develops on trees in decline due to parasitic fungus *Cryptonectria parasitica* attack. (Karadžić et al, 2002).



Lopharia spadicea (Pers.) Boidin



Steccheruinum basibadium Banker

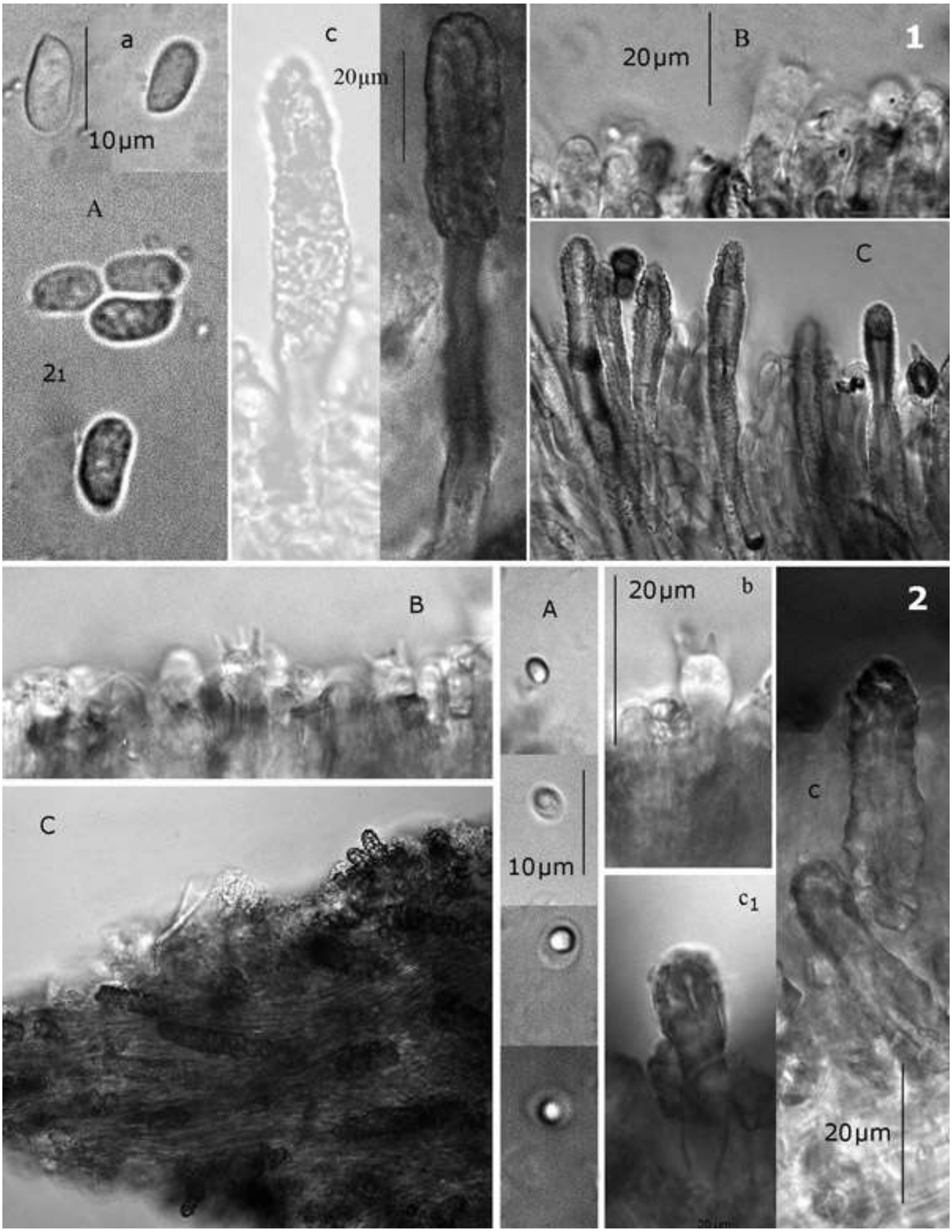


Figure 1-2: *Lopharuia spadicea* i *Steccheruinum basibadium* (Foto B. Perić)

1. *Lopharuia spadicea* A - basidiosporae, a - (in water), a1- (u KOH 5%) ; B – basidia (in water); C – pseudocistidae, c – pseudocistidae in the top (in KOH 5%)
2. *Steccheruinum basibadium*: A – basidiosporae (in KOH 5%), B – basidia (in water); C – pseudocistidae, c – pseudocistidae in the top (in KOH 5%), c1- pseudocistidae in the top (in metil blue)

The appearance of carpophores of fungi that cause decay is clear indicator of bad health condition of the host. It is characteristic of decay process in the late phase, meaning high degree of damages and degradation of certain trees, with very changed physical characteristics of woods, i.e. reduced mechanical stability. Presence of decay represents a consequence of numerous harmful environmental conditions and anthropogenic damages in cities, but also of old age of some of trees on green areas that are at the end of their lifetime.

Other 13 species are developed as typical saprobes. *Bjerkandera adusta* and *Irpex violaceus* were noted on stumps. *Coriolopsis galica*, *Cylindrobasidium evolvens, Hapalopilus rutilans, Hymenocheate rubiginosa, Grandinia crustosa, Lenzites warnieri, Lopharia spadicea, Oligoporus subcaesius, Peniophora incarnata, Perenniporia ochroleuca, Pulchericum caeruleum, Steccherinum basibadium* were noted on fallen branches or twigs. *Irpex violaceus* and *Grandinia crustosa* are the species that were noted on conifers, on *Pinus halepensis*. All species, except *Oligoporus subcaesius,* which causes brown rot, cause white rot. *Cylindrobasidium evolvens, Grandinia crustosa, Lopharia spadicea, Oligoporus subcaesius* and *Steccherinum basibadium* are the species new for Montenegro.

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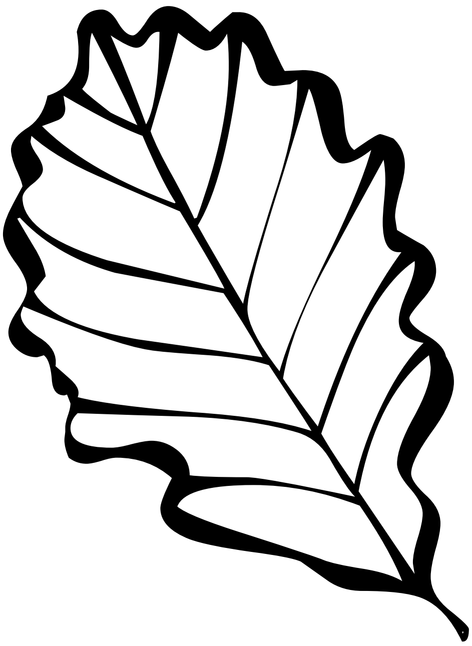

VARIABILITY IN WILD PEAR (*Pyrus pyraster* Burgsd.) POPULATIONS IN SERBIA BASED ON LEAF MORPHOLOGICAL CHARACTERISTICS

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Abstract: *The variability among and within nine populations of Wild pear (Pyrus pyraster Burgsd.) in Serbia were examined, based on eight leaf morphological characters. Examined morphological characters were: total leaf length (TLL), length of leaf blade (LBL), length of leaf petiole (LPL), leaf width (LW), leaf width / total leaf length ratio (LW/TLL), ratio between leaf width and leaf blade length (LW/LBL), ratio between leaf width and leaf petiole length (LW/LPL) and ratio between leaf petiole length and leaf width (LPL/LW). The results of analysis of variance (Nested design) emphasize the significance of length of petiole (LPL), ratio between leaf width and total leaf length ratio (LW/TLL) and ratio between leaf width and leaf petiole length (LW/LPL) in discrimination of examined populations. Contribution of variability within population was mostly higher then contribution of variability among examined populations. Extremely weak contribution of variation within and among population was observed for total leaf length and leaf blade length. Cluster analysis showed grouping of populations in three clearly distinct groups. That was confirmed by principal component analysis. It also showed that populations could be deferred by fewer characters, such as length of leaf blade (LBL) (in spite weak influence of differences among population), length of leaf petiole (LPL) and ratio between these two characters (LPL/LBL). Results of this study could be utilized in further work on variability studies and breeding in this noble and rare tree species.*
Key words: *Wild pear, leaf morphology, multivariate analysis*

INTRODUCTION

Wild pear (Pyrus pyraster Burgsd.) is considered as rare species that is present in almost all parts of Europe except far north. It distribution is scattered. It usually grows solitarily, or in small groups. Wild pear is a rare, scaterelly distributed species in Serbia too. It grows mostly as an individual tree, rarely in small groups (Ratkinic et al., 2004). Usually it could be found on meadows, farmland hedges, at the edges of forests, river floodplains etc. It is heliofilic species, tolerant to different soil conditions, except for soils with low pH. Best growth occurs on fresh, clacareos soils (Stephan et al., 2003). It is also tolerant to drought due to its tap roots. Thus, wild pear is perspective woody plant species concerning the expected changes of global climate (Paganova, 2003). Wild pear grows up to 20 (even more than 22) meters high, with 80 (even 130) cm of diameter at the breast height. It is vigorous with well fruit production. The fruits of Wild pear are small, mostly with characteristic shape, very tasty and nourishing (Bukvic and Fotiric, 2007). It is good for afforestration of dry sites, or salty soils in forms of shelterbelts or small stands for gaming purpose or honey production (Korac, 1988). It is also used as a scion in grafting of domestic pear, while wood assortments are highly valued on global market. In Serbia, some small population remained which represent vary valuable material for breeding of this species. Wild pear tree is rather variable species, and it is many varieties defined within it (Rotach and Baume, 2004; Paganova, 2001; Paganova, 2003; Eudtmann, 1999; Krstic and Vojnovic, 2002; Paganova, 1996). Its variability is also analysed by molecular markers (Kimura, 2007; Wagner, 1995).

MATERIAL AND METHODS

During the September 2007 the leaves were collected from 9 populations in Serbia (Dijana, Vranjak, Bodjanske šume, Karadjordjevo, Bela Palanka, Golija 1, Golija 2, Zlatibor and Jastrebac) – with different number of trees per population. The tree age varyed from 20 to 70 years. The number of collected leaves per tree varied also, so the samples were unequal. After the collection of leaves following characters were measured: total leaf length (TLL), length of leaf blade (LBL), length of leaf petiole (LPL), leaf width (LW). Based on these measured characters, following morphological characters were derived: leaf width / total leaf length ratio (LW/TLL), ratio between leaf width and leaf blade length (LW/LBL), ratio between leaf width and leaf petiole length (LW/LPL) and ratio between leaf petiole length and leaf width (LPL/LW). Variability of examined characters was described by parameters of descriptive statistics, as well as by contribution of expected variances to the total variation.

DATA ANALYSIS

The variability of rooting characters was examined by two-way ANOVA, nested design:

$$X_{ijm} = \mu + g_i + y_{j(i)} + \varepsilon_{m(ij)} ,$$

where X_{ijm} stands for measured value, μ - average value, g_i - effect of genotype (G), $y_{j(i)}$ - effect of year within i^{th} genotype (Y), and $\varepsilon_{m(ij)}$ - effect of uncontrolled variation. Samples were unequal. The results of two-way ANOVA, nested design, were used in calculation of expected variances for examined sources of variation (Kirk, 1968). Negative expected variances were considered to be zero. The effect of examined sources of variation were described by coefficients of variation: $Cv = \frac{\sigma_A}{\overline{X}} * 100\%$, where σ_A - stands for expected standard deviation of A source of variation. LSD-test among examined provenances was based on one-way ANOVA, with average values of trees as repetitions.

Principal component analysis and cluster analysis were used in order to reduce the data amount and to enable presentation of relationship among examined genotypes. The principal component analysis was also used for grouping of examined characters according to their loadings with principal components, selected to meet criterion: $\lambda > 1$ (Thurstone, 1969). Principal component analysis was based on correlation matrix and gained principal components were not rotated. Also, the examined genotypes are grouped by cluster analysis based on standardized genotype means, using unweighted pair-group average linkage (UPGMA).

The program package STATISTICA 7.1 (StatSoft Inc. 2006) was used for the statistical analysis.

RESULTS AND DISCUSSION

All examined leaf morphological characters showed significant variation among examined populations. However, variation among genotypes within populations for total leaf length (TLL) and length of leaf blade (LBL) was not significant. Their coefficient of variation were relatively high (9.3 and 15.0%, respectively), but the contribution of controlled sources of variation to the total variation for these two characters was minor (less then 0.02). Thus, these two characters could not be recommended for the further population studies, except in order to calculate some derived characters (Tab. 1).

This research shows that among examined populations occur considerable leaf morphological differences, which implies also considerable genetic differences. Rather high variability was indicated within populations too, suggesting high level of heterozygosity. However, the existence of genetic drift caused by inbreeding could be assumed because the wild pear is rather rare tree species, with scattered range, where it has been represented mostly by solitary trees. This situation in Serbia, confirmed by Ratknic et al. (2004) could have, at least partially, anthropogenic origin.

There was a difference among examined characters in their variability among and within populations. The poor variability among population and strong variability within populations were characteristics of leaf width (LW) and leaf width / length of leaf blade (LW/LBL). According to F-test, contribution of examined sources of variation to the total variation and coefficients of variation the most distinctive characters for examined populations were: leaf width (LW), length of leaf petiole / leaf blade length ratio (LPL/LBL) and leaf petiole length / leaf width ratio (LPL/LW). Last two had also relatively high coefficients of variation in comparison to coefficient of variation for error.

Table 1. Analysis of variance and population average values for examined characters of Wild pear

1)	TLL[2)]	LBL	LPL	LW	LW/TLL	LW/LBL	LPL/LBL	LPL/LW
df_{among}	8	8	8	8	8	8	8	8
df_{within}	109	109	109	109	109	109	109	109
df_{error}	3129	3129	3129	3129	3129	3129	3129	3129
dft_{total}	3246	3246	3246	3246	3246	3246	3246	3246
MS_{among}	44495.17	16165.90	8925.75	1910.66	0.568	0.337	7.575	9.478
MS_{within}	28520.21	5858.87	1040.83	586.57	0.077	0.212	0.648	0.834
MS_{error}	27494.45	5697.51	126.16	58.74	0.012	0.028	0.026	0.092
F_{among}	1.560^{ns}	2.759^{**}	8.576^{**}	3.257^{**}	7.410^{**}	1.586^{ns}	11.694^{**}	11.358^{**}
F_{within}	1.037^{ns}	1.028^{ns}	8.250^{**}	9.986^{**}	6.646^{**}	7.568^{**}	24.691^{**}	9.065^{**}
$\sigma^2_{among}/\sigma^2_{total}$ [3)]	0.168	0.521	12.589	4.760	9.335	1.082	29.226	17.437
$\sigma^2_{within}/\sigma^2_{total}$	0.135	0.102	18.195	23.405	15.406	19.026	32.703	18.681
$\sigma^2_{error}/\sigma^2_{total}$	99.697	99.377	69.216	71.835	75.260	79.892	38.071	63.882
Cv_{among} (%)	8.176	12.694	12.536	5.492	8.261	2.237	15.218	15.853
Cv_{within} (%)	7.320	5.616	15.071	12.179	10.612	9.383	16.098	16.409
Cv_{err} (%)	199.025	175.261	29.396	21.336	23.456	19.227	17.369	30.344
	TLL	LBL	LPL	LW	LW/TLL	LW/LBL	LPL/LBL	LPL/LW
Dijana	77.51	37.32	40.22	32.21	0.424	0.870	1.084	0.853
Vranjak	84.52	40.23	44.28	33.51	0.403	0.845	1.110	0.786
Bodjanske sume	102.63	39.84	44.14	35.47	0.429	0.899	1.115	0.838
Karadjordjevo	85.40	41.91	43.80	35.80	0.428	0.858	1.052	0.868
Golija 1	83.02	46.96	36.06	38.36	0.467	0.858	0.845	1.030
Golija 2	94.85	56.98	37.87	38.14	0.462	0.834	0.818	1.069
Brza Palanka	79.27	44.14	35.14	36.26	0.463	0.831	0.802	1.093
Zlatibor	75.74	42.27	33.47	38.22	0.507	0.906	0.804	1.179
Jastrebac	66.89	37.97	28.93	35.33	0.537	0.938	0.764	1.283
$LSD_{0.05}$	24.43	11.18	4.87	3.74	0.041	0.069	0.119	0.137

1) df – Degree of freedom, SS – Sum of squares, MS – Mean squares, Cv – coefficient of variation (%), among – among populations, within – within populations, $LSD_{0.05}$ – the least significant difference at α=0.05 (according to one-way ANOVA)
2) Character labels: TLL - total leaf length (mm), LBL - length of leaf blade (mm), LPL - length of leaf petiole (mm), LW - leaf width (mm), LW/TLL - ratio between total leaf length and leaf width, LW/LBL - ratio between leaf width and leaf blade length, LPL/LBL - ratio between leaf petiole length and leaf blade length and LPL/LW - ratio between leaf petiole length and leaf width.
3) $\sigma^2_{among}/\sigma^2_{total}$ - contribution of within-population expected variance to the total variance, $\sigma^2_{within}/\sigma^2_{total}$ - contribution of within-population expected variance to the total variance, $\sigma^2_{error}/\sigma^2_{total}$ - contribution of residual expected variance to the total variance

Studies on variability of morphological characters in wild pear are rather aboundant (Rotach and Baume, 2004; Paganova, 2001; Paganova, 2003; Eudtmann, 1999; Krstic and Vojnovic, 2002; Paganova, 1996). However, multivariate approach in statistical analysis is almost rare in description of variation among populations of Wild pear by morphological characters (Kim et al., 2004 on Asian pears). In our study principal component analysis and cluster analysis allowed condensation of complex data and simplification of presentation of relationship among examined characters and among and within examined populations.

Table 2. Correlations (loadings) of examined characters with the first two principal components

	PC1[2)]	PC2
TLL[1)]	-0.618	0.571
LBL	0.174	0.953
LPL	-0.975	0.112
LW	0.571	0.702
LW/TLL	0.981	-0.109
LW/LBL	0.454	-0.662
LPL/LBL	-0.942	-0.290
LPL/LW	0.993	0.031
Eigenvalue (λ)	4.731	2.275
(λ /Σ λ) *100%	0.591	0.284

[1)] Character labels: TLL - total leaf length (mm), LBL - length of leaf blade (mm), LPL - length of leaf petiole (mm), LW - leaf width (mm), LW/TLL - ratio between total leaf length and leaf width, LW/LBL - ratio between leaf width and leaf blade length, LPL/LBL - ratio between leaf petiole length and leaf blade length and LPL/LW - ratio between leaf petiole length and leaf width

[2)] PC1 – the first principal component, PC2 – the second principal component

Relationship among examined characters were analyzed by coefficients of correlation (loadings) of examined characters with the first two principal components. These two principal components explain near 90% of total variation among populations. Examined characters are grouped in two groups: one where dominates leaf width / total leaf length ratio (LW/TLL), and the second where dominates length of leaf blade (LBL). Characters LPL/LBL and LPL/LW are in the first and LW in the second group. As there is considerable correlation between LPL/LBL and LPL/LW, it could be said that majority of information about differences among populations that is explained by LPL/LBL is already explained by character LPL/LW. As LPL/LW and LW are in different groups, low correlation among these two characters could be expected. Thus, these two characters could be recommended for further population studies.

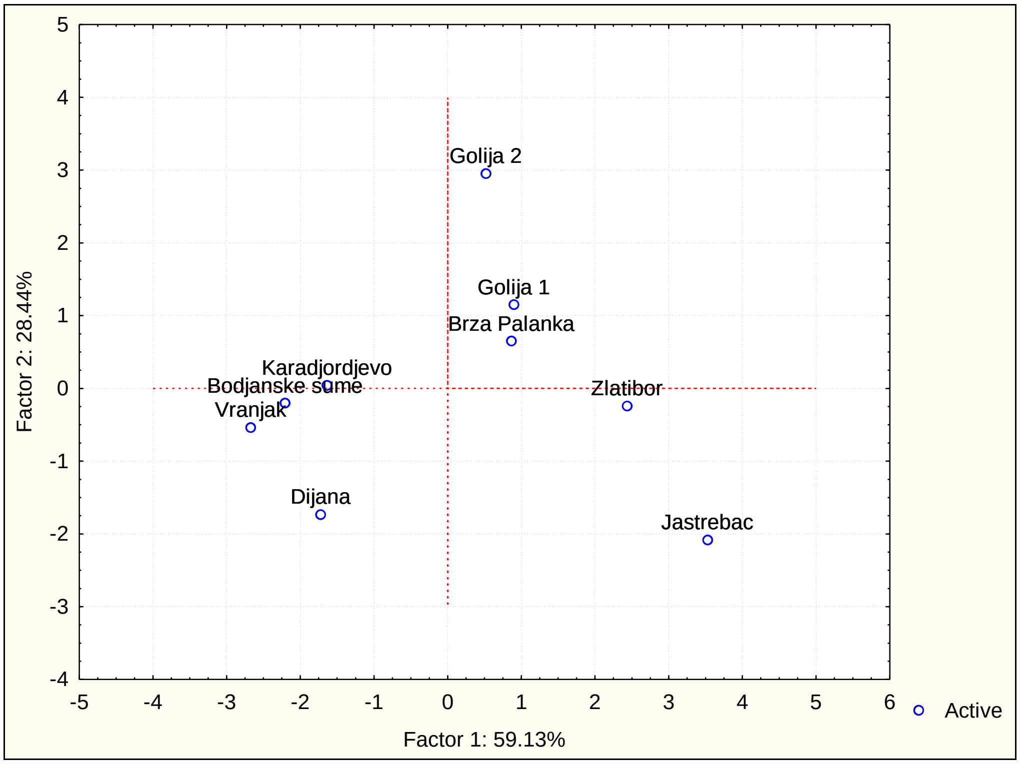

Figure 1. Relationship among examined populations of *Pyrus pyraster* Burgsd. according to the first two principal components

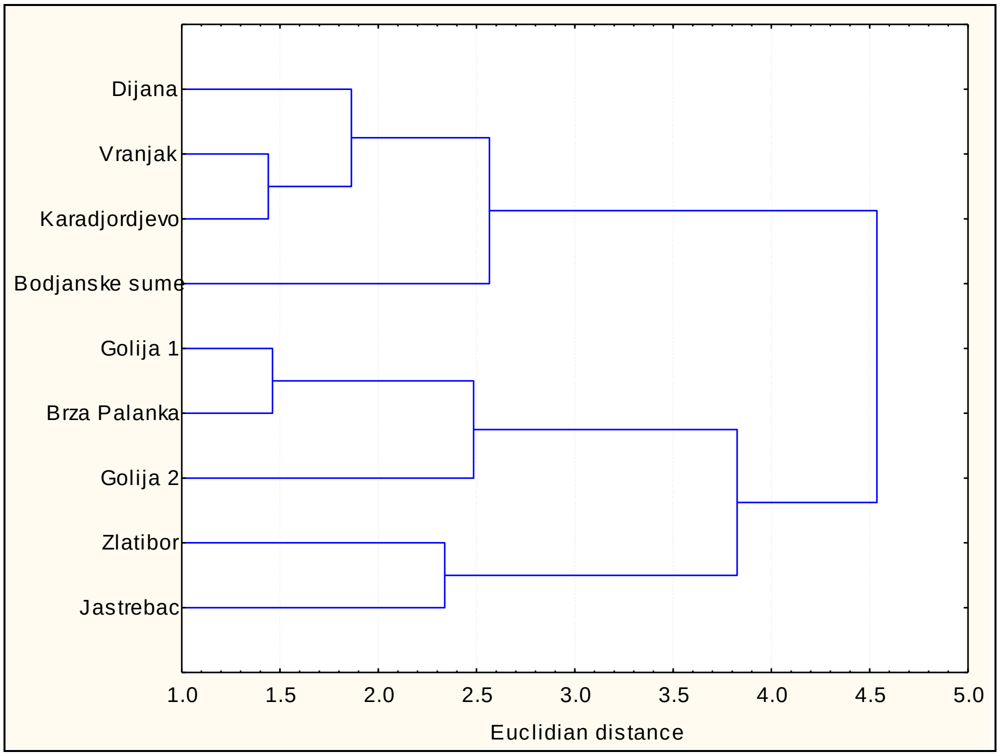

Figure 2. Cluster analysis, UPGMA linkage method, for examined populations of *Pyrus pyraster* Burgsd.

Cluster analysis showed grouping of populations in three clearly distinct groups. That was confirmed by principal component analysis.

Visual presentation of scores of the first two principal components, as well as cluster analysis emphasizes similarities among Diana, Vranjak, Bodjani and Karadjordjevo populations. The similarity for all four populations is that these populations were growing on deep salty soil. Populations Jastrebac and Zlatibor are found in the mountainous region on shallow soil. Populations Golija1, Golija2 and Brza Palanka were found also in the mountainous region, but the soil was not as shallow as for previous two groups (Fig. 1, 2).

It also showed that populations could be deferred by fewer characters, such as length of leaf blade (LBL) (in spite weak influence of differences among population), length of leaf petiole (LPL) and ratio between these two characters (LPL/LBL). The first group was characterized by rather high LPL/LBL ratio, the second by rather long leaf blade (LBL), while the third group of populations had lower LPL/LBL ratio (Tab. 1).

Considering valuable wood, eatable fruits, utilization in pharmacy, honey production and ornamental value this species should attract more interest in its breeding and improvement. Results presented in this study could be utilized in that way.

CONCLUSIONS

The examination of morphological leaf characters of Wild pear (*Pyrus pyraster* Burgsd.) in Serbia showed significant variability among and within examined populations. Characters LPL/LW and LW showed high variability among and within populations, and relatively low correlation between two of them. That recommends them for further population studies. These results suggest the existence of interesting diversity and differentiated “gene pool” of Wild pear in Serbia. Results of this study could be utilized in further work on variability studies and breeding in this noble and rare tree species.

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MULTI-ANNUAL INVESTIGATION OF POPLAR CLONES SUSCEPTIBILITY TO *Dothichiza populea* Sacc. et Br. IN THE FIELD

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Summary: *Susceptibility of 93 poplar clones to fungus Dothichiza populea Sacc. et Br. was studied in the period between 2003 and 2008. Investigations were based on the average number of spontaneous necroses in the cortical tissue of two years old plants. Clones with less than two necroses in the cortical region were characterized as less susceptible (52 clones), with 2-5 necroses as moderate susceptible (26 clones), while clones with five or more necroses comprised a group of very susceptible ones (15 clones). A group of 64 clones whose susceptibility was evaluated previously (Avramović et al., 1999) was included in this investigation. These clones retained a stable susceptibility to the pathogen, and previous results concerning stron*
Key words: *poplar, Dothichiza populea, susceptibility of clone*

INTRODUCTION

It is known that selection and breeding processes in plant production strive for creation of families and genotypes *g influence of genetic factors on poplar resistance to D. populea have been confirmed.* with superior characteristics over clones widely used in practice. Fast growth and increment of poplars are genetically determined and they could be deteriorated by pathogen agents. The most important pathogen is fungus *Dothichiza populea* Sacc. et Br. causing withering away of the poplar bark. The most suitable solution of problems caused by *D. populea* in cultivation of poplars is selection or creation of genotypes with low susceptibility to the pathogen attack (Herpka and Tomović, 1978; Herpka and Guzina, 1979; Marinković, 1980; Herpka, 1982; Tomović and Guzina, 1985; Tomović, 1987, 1990; Avramović, 1995, 1999). Until now, in the Institute of Lowland Forestry and Environment, hybridization and selection yielded in numerous genotypes of poplars characterized with especially dense growth and other desirable characteristics. A special attention was paid to monitoring and studying of susceptibility of the clones to *D. populea* in the past decades. The latest studies of susceptibility of a group of poplar clones to the pathogen were performed in the Institute in the late nineties of the past century (Avramović et al., 1999). However, long-term programs of selection and breeding of poplars in the Institute anticipate a continuous determination and monitoring of susceptibility of the clones to the pathogen, resulting in continuation of investigations in the following years. Hence, collaborators of the Institute tested susceptibility of 93 poplar clones to *D. populea* in the period between 2003 and 2008. A relatively large group consisting of 64 clones was included in similar investigations performed in the period between 1997 and 1999 (Avramović et al., 1999). Results of such investigations enable sparing expenses and time in the early phase of selection needed to provide basic information about the potential of clones in their susceptibility to the fungus.

In the present work, results of several years lasting analysis of poplar clones susceptibility to *D. populea* were presented. These investigations are going to be continued and expanded in order to include new clones.

MATERIAL AND METHODS

A series of 93 black poplar clones (section *Aigeiros*) originating from the genetic collection of the Institute's Experimental Estate was selected for this investigation. Some of the clones are foreign assimilated cultivars widely used in practice during the past decades (clones I-214, I-45/51, Ostia and Robusta). Other clones were introduced gradually in the production in our country in the period between 1980 and 2004 (clones 725, 55/65, S 1-8, S 1-3, S 11-8, Pannonia, 478, S 1-7). Furthermore, there are promising clones still in the selection process with other desirable characteristics. Presence of clones previously tested and foreign assimilated cultivars in the evaluation procedure enables the comparison between new and old selections, and determination of superiority of new clones over the widely used ones in their resistance to the pathogen.

In order to investigate susceptibility of the clones to *D. populea,* experimental stoolbed was established in spring of 2001 at the Institute's Experimental Estate. Ten cuttings of each clone were planted on two neighboring parcels, and the plant spacing was 1,50 x 0,40m. After the first vegetative period (in spring of 2002) sprouts were cut down at one parcel, while on the other seedlings were cultivated until spring of 2003 (age of two years). Systematic monitoring of susceptibility of the clones to *D. populea* began in 2003, and it was based on number of spontaneous necroses on two years old sprouts. Aimed to conduct a continuous evaluation of susceptibility from year to year, roots were left in the ground to produce new sprouts in the following years. One-year-old sprouts at the adjacent parcel were grown until next spring (Figure 1).

Figure 1. Experimental stoolbed intended for investigation of poplar clones susceptibility to *Dothichiza populea* (March 2008)

Necroses were diagnosed in two years old sprouts after removal of thin layers of the bark. A particular attention was paid to topographically sensitive zones of the bark. Necroses occur the most frequently in these zones, which occupy lower part of the sprouts (up to 1 m above the ground), spots of insertion and trimming of branches, and the level of "vegetative ring". For each clone three sprouts were sampled, and number of necroses was determined in the beginning of March in the period between 2003 and 2008 (Figure 2).

An average number of necroses in distinct clones were calculated for each year, as well as for all six years. These values were used for classification of clones into groups according to their sensitivity to the disease.

Figure 2. Determination of the number of necroses in the bark of two years old sprouts (March 2008)

RESULTS AND DISCUSSION

Majority of the clones (52) are less susceptible to the disease (Table 1). In the cortical tissue of these clones more than two necroses per plant were not found. These clones were characterized by high variability of the average number of necroses from year to year. For example, in the bark of clones 101/88-13, 182/81, 101/88-40 and B-352, five or more necroses per plant were detected in one year, while in other years necroses were sparse or missing. Table 1 provides numerous examples of such variability. This variability could be explained partly by environmental conditions, which were favorable for the fungus and unfavorable for the host or vice versa in some years. Studied plants grew under different environmental conditions, since uniformity of the conditions can not be achieved in the nature. Various environmental conditions directly affected physiological processes in plants, as well as the degree of the pathogen attack, i.e. number of necroses formed on sprouts. Speaking of this group of clones, it should be emphasized that 36 clones have already been evaluated and analyzed in the former period (Avramović et al., 1999). Comparison of results shows that 16 clones were classified in the group of sensitive ones (with 4-9 necroses per sprout). More surprisingly, clones S 1-3, 182/81, B-17 and 88/54 showed low susceptibility in our investigation, while in previous investigations they were considered as very susceptible clones (more than 10 necroses per sprout). It should be emphasized that the same methodological procedure was used for determination of necroses in the cortical region in both our and previous investigations, but criterions used for classification of the clones into groups were different. The criterions used by Avramović et al. (1999) were stricter, since group of sensitive clones contained members with 4 or more necroses which occurred only in one year or at one sprout, and clones with 10 or more necroses which occurred only in one year or at one sprout were classified as very susceptible clones. Thus, due to differences in criterions used for evaluation of susceptibility of the clones, some clones showed lower susceptibility in our work. Overall, studied group of clones showed low susceptibility to *D. populea* during nine years. Such results were expected, and could be explained by a relatively short period of monitoring and evaluation. Majority of the clones are not used in afforestation of great areas, and pressure produced by the pathogen population is low, which also contributes to results obtained in this work.

In our opinion, group of less susceptible clones (Table 1) could be recommended for wide use in establishment of poplar plantations. Moreover, some of these clones have already been registered for practical use, because of their good characteristics (increment, stem straightness, etc.) (clones 55/65, S 1-3, Pannonia, S 1-7).

Table 1. Average number of necroses caused by *D. populea* in the cortex of two years old sprouts in stoolbed in the period between 2003 and 2008

Clone	Taxonomical classification	2003.	2004.	2005.	2006.	2007.	2008.	**x**
Group I– less susceptible clones (below 2,0 necroses per sprout on average)								
88-20	hybrid	0,3	-	0,3	0	0	-	**0,2**
161/81	hybrid	-	0,3	-	-	-	0	**0,2**
4489	*P. deltoides*	0	0	2,0	0	0	0	**0,3**
9/21	*P. deltoides*	0	0	0	0,3	0	1,3	**0,3**
88-4	hybrid	0	0	1,0	0,7	0	0,3	**0,3**
139/81	hybrid	1,3	-	0	0	0	-	**0,3**
46/94	*P. deltoides*	0	-	1,0	-	0,3	-	**0,4**
185/81	hybrid	0	0	1,0	0,3	1,0	0,3	**0,4**
S6-7(3)	*P. deltoides*	0,7	0,7	1,6	0	0	0,7	**0,6**
121/81	hybrid	0,7	0,2	2,0	0,7	0,2	0,6	**0,7**
88-17	hybrid	0,3	0,3	0,7	0	0	2,7	**0,7**
438	*P. deltoides*	0	0,3	1,0	2,3	0,7	0,7	**0,8**
S6-21	*P. deltoides*	0	1,0	1,0	1,3	0,3	1,0	**0,8**
135/81	hybrid	1,0	0,2	0	1,0	0,7	2,0	**0,8**
4/94	*P. deltoides*	-	0,3	0,7	0,3	-	2,0	**0,8**
35/94	*P. deltoides*	-	-	2,4	0	0	-	**0,8**
101/88-60	hybrid	0	0	2,3	2,0	0	0,7	**0,8**
88-82	hybrid	0	0	-	1,7	-	1,7	**0,9**
43/94	*P. deltoides*	0	0,3	3,0	0,7	0,2	1,0	**0,9**
B-66	*P. deltoides*	0	1,0	3,0	1,0	0,3	0,3	**0,9**

S1-7/92	*P. deltoides*	2,0	0	2,3	0	1,0	0	**0,9**
1113	*P. deltoides*	0,3	1,0	1,3	0,7	0,3	1,7	**0,9**
125/81	hybrid	0	1,0	1,7	0	0	3,0	**1,0**
B-81	*P. deltoides*	0,7	1,2	1,7	0,5	0,8	1,3	**1,0**
1247	*P. deltoides*	0,7	0,7	1,0	1,2	1,5	0,8	**1,0**
4494(2)	*P. deltoides*	-	1,3	0	0	-	2,7	**1,0**
88-35	hybrid	0,3	1,7	2,0	0,7	0,7	1,3	**1,1**
101/88-21	hybrid	0,7	0	2,3	1,3	0,7	1,3	**1,1**
S6-1	*P. deltoides*	0	1,0	1,3	1,3	0	2,7	**1,1**
S1-3	*P. deltoides*	0	0	2,3	1,7	0,7	2,3	**1,2**
101/88-51	hybrid	0	0	1,0	2,0	0	4,0	**1,2**
B-178	hybrid	1,0	0	1,7	2,0	1,7	1,0	**1,2**
45/76-28	*P. deltoides*	0,3	1,7	4,5	0	0,7	0,1	**1,2**
88-66	hybrid	0,3	1,7	1,7	0,7	1,0	2,0	**1,2**
B-75	*P. deltoides*	1,0	0	4,9	0,3	0	-	**1,2**
88-54	hybrid	0	0	4,0	0,7	1,0	2,0	**1,3**
12/94	*P. deltoides*	0	0,3	3,1	2,5	0,8	1,3	**1,3**
101/88-13	hybrid	0,3	1,0	5,7	1,3	0	0	**1,4**
88-5	hybrid	1,0	0	1,7	1,3	1,3	3,0	**1,4**
182/81	hybrid	0	0	1,3	0,7	0,3	6,3	**1,4**
205/81	hybrid	0,3	1,0	4,3	1,3	1,0	1,0	**1,5**
Pannonia	*P.* x *ea*	2,0	1,6	3,9	0,6	0,8	0,2	**1,5**
101/88-40	hybrid	5,6	1,3	0,3	0	1,0	1,0	**1,5**
50-3/94	-	-	-	3,6	0,3	0,6	1,3	**1,5**
B-17	*P. deltoides*	1,3	1,2	4,7	1,3	0	0,7	**1,5**
934	*P. deltoides*	0	0	4,3	1,7	1,7	1,7	**1,6**
55/65	*P. deltoides*	1,0	0	3,6	2,3	0,4	2,4	**1,6**
665	*P. deltoides*	1,0	0,3	5,0	1,0	1,7	1,3	**1,7**
B-352	hybrid	1,5	0,7	5,1	0	0	2,7	**1,7**
S6-15	*P. deltoides*	1,8	0,7	2,3	1,9	-	-	**1,7**
40/94	*P. deltoides*	-	0,3	-	3,0	-	-	**1,7**
19/94	*P. deltoides*	-	-	2,8	1,0	0,3	3,2	**1,8**
9/31-22	-	-	-	3,7	1,7	0,5	1,7	**1,9**
Group II – moderate susceptible clones (2,0-5,0 necroses per sprout on average)								
54/76-11	*P. deltoides*	1,7	0	4,5	1,7	2,0	-	**2,0**
478	*P. deltoides*	1,0	2,2	4,3	1,2	1,0	3,0	**2,1**
PE 19/66	*P. deltoides*	2,0	1,4	5,3	0,7	0,6	3,2	**2,2**
10-8/96	*P. nigra*	2,7	0,3	7,7	0,7	1,3	0,7	**2,2**
181/81	hybrid	0,3	2,2	1,0	2,3	4,3	3,7	**2,3**
44/94	*P. deltoides*	-	-	2,8	0	4,1	-	**2,3**
33/94	*P. deltoides*	-	-	0,4	1,8	0,3	6,9	**2,4**
pe 4/68	*P. deltoides*	7,0	1,8	1,3	4,0	0,5	0,3	**2,5**
129/81	*P.* x *ea*	1,0	0,8	3,3	0,3	5,0	5,3	**2,6**
32/76-6(5)	hybrid	2,0	1,3	5,0	0,7	4,0	2,3	**2,6**
B-229	*P. deltoides*	4,0	0,4	5,7	1,2	2,3	2,2	**2,6**
725/95	*P. deltoides*	0	0,3	7,4	1,7	0,3	5,7	**2,6**
45/94	*P. deltoides*	0,3	1,3	5,1	1,2	3,0	5,0	**2,7**
88-22	hybrid	6,3	0	4,3	1,7	2,7	1,7	**2,8**
S11-8	*P. deltoides*	4,0	0,4	3,6	1,4	2,1	5,8	**2,9**
54/76-28	*P. deltoides*	0,7	3,2	6,3	0	1,3	6,7	**3,0**
54/76-2	*P. deltoides*	5,7	3,0	3,2	0,7	2,0	3,7	**3,1**
41/94	*P. deltoides*	-	-	3,3	1,2	4,4	3,3	**3,1**
S1-8/95	*P. deltoides*	2,0	0,3	2,8	2,4	3,2	7,8	**3,1**
11/94	*P. deltoides*	-	-	-	2,9	-	3,4	**3,2**
38/76-3	*P. deltoides*	3,3	0	5,3	2,7	5,2	-	**3,3**
S1-20	*P. deltoides*	0,3	1,3	6,3	4,7	3,3	4,3	**3,4**
55/76-7	*P. deltoides*	0	0,3	9,7	5,3	4,3	1,3	**3,5**
I-45/51	*P.* x *ea*	1,0	5,3	4,0	6,0	0,7	4,3	**3,6**
5/94	*P. deltoides*	4,0	2,7	3,7	1,7	6,0	5,8	**4,0**

Group III – very susceptible clones (over 5,0 necroses per sprout on average)								
237/81	hybrid	7,3	0	6,3	4,0	5,7	6,7	**5,0**
9111/93	*P. nigra* x *P. maximowiczii*	-	-	-	4,2	6,1	-	**5,2**
260/81	*P. deltoides*	6,0	0,3	9,3	10,0	9,3	6,0	**6,8**
70/76-10	*P. deltoides*	9,7	2,3	10,0	8,7	4,0	10,0	**7,5**
P. nigra	*P. nigra*	5,3	0,7	10,0	10,0	10,0	9,3	**7,6**
269/81	*P.* x *ea*	6,7	2,3	10,0	10,0	10,0	10,0	**8,2**
261/81	*P.* x *ea*	3,7	8,7	10,0	-	9,3	10,0	**8,3**
10-32/97	*P. nigra*	10,0	2,8	10,0	7,6	9,8	10,0	**8,4**
10-1/99	*P. nigra*	6,3	5,0	10,0	10,0	9,0	10,0	**8,4**
I-214/97	*P.* x *ea*	10,0	7,7	9,4	9,8	9,8	6,4	**8,9**
Zel. Robusta	*P.* x *ea*	10,0	9,7	10,0	10,0	9,0	10,0	**9,8**
Ostia	*P.* x *ea*	10,0	9,3	10,0	10,0	10,0	10,0	**9,9**
265/81	*P.* x *ea*	10,0	9,3	10,0	10,0	10,0	10,0	**9,9**
251/81	*P. deltoides*	10,0	10,0	10,0	10,0	10,0	10,0	**10,0**
256/81	*P.* x *ea*	10,0	10,0	10,0	10,0	10,0	10,0	**10,0**

A group of moderate susceptible clones (2-5 necroses per sprout) contained 25 clones. These clones were also characterized by high variability of the necroses abundance in studied years. Majority of clones had 5 or more necroses per sprout in some years, while in other years these values were considerably lower. This group contains 14 clones whose susceptibility to *D. populea* was examined previously by Avramović et al. (1999). Comparison of results showed that eight clones were classified as "moderate susceptible" in both experimental periods, while six clones were denoted as "very susceptible" in previous investigation. These differences arose from differently defined criterions for classification of clones into groups according to their susceptibility. We concluded that clones from this group probably have greater demands for environmental conditions in order to reach optimal growth, in comparison with less susceptible clones. Optimal conditions (favorable soil, optimal humidity, space between plants, and absence of harmful organisms) could decrease number of necroses, and tendency of these clones to be endangered by bark necrosis. In our opinion, moderate susceptible clones could be used for afforestation under optimal environmental conditions.

Similar variability was evident in other very susceptible clones (15). Presence of 10 or more necroses per sprout in the cortical tissue of these clones (except of clones 237/81 and 9111/93) in at least one year points out their high susceptibility to the pathogen. Avramović et al. (1999) confirmed high susceptibility of 11 clones previously. Clones from this group are not recommended for wide use in practice, because the pathogen could endanger vitality and survival of plants in poplar nurseries and plantations.

Clones without necroses in the cortical tissue were not found in this investigation (Table 1). Even clones with the lowest susceptibility (clones 88-20, 4489, 9/21, 88-4, 139/81) had necroses in one of the studied years. Clones without necroses in the cortical tissue have been clearly separated as a group of practically unsusceptible clones by Avramović et al. (1999). Three clones from this group (4494(2), 161/81, and 101/88-51) showed low susceptibility in our investigation. Our results have confirmed earlier findings that the fungus attacks genus *Populus*, and the clones could be more or less susceptible to the disease; there are not resistant clones.

Compared with previous results (Herpka and Tomović, 1978; Herpka, 1982; Tomović and Guzina, 1985; Avramović et al., 1995, 1999, Keča, 2001), majority of studied clones showed a stable susceptibility to the bark cancer. Results presented in this work showed significant differences in their resistance to the disease. In his doctoral thesis, Tomović (1990) proved genetically determined stability of clone susceptibility to *D. populea*. This author examined susceptibility of some American black poplar clones and their hybrids with euramerican hybrids for several years. He found high coefficients of heritability (in both broader and narrower sense) and high correlations between parents and descendants, suggesting a genetic control of this trait. However, contribution of environmental factors to the disease intensity should not be neglected. According to Tomović (1990), establishment of polyclonal plantations using several resistant descendants and genotypes could provide higher biological stability than in monoclonal plantations. The pathogen is capable to adapt itself relatively fast to clones selected for massive use. Hence, permanent breeding of poplars is necessary in intensive production in order to improve their resistance to the pathogen.

In present paper, we found it useful to present and analyze susceptibility of Pannonia clone, an acknowledged clone used for afforestation for several years. In addition, we presented candidates submitted for acknowledgment: B-229, PE 19/66, B-81, 182/81 and 129/81. Our analysis is based on results presented in Table 1 and other sources and papers. Institute of Lowland Forestry and Environment in cooperation with Public Organization „Vojvodinašume" established several plantations using clones submitted for acknowledgment in spring of 2004, in order to examine their characteristics and productive capacity. Poor establishment of seedlings and occurrence of *D. populea* appeared in dependence on technological procedure, planting style and type of the planting material used. The most problematic clones were B-229 and PE 19/66, belonging to *P. deltoides*. In these clones, dying back of plants spread over 40-70% of seedlings and most of them were attacked by the fungus. The fungus was less abundant in seedlings of clone B-81 (5-10% of seedlings was infected). Absence of fungal attack and dying back of seedlings in clones Pannonia, 182/81 and 129/81 was very indicative (unpublished data). To our knowledge, this is the first report about intensive attack of the fungus in young plantations of these clones, resulting in declining of numerous plants. Susceptibility of clones PE 19/66 and B-229 to *D. populea* found in these plantations was confirmed by this work. Our investigation classified these clones as sensitive ones (2-5 necroses per sprout), while clones Pannonia, B-81 and 182/81 belong to the group of less sensitive clones (Table 1).

Avramović (1995) reported high susceptibility of clone PE 19/66 to the fungus. This author studied susceptibility of 18 black poplar clones cultivated in the climate chamber. Significant differences between clones with respect to artificial necroses on the cuttings were also found, indicating clear differences in their susceptibility to the fungus. High susceptibility was recorded in clones I-214, PE 19/66 and Zelena Robusta, while low in clones S 6-3, S 1-8 and 438. This author have classified this clone as sensitive (of 125 studied clones) on the basis of number of spontaneous necroses in the cortical tissue with 4-9 necroses on sprouts (Avramović et al., 1999).

Pap (2005) studied susceptibility of five poplar clones (I-214, Pannonia, B-229, B-81, 182/81) using an artificial inoculation of the mycelium into the cortical tissue of two years old seedlings. Results obtained from a two years lasting trial pointed out a relatively low susceptibility of clones Pannonia, B-81 and 182/81, and considerably higher susceptibility of clones B-229 and I-214 to the pathogen. Clone B-229 was previously considered as less susceptible (Tomović, 1990). In contrast to his results, results of Pap (2005) and those presented in our paper suggest that this clone belongs to the group of clones susceptible to *D. populea*.

Susceptibility of clones PE 19/66 and B-229 obtained in former investigations and experimental plantations requires a caution related to use of these clones. They could be used in afforestation programs, but optimal growth conditions for seedlings are necessary (favorable soil, optimal humidity, suppression of insects, optimal period of planting).

Investigations of susceptibility of clone Pannonia to *D. populea* have begun in the middle eighties of the past century. Until now, numerous authors emphasized superiority of Pannonia clone over other clones studied (Tomović and Guzina, 1985; Tomović, 1987; Gergácz et al., 1988; Szontag, 1990; Tomović, 1990; Avramović, 1999; Keča, 2001; Pap, 2005). We concluded that this clone preserved a stable low susceptibility to the pathogen in a relatively long period of its use in practice.

CONCLUSIONS

Studied poplar clones exhibited a relatively high variability of their susceptibility to *Dothichiza populea.*

Majority of clones (52) exhibited low susceptibility, 26 clones showed moderate susceptibility, while 15 clones were highly susceptible to the fungus.

Clones identified as less or moderate susceptible (B-81, 4494(2), 665, B-229, PE 19/66, 182/81, etc.) are recommended for selection and acknowledgment according to their resistance to the pathogen.

Degree of poplar clones susceptibility was more or less stabile in studied years. Unfavorable growth conditions increase a tendency to fungal attack.

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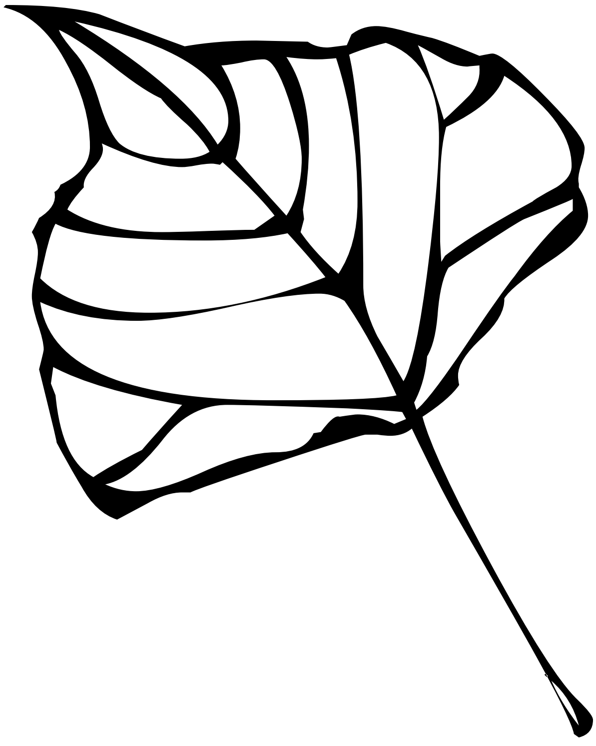

THE INFLUENCE OF HEAVY METALS ON SPRUCE SEED GERMINATION

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Summary: *Sprouting is a complex biochemical process, which includes beginning of growth and mobilization of reserve substances in the seed in order to be used by a growing embryo. How the presence of heavy metals affects the process of spruce seed sprouting, was tested in the laboratory of the Forestry Institute, where an experiment was made with the following metal solutions: lead, copper, cadmium and zinc. Petri vessels with double layer of filter paper on the bottom were moistened with solutions of compounds containing these metals in three different concentrations (3 ppm, 33 ppm and 100 ppm) and then the seed of Picea abies was sown.*

The spruce seed which developed on substrates with addition of heavy metals solutions in three different concentrations showed significant tolerance to presence of all metals, but percentage of sprouted seeds depended on the type of metal and its concentration.

The seed of P. abies demonstrated tolerance to presence of heavy metals in concentration of 3ppm and 33 ppm, where sprouting was registered in all variants. At the least concentrations (3 ppm), results were uniform at the end of testing period for Cu, Pb and Cd, while in the presence of Zn germination ability of even up to 92% was recorded. In the conditions of largest concentrations (100 ppm), the seed did not sprout regardless of the type of metal.

Key words: *heavy metals, sprouting, seed, spruce*

INTRODUCTION

Germination as a complex biochemical process encompasses the process of growth and mobilization of the reserve substances in the seed with the aim of the use of them by the growing embryo. Germination starts with absorption of water by the seed (imbibition), and finishes with the beginning of the embryo axis elongention, usually the radiculum Nešković et *a*l. (2003). Germination becomes visible only when the top of the rootlet released itself through micropilum which opens up under its pressure, and endosperm, i.e. nutrient matters bulge. It happens at a fast pace in the spruce seed, as early as three or four days after bulging Stilinović (1991).

Numerous factors present in the soil, such as acidity level, presence of the heavy metals, etc. have an adverse effect on the seed germination process. According to Kadović & Knežević (2002), the layers of the forest layer and ground organic-mineral layers, usually contain the greatest concentrations of heavy metals. The main source of these elements in the ground layers of soil is usually air pollution. The authors report that it particularly applies to lead, copper and zinc in the coniferous forests. The research on the influences of these metals on spruce seed germination has been conducted in the laboratory of Institute for Forestry.

Heavy metals are present in traces and in all unpolutated soil as the result of the decay of the parent substratum, as the elements in traces. Owing to it, they are widely spread in the soil, plants and animals. Some heavy metals are essential for plants as microelements (Cu, Mn, Fe and Zn), since they are part of the metabolic processes. Although they are useful in the small quantities, these elements become harmful when the assessible forms in soil are present in the great quantities, and become toxic to plants and microorganisms when they are present in the greater quantities in the soil. They can influence the microbial processes in the soil through litter decompostion, enzymatic activity, change of the microbial population, by the alternations in the number, composition and diversity of the microorganisms Rudawska (2000), i.e. they have an adverse effect on plant germination and growth, which had a negative longtime effect on the forest ecosystem Veselinović (2006). Accumulated heavy metals slowly remove from the soil, by elutriation, plant consumption or erosion and deflation. Behaviour of the heavy metals in the soil depends upon the soil properties, most notably of pH values, capacity of cation changes

(CEC), organic matter content, type and content of clay, iron oxides, manganum and alluminium Kadović & Knežević (2002).

MATERIALS AND METHODS

Spruce seed used in the experiment originates from FMU Rasina Kruševac, was collected in the seed facility registrated as C 01.02.01.10. Seed purity is 98.6%,of inert matter is 1.4%. Germination energy after 10 day is 75.6%; germinated seed vitality is 81.0%; useless debris is 19.0%. Moisture content (against method of drying at 105^0 C) is 7.02%. Pure seed mass in the sample of 1,000 seeds is 7.4 g.

By the laboratory analysis of the seed health condition neither the presence of the pathogenic, which cause the lodging of the plants, nor of the insect were identified.

The experiments with solutions of four metals (Pb, Cu, Cd i Zn) was set in the laboratory. Petri dishes with double layer of filter paper at the bottom are moisted with the solutions of the compounds containing lead, copper, zinc, or cadmium in three different concentrations (3 ppm, 33 ppm and 100 ppm) Dunabeitia et *al.* (2004). These solutions are prepared of the salts of these metals in the forms of the following compounds: $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $CdSO_4 \cdot 8H_2O$ and $Pb(COOH)_2$, and prior to the pouring the filter paper is sterilised in the autoclave at temperature 120˚ C, pressure 1.5 at,and for 20 minutes. pH is 5.5 for all solutions, except for copper in which pH is 4.5. Petri dihes in which filter paper was soaked only by sterilised water are used as control variant. Four seeds, which are previously sterilised in 30% H_2O_2, for 20 minutes and then rinsed by sterile water three times are set in each dish. All Petri dishes containig seeds are put in the thermostat, at temperature 22 ± 1^0 C and germination was monitored for four weeks.

RESULTS

The type and concentration of metal had an important influence on the percentage of the germinated seeds *P. abies.* (Figures 1-4).

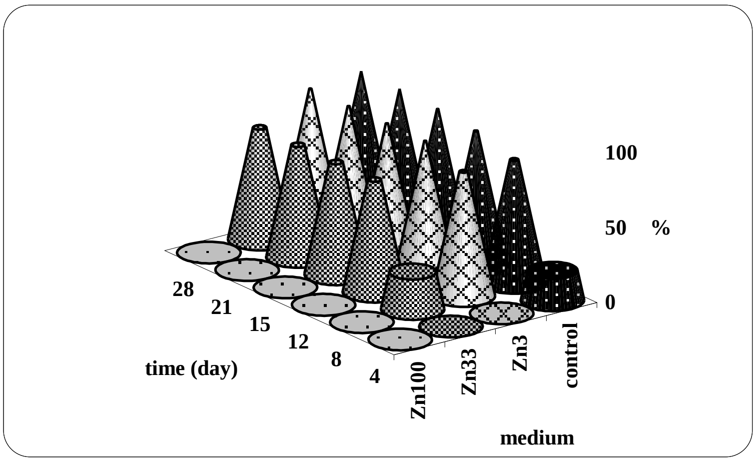

Figure 1. Seed germination (%) *P. abies* in the control and at different zinc concentrations (ppm)

Zinc presence at the smallest concentration did not influence the decrease of the number of the germinated seeds in the studied experiment. Concentration 33ppm influenced the decrease of the number of the germinated seeds. Only the greatest concentration of this metal (100 ppm) completely disabled the seed germination. Zinc is characterized by a high biological accessability to the plants, because of which the greatest concentrations of this element are mainly found in the root system and young plants, and considerably less in the bark. It is one of the ferment components and has a role in the synthesis of auxine. Plants contain small quantity of it, and the high content of it is toxic. When it is present in sufficient quantity, it increases the plant resistance to drought and diseases Nešković et *al.* (2003). Copper presence at these concentrations had the similar influence on the seed germination as the zinc presence (Figure 2).

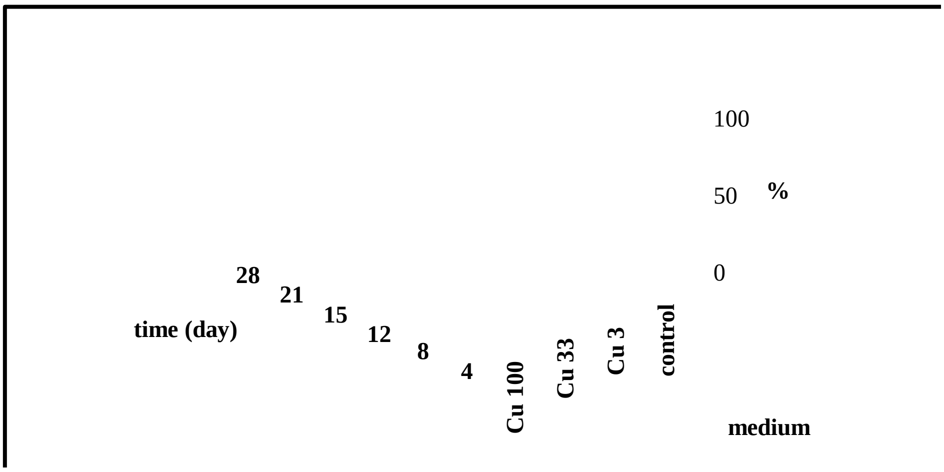

Figure 2. Seed germination in the control and at different copper concentrations (ppm)

Copper presence at concentrations 3ppm and 33ppm caused the smallest number of the germinated seeds in the comparison with zinc presence at these concentrations, although the measured pH value was 4.5. Copper is one of the least mobile heavy metals in the soil. Solubility, mobility and accessibility to the plants, to the greatest level depend on pH environment. Copper accessibility is drastically reduced at pH higher than 7, and it becomes easily accessible at pH lower than 6, and particularly lower than 5 Kadović & Knežević (2002). In plants copper has a role in ferment oxidation and synthesis of proteins, and in this way protects chlorophyll from destruction. In the cases of the lack of this element, young leaves become chlorotic.

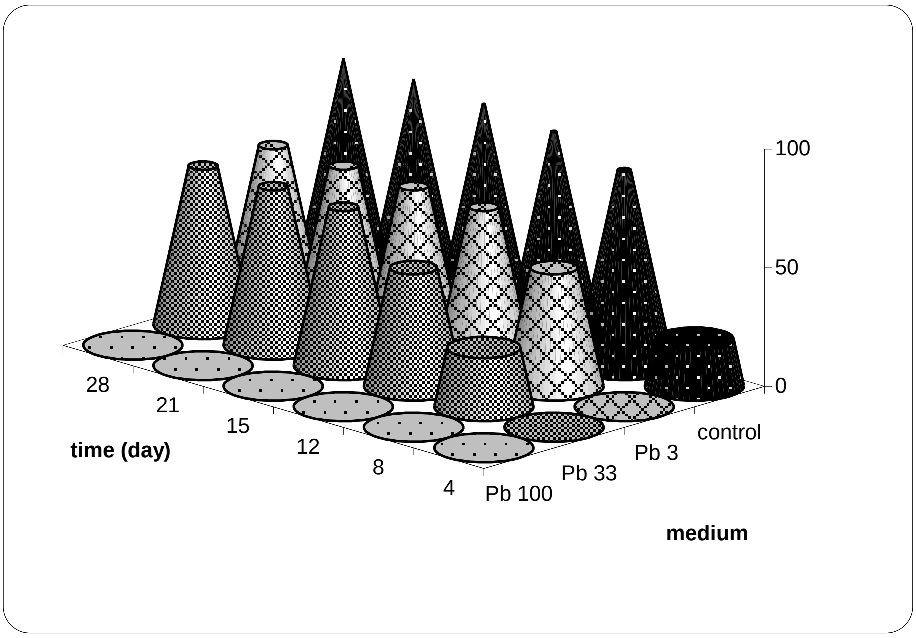

Figure 3. Seed germination in the control and at different lead concentrations (ppm)

Lead presence in the soil had the similar effects on the seed germination as copper presence (Figure 3), i.e. smaller number of the germinated seeds in the comparison with the control variant was identified when zinc was added. Soil to which lead was added at 33ppm concentration did not postpone the beginning of germinantion, which was reported when copper was present at this concentration. Lead is widely present in the soil. Nature content in the soil originates from parent rock or pollution, and often is greatest in the surface. Lead is bound to the organic matter and together with the solution of the organic matter migrates through the profile. The low mobility of it in the soil increases with the increase of acidity. Plants are mainly very sensitive to lead presence Kadović & Knežević (2002). Lead phytotoxicity is relatively lower in the comparison with cadmiun, which is explained by the limited lead accessibility to the plants of the soil solution.

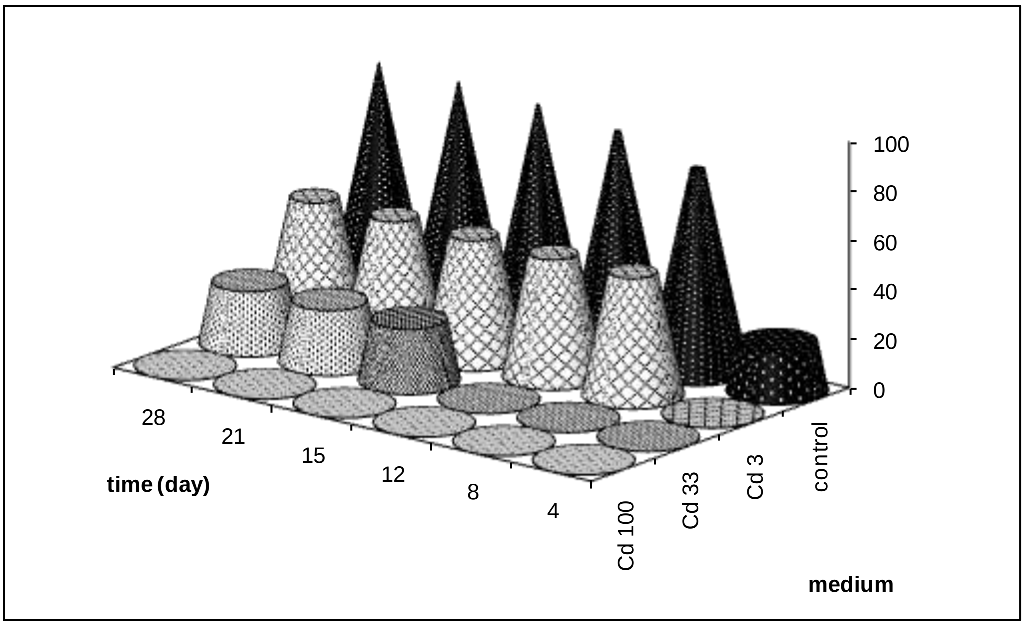

Figure 4. Seed germination in the control and at different cadmium concentrations (ppm)

Cadmium had the most toxic effect on the spruce seed germination of the studied heavy metals. At 3ppm concentration the smaller number of the germinated seeds was observed, and at higher concentration (33ppm), the negative effect was reflected in the considerably smaller number of the germinated seeds as well as in the postponement of the beginning of the germination. At the greatest concentration 100ppm no germinated seeds were reported. Plants assimilated cadmium passively by the root system and by assimilation organs. It is mostly accumulated in the root, and in the overground part there is medium or high content of it. When cadmium content is increased in the air, there is the greater assimilation of it through the needles. It is more intensively assimilated and transported in the overground parts than lead. Greater cadmium concentrations inhibitate iron metabolism, cause chlorosis and in this way reduce the intensity of photosynthesis, and lead to the transpiration inhibition. This element is part of the oxidation processes and influence plant morphogenesis (Sarić, 1979; Nešković et *al.*, 2003). Heavy metals and cadmium are not essential to plants, i.e. they are not elements which are necessary for the functioning of the plant organism Kadović & Knežević (2002).

DISCUSSION

Heavy metals had different influence of the germination of *Picea abies* seeds. *P. abies* seed exibited a great tolerance to heavy metal presence at concentration 33ppm and germinated in all variants. At the lowest concentrations (3 ppm) at the end of the studied period the results were balanced for Cu, Pb and Cd, whereas at Zn presence the germination was as much as 92%. The highest concentrations of these metals had an adverse effect on the germination and, as a result, at the concentration 100ppm of all studied heavy metals no germinated seeds were reported.

Spruce is a species which besides Scots pine and Austrian pine is most often used for afforestation in Serbia because of the small requirements from the the environment. Spruce often perseveres in very unfavourable environmental conditions, in the areas where the acid rains are reported or nitrogen, potassium or phosphorous depositions (Gronbach & Agerer, 1986; Hagerberg et *al.* 2003). Although it belongs to the species which prefer acid soils Jonsson et *al.* (1999), it also adapts to the limestone bases. Numerous studies of the spruce development under the unfavourbale environmental conditions have pointed to the fact that its survival has been enabled mostly by ectomycorrhizal formations on the root of these trees. Ectomycorrhiza influences on the increase of the growth and easy adoption of the seedlings in the endangered places, since ectomycorrhizal fungi are obligatory bound to the root of the host plant and increase the plant resistance to different environmental stresses Goldbold et *al.* (1998).

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CHAITOPHORUS POPULIFOLII (APHIDIDAE: HOMOPTERA) NEW APHID SPECIES ON POPLARS IN SERBIA

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Abstract: *During investigation of aphid fauna on poplars in Serbia, the presence of Chaitophorus populifolii was observed on two poplar clones. It is new species for aphid fauna of Serbia. The finding is very interesting as this species is widely distributed in USA, Canada and Mexico where it can be found on Populus spp. especially on Populus angustifolia and Populus balsamifera. This is also second finding in Europe where it was introduced with poplal cuttings from north america during 80`s of last centery .In Serbia Chaitophorus populifolii was observed on Populus x euramericana cl. I-214 -Kać, i Populus deltoides cl.* S_1*-8, -Novi Sad*
Key Words: *Aphididae, plant lices, Chaitophorus populifolii, poplars*

The species *Chaitophorus populifolii* was identified during the research of the aphid entomofauna on poplars in Serbia. This is a new species for entomofauna of Serbia. It was introduced to Europe from North America in the eighties of the last century, with the cuttings of balsam poplars. The aphids were collected during the period 1997 - 2004. This paper presents the basic morphological characteristics and the species biology.

The aphid family (Aphididae) because of the density and distribution of the species classified as aphids, the number of plant species they colonise, specificity of development and the way of living, specific morphological and anatomic traits, as well as the consequences of feeding on the host plants, has been arising and still arises the interest of the entomologists and also of the experts dealing with plant production. The literature reports on a series of aphid species which develop and feed on the parts of different species of forest trees, and their harmful significance is especially emphasised.

Poplars have a special place, being very important in the production of wood of fast growth and wide usability. However, it is important to determine which aphid species occur on poplars in our country, their distribution and scope of occurrence on the plants and to determine the species and genotypes of poplars infested by aphids.

To date, about 120 species of aphids which feed on poplars have been registered. They are divided into 24 genera (Blackman, R.L. and Eastop, V.F.,1994): . Some of them are specific only for poplars, while there are also those which do not feed only on poplars, but they can be found also on other plants.

In Serbia to date, 16 aphid species have been identified on poplars (Boža, P. 1983;1983b; Eastop. V. & Tanasijević, N.,1968; Janežić, F., 1982; Petrović, O. ,2003)

Aphids were collected on poplars in the period 1997 – 2004. Their presence and representation were determined in the nurseries, plantations, tree rows, parks and in natural poplar stands on the territory of central Serbia, Vojvodina, and Montenegro, but the most detailed research was performed in the area of the Experimental station of the Institute of Lowland Forestry and Environment, Faculty of Agriculture Novi Sad, as well as in the vicinity of Novi Sad..

The collection of the material was based on the standard entomological methods.

The determination was performed based on the methods of the following authors: Blackman & Eastop (1984); Heie, (1982); Szelegiewicz, (1961).

Subfamily: Chaitophorinae

Viviparous females can be winged and wingless. The body is most often covered with long hairs, which can be pointed, blunt, spatulate or forked. They do not have wax glands. The front edge is straight or gently convex without tubercles. Antennae consist of 4-6 segments. Primary rhinaria in some species are surrounded with short hairs. Secondary rhinaria are round, and are absent in wingless females. Rostrum is relatively short. Cornicles are short, stumplike and net structure. Cauda is semicircular in form and knoblike. Aphids of this subfamily live on broadleaf trees or on herbaceous monocotyledonous plants. They never produce galls. Some species of this subfamily are myrmecophilous, (Heie,1982).

Genus Chaitophorus

This is the genus with the greatest number of species distributed in the Holarctic region. They colonise primarily the plants in the family *Salicacae*. Some of the species feed on *Populus,* and some on *Salix* species. They are highly specialised and to date no species have been identified to feed on the plants in both genera. Aphids in this genus are classified as small to medium size (1.5 – 2.5 mm). Their body is densely covered with hairs (Cheta) by which they were named. Their cauda is usually knoblike. They are mostly myrmecophilous species.

Chaitophorus populifolii Essig**,** 1912
Syn: Chaitophorus balsamiferinus Hille Ris Lambers; *Chaitophorus populellus* Gillette and Palmer; *Chaitophorus essigi* Gillette and Palmer

Photo 1. *Chaitophorus populifolii* (Essig)

Widely distributed in USA, Canada and Mexico. Colonies found on Populus spp., particularly *Populus angustifolia* and *Populus balsamifera* (Blackman, R.L. and Eastop, V.F.,1994): . Introduced to Europe on the cuttings of balsam poplars (Blackman, R.L. and Eastop, V.F.,1994): .

Morphological haracters:

Aptera of individuals in this species are pale yellow. In some cases there is some pigmentation of head, pro and mesothorax, as well as of abdominal tergites of the 7th and 8th segments. Sometimes, in some individuals there are pairs of dark coloured and incomplete pleural bands.

Oviparous as well as winged and wingless males appear in the period from September to November.

In the world, *Chaitophorus populifolii* is identified on the following poplar species *Populus angustifolia, Populus balsamifera, Populus fremontii Poulus deloides, Populus grandidentata, Populus trichocarpa* (Blackman, R.L. and Eastop, V.F.,1994): .

In Serbia, this aphid is identified on the following two species of poplars:

- *Populus* x euramericana cl. I-214, Kać, Experimental estate of the Institute of Lowland Forestry and Environment, 07.04.1998, and
- *Populus* deltoides cl. S_1-8, Novi Sad, Experimental estate of the Institute of Lowland Forestry and Environment, bed - 09.04.1999.

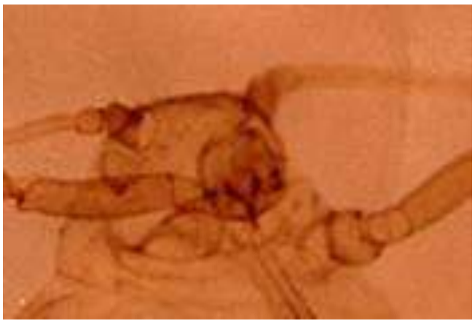

Photo 2: Head of *Chaitophorus populifolii* (Original)

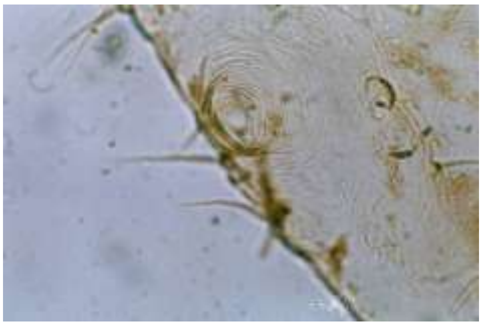

Photo 3: Cornikula *Chaitophorus populifolii* (Original)

Photo 4: Last segment of Rostrum *Chaitophorus populifolii* (Original)

Photo 5: Procesus terminalis *Chaitophorus populifolii* (Original)

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ELEMENTS OF GROWTH AND STAND STRUCTURE OF TWO BLACK POPLAR CLONES (SECTION *AIGEIROS* DUBY) DEPENDING ON PLANTING DISTANCE

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Abstract: *The results on evaluation of the significant influence of clone and planting distance (as two basic factors of poplar tree production) on tree and stand growth of two black poplar clones (S_{6-7} i Pannonia) that were planted with three planting distances (3 × 3 m, 4,25 × 4,25 m i 6 × 6 m) are presented in this work. Experimental stand was planted on fluvisol soil type in a random block design with three replications in a protected part of Danube aluvial plain in the vicinity of Novi Sad.*
Significant influence of clone and planting distance on obtained mean values of growth elements (d_g, $d_{g20\%}$, g_s, G) was observed five years from planting. Clone S_{6-7} had higher mean growth value in comparison to Pannonia clone. Greater planting distances caused higher mean values of tree growth, but smaller basal areas per hectare in relation to smaller planting distances. Significant influence of both factors (clone and planting distance) was maintained for seven years after stand establishment, but in such a way that the significance of clone factor decreased, while that of planting distance increased. Current increase of growth elements for the period from fifth to seventh year revealed significant influence of factors associated with planting distance, while that of clone remained insignificant.
Clone S_{6-7} had higher variability of diameters at breast height and basal areas in relation to Pannonia clone at all planting distances which was also manifested in the form of distribution. Higher planting distance caused increase of absolute variability, and decrease of coefficient of flat structure of diameters at breast height and basal areas in both clones. Increase in diameter increment and increase of basal areas with slight variability expressed as correlation coefficient in both clones occurred when planting distance was increased.
Significant differences between clones and planting distances were determined by comparison of diameter structures, structures of basal areas per diameter classes and structure of diameter increment and basal area increment (Kolmogorov-Smirnov non-parametric test). Differences were more obvious in relation to comparison of mean values which pointed out to justification of their application in this kind of investigations.
Key words: *poplar, clones, planting space, growth elements, stand structure*

INTRODUCTION

Poplar stands are mainly established with the number of trees expected to be the same at the end of production cycle. In such procedure tree crowns have free standing position so called "solitary growth" after planting, and later on canopy closes due to increased crown dimensions. Canopy closure leads to pronounced competition of trees for space and growth, which causes change in increase of width along the tree trunk (Stamenković and Miščević, 1970; Miščević and Stamenković, 1972; Vučković, 1989).

Previous investigations revealed strong influence of growth space on tree breast diameters. With greater planting distances young trees keep their so called "free upright position" longer, and planting density has no influence on their thickness growth. During that period significant i.e. determining factor influencing the diameter growth are the clone and soil characteristics (Bartek, 1980; Marković, 1980; Marković and Rončević, 1989; Marković, et al., 1997, 1998; DeBel and Harrington, 1997; Rončević, et al., 1998).

Growth of diameters is more intensive under favorable conditions, and differentiation of diameter growth at different planting densities occurs earlier. On habitats with poorer production potential the diameter growth is lower, and differences in diameters at close planting densities are determined later (Marković, 1980, 1985; Marković and Pudar, 1990; Pudar, 1982, 1986).

More intensive diameter growth of individual poplar genotypes leads to early significant influence of planting density on tree diameter growth i.e. dimensions of mean tree in the stand (Marković, et al., 1998).

Obtained tree dimensions in a stand at a certain age are the results of number of factors, both exogenous (climatic region, soil characteristics, hydrology regime, tree species, i.e. variety, stand density), and endogenous (internal stand construction, and mutual influence of trees one upon another). Influence of so called "exogenous" factors can be defined as certain trial design by taking basic principal of statistical method into consideration.

The aim of this paper was to quantify the influence (effect) of different growth of two poplar clones and their reaction to different stand densities during early developmental period, when relations between mean values are still not clearly differentiated. Besides mean values, the usage of numerical parameters of the stand structure is more pronounced with the aim of more precise estimation.

MATERIAL AND METHODS

Investigations were conveyed in the stand of two black poplar clones (Section *Aigeiros* (Duby)): S_{6-7} (*Populus deltoides* Bartr ex Marsh, clone in the breeding process) and Pannonia (*Populus* × *euramericana* (Dode) Guinier, variety registered in Serbia in 1998) with three planting distances (3 × 3 m, 4.25 × 4.25 m i 6 × 6 m), i.e. stand densities (1.111, 555 and 278 trees per hectare). Trial stand was established with so called "deep" planting using a two-year old nursery plants of type 2/0 (stick) on sandy-loamy fluvisol in the protected area of alluvial Danube plane in the vicinity of Novi Sad. Trial was set in a random block design with three replications including from 48 (distance 6 × 6 m) to 90 (distance 3 × 3 m) trees per replication.

All diameters at breast height (excluding those tree rows at the edge of the stand) were measured in the 5th and 7th year.

Data were processed using standard methods of statistical analysis. Mean values of growth elements were compared using analysis of variance and test of least significant differences at risk level of 5%. Fixed model of two way factorial analysis of variance was applied:

$$X_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{k\,(ij)}$$

Effect of tested sources of variation was estimated on the basis of their expected variances (σ_i^2) in total variation.

Comparison of distribution of diameteres at breast height and basal areas per diameter classes, and distribution of diameter growth and growth of basal area was done using no-parametric Kolmogorov-Smirnov test. For the mentioned elements the numerical structure parameters were defined: arithmetic mean - x_a, standard deviation – s_d, variation coefficient – c_v, minimum – x_{min}, maximum – x_{max}, assimetry coefficient – α_3, and coefficient of flatness – α_4. Linear models were used for definiton of diameter increment dependence on their diameters at breast height, and model parameters were obtained using method of least squeres.

RESULTS OF INVESTIGATION

Significant influence of both studied factors (clone and stand density) on stand growth elements (d_g, $d_{g20\%}$, g_s, G) was determined in the 5th year of stand development. The greatest F-quotient was determined for total basal area per hectare (G), as the consequence of great difference in the initial number of trees per hectare at stand establishment. Interaction clone × planting distance did not prove significant for all studied growth elements (table 1).

Contribution of expected variances (σ_i^2) in total variation was greater for factor „planting distance" for mean basal area diameter (d_g), mean (g_s), and total basal area per hectare (G), and for mean basal area diameter 20% of the thickest trees in the stand ($d_{g20\%}$) factor „clone" doubled in relation to factor „planting distance", which pointed out to differences existing in diameter structures of these two poplar clones.

Clone S_{6-7} had greater mean growth elements in relation to clone Pannonia at all planting distances, with mean diameter (d_g, $d_{g20\%}$) and basal area (g_s) values being greater at greater planting distances. Also, for clone S_{6-7} significant differences between mean values of the rarest (6 × 6 m) and the densest (3 × 3 m) spacing were determined in the 5th year, while for clone Pannonia differences were not significant (table 2).

Table 1. Results of two-way factorial analysis of variance for stand growth elements

Growth elements		*5 year*			*7 year*			*Current increment*		
		Clone	*Planting distance*	*Clone × Plan.dist.*	*Clone*	*Planting distance*	*Clone × Plan.dist.*	*Clone*	*Planting distance*	*Clone × Plan.dist.*
F-quotient	d_g	8.726^{*}	6.063^{*}	0.161^{ns}	6.35^{*}	7.953^{**}	0.031^{ns}	0.043^{ns}	8.831^{**}	1.066^{ns}
	$d_{g20\%}$	21.648^{***}	5.887^{*}	0.253^{ns}	15.926^{**}	7.057^{**}	0.207^{ns}	0.0491^{ns}	4.2624^{*}	0.0115^{ns}
	g_s	8.001^{*}	5.651^{*}	0.297^{ns}	5.922^{*}	7.446^{**}	0.092^{ns}	1.25^{ns}	9.615^{**}	0.306^{ns}
	G	20.906^{***}	92.424^{***}	0.43^{ns}	11.954^{**}	89.152^{***}	0.05^{ns}	0.034^{ns}	33.342^{***}	0.734^{ns}
σ_i^2 [%]	d_g	32.4	42.5	0.0	21.2	55.1	0.0	0.0	71.9	0.6
	$d_{g20\%}$	56.7	26.8	0.0	45.2	36.7	0.0	0.0	52.1	0.0
	g_s	31.0	41.3	0.0	20.7	54.4	0.0	0.0	62.5	0.0
	G	9.5	87.6	0.0	5.7	91.2	0.0	0.0	91.5	0.0

Table 2. Mean values for tree and stand growth elements and t-test results

Factor		*Growth element*											
Clone	*Planting distance*	d_g *[cm]* 5 year	d_g *[cm]* 7 year	i_{dg} *[cm·year⁻¹]*	$d_{g20\%}$ *[cm]* 5 year	$d_{g20\%}$ *[cm]* 7 year	$i_{dg20\%}$ *[cm·year⁻¹]*	g_s *[cm²]* 5 year	g_s *[cm²]* 7 year	i_g *[cm²·year⁻¹]*	G *[m²·ha⁻¹]* 5 year	G *[m²·ha⁻¹]* 7 year	I_G *[m²·ha⁻¹·year⁻¹]*
S_{6-7}	3 × 3 m	14.7 *bc*	17.5 *bc*	1.41 *bc*	18.2 *bc*	21.6 *bcd*	1.69 *a*	169 *bc*	241 *bc*	36.0 *cd*	17.14 *a*	22.71 *a*	2.79 *ab*
	4.25×4.25 m	16.7 *ab*	20.0 *ab*	1.62 *abc*	20.8 *ab*	24.5 *ab*	1.85 *a*	221 *ab*	314 *ab*	46.6 *abc*	11.62 *b*	16.24 *b*	2.31 *bc*
	6 × 6 m	17.9 *a*	21.3 *a*	1.73 *ab*	21.8 *a*	26.1 *a*	2.19 *a*	253 *a*	360 *a*	53.5 *a*	6.61 *d*	9.27 *c*	1.33 *d*
Pa-nno-nia	3 × 3 m	13.2 *c*	15.9 *c*	1.35 *c*	15.5 *c*	18.9 *d*	1.69 *a*	137 *c*	200 *c*	31.2 *d*	13.65 *b*	19.69 *a*	3.02 *a*
	4.25×4.25 m	14.9 *bc*	17.9 *bc*	1.53 *bc*	17.1 *c*	20.7 *cd*	1.79 *a*	175 *bc*	253 *bc*	39.3 *bcd*	9.20 *c*	13.35 *b*	2.07 *c*
	6 × 6 m	15.5 *abc*	19.3 *ab*	1.93 *a*	18.0 *c*	22.3 *bc*	2.16 *a*	189 *bc*	296 *ab*	53.2 *ab*	4.36 *d*	6.84 *c*	1.24 *d*

Significant influence of both studied factors on growth elements remained seven years upon stand establishment; significance of factor clone decreased, while that of planting distance increased (table 1). Mean values of growth elements at the rarest spacing (6 × 6 m) differed significantly from the densest spacing (3 × 3 m) in both clones (table 2).

Current annual increment of analyzed growth elements for period from 5 to 7 years differed significantly at different planting distances, and studied clones achieved close values of current increment of growth elements (i_{dg}, $i_{dg20\%}$, i_g, I_G) in the period from 5-7 years (table 1, 2).

Current increment of mean basal area diameter (i_d) and mean basal area (i_g) differed significantly between the rarerst and the densest spacing. Although at rarer spacing current increments of mean dominant tree were greater in both clones differences were not significant. Current increment of basal area per hectare in both clones was the highest at the densest spacing (3 × 3 m). In clone S_{6-7} differences were not significantat at planting distances 3 × 3 m i 4.25 × 4.25 m, which pointed out to the fact that denser spacing in this period of development already significantly decreased the current increment.

Comparison of distribution of number of trees and basal areas per diameter classes using non-parametric Kolmogorov-Smirnov test in 5th year from stand establishment determined significant differences among all applied planting distances in clone Pannonia. In clone S_{6-7} significant differences in basal area structure were determined at all planting distances, while in diameter structure between planting distance of 4,25×4,25m and 6×6m differences were not significant (table 3, 4, graphs 1a, 2a).

Table 3. Results of non-parametric Kolmogorov-Smirnov test for distribution of number of trees and basal area per diameter classes in the 5th year

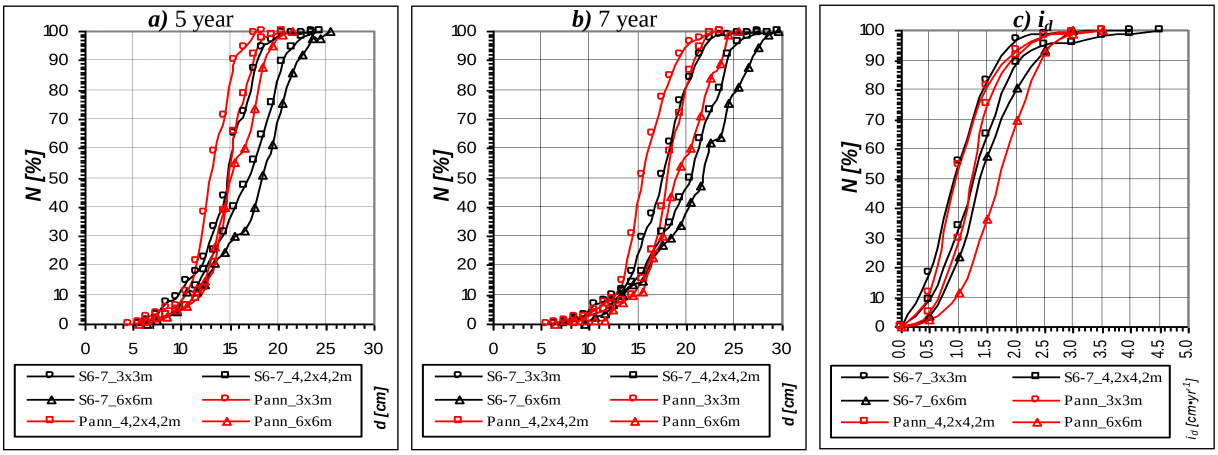
			Diameter structure (N%)					
	Clone		S_{6-7}			Pannonia		
		Plan. distance	3×3m	4.25×4.25m	6×6m	3×3m	4.25×4.25m	6×6m
Basal area structure (G%)	S_{6-7}	3×3m	-	0.32755^{***}	0.477551^{***}	0.281429^{***}	0.108709^{ns}	0.144878^{ns}
		4.25×4.25m	0.429499^{***}	-	0.15^{ns}	0.51^{***}	0.370792^{***}	0.231287^{**}
		6×6m	0.569091^{***}	0.196531^{*}	-	0.62^{***}	0.520792^{***}	0.361287^{***}
	Pann o-nia	3×3m	0.333333^{***}	0.623995^{***}	0.759899^{***}	-	0.372178^{***}	0.355446^{***}
		4.25×4.25m	0.103939^{ns}	0.502449^{***}	0.65^{***}	0.324545^{***}	-	0.19802^{*}
		6×6m	0.175531^{ns}	0.326531^{***}	0.468367^{***}	0.449907^{***}	0.257551^{**}	-

Table 4. Results of non-parametric Kolmogorov-Smirnov test for distribution of number of trees and basal area per diameter classes in the 7th year

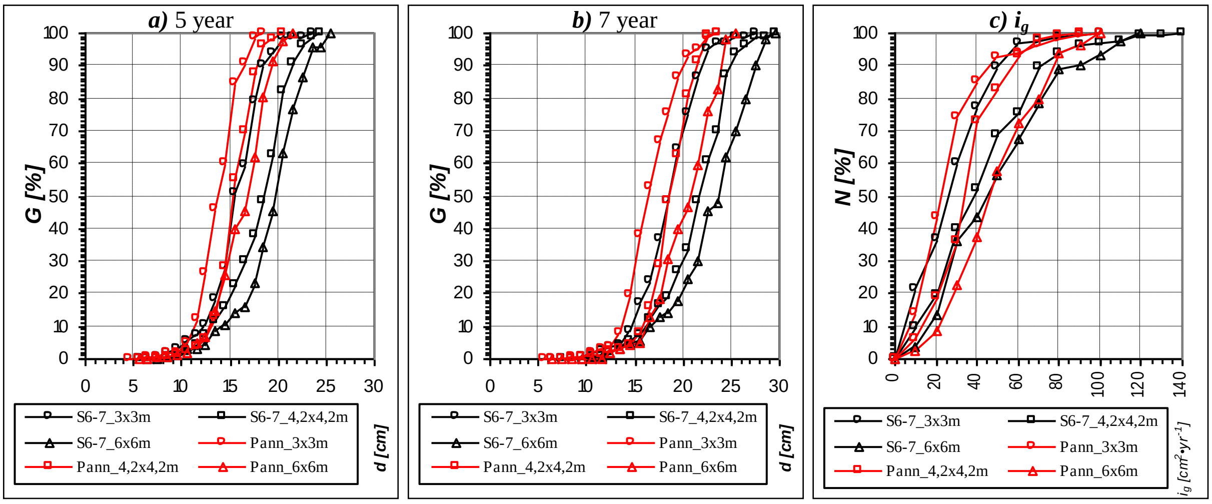
			Diameter structure (N%)					
	Clone		S_{6-7}			Pannonia		
		Plant.distance	3×3m	4.25×4.25m	6×6m	3×3m	4.25×4.25m	6×6m
Basal area structure (G%)	S_{6-7}	3×3m	-	0.341584^{***}	0.461608^{***}	0.276824^{***}	0.15703^{ns}	0.237624^{**}
		4.25×4.25m	0.42217^{***}	-	0.175102^{ns}	0.496939^{***}	0.36^{***}	0.114554^{ns}
		6×6m	0.574257^{***}	0.262427^{**}	-	0.602041^{***}	0.480816^{***}	0.258436^{**}
	Pann o-nia	3×3m	0.283762^{***}	0.591959^{***}	0.682475^{***}	-	0.413061^{***}	0.46858^{***}
		4.25×4.25m	0.09741^{ns}	0.467875^{***}	0.612061^{***}	0.367071^{***}	-	0.25604^{**}
		6×6m	0.292376^{***}	0.151753^{ns}	0.366139^{***}	0.48^{***}	0.338081^{***}	-

Comparison of diameter structure and structure of basal areas seven years after stand establishment confirmed differences between applied planting distances for each clone, the same as those found after 5th year. In the 7th year after stand establishment significant differences among all applied planting distances in all studied clones were determined. Also, differences in structures between the densest spacing in clone S_{6-7} (3×3m) became significant in relation to the rarest spacing in clone Pannonia (6×6m) (table 4, graphs 1*b*, 2*b*).

By comparison of structure diameter and basal area increments at different planting distances significant differences were determined in clone Pannonia, while in clone S_{6-7} differences between planting distances 4,25×4,25m and 6×6m were not significant. This was in accordance with differences in diameter structure and structure of basal area per diameter classes. At planting distance 3×3m significant differences were not found in structure of diameter and basal area increment between both studied clones. However, structures of diameter increment at the rarest spacing differed significantly, and in structure of basal area increment differences became significant at intermidate spacing (4,25×4,25m) (table 5, graphs 1*c*, 2*c*).



Graph 1. Sumarized curve of diameter structure five and seven years after stand establishment and curve of structure of diameter increment for period from 5th to 7th year after stand establishment



Graph 2. Sumarized curve of basal area structure per diameter classes five and seven years after stand establishment, and curve of structure of basal area increment for period from fifth to seventh year

At the smallest density absolute variability of diameters at breast height increased in both clones in 5th year after stand establishmnet, while relative variability remained at similar values due to increase in mean diameter (table 6). Variability of clone $S_{6\text{-}7}$ was greater in relation to clone Pannonia at all three stand densities. In all treatments left assimetry of diameter structure was present after five years. Smaller stand density had smaller coefficient of flatness of diameter structure (expressed platicurtic distribution), while clone Pannonia had greater coefficient of flatness in relation to clone $S_{6\text{-}7}$.

Seven years after stand establishment very similar values of numerical parameters of diameter structure were determined, the same as after 5th year, but absolute variability increased, and relative variabilty decreased in relation to the 5th year (table 6).

Table 5. Results of non-parametric Kolmogorov-Smirnov test for the structure of diameter increment and basal area increment, for the period from 5th to 7th year after stand establishment

			Structure of diameter increment (i_d)					
	Clone		S_{6-7}			Pannonia		
		Plant distance	3×3m	4.25×4.25m	6×6m	3×3m	4.25×4.25m	6×6m
Structure of basal area increment (i_g)	S_{6-7}	3×3m	-	0.21627**	0.3196***	0.064994 ns	0.25731***	0.467565 ***
		4.25×4.25 m	0.247432 ***	-	0.103331 ns	0.207298 **	0.099728 ns	0.28631***
		6×6m	0.333627 ***	0.128678 ns	-	0.310629 ***	0.175504 ns	0.210534 *
	Pannonia	3×3m	0.144034 ns	0.346532 ***	0.412729 ***	-	0.248338 ***	0.451165 ***
		4.25×4.25 m	0.238734 ***	0.207185 **	0.292792 ***	0.382769 ***	-	0.386038 ***
		6×6m	0.396242 ***	0.17381 ns	0.134551 ns	0.520342 ***	0.355994 ***	-

Table 6. Numerical indicators of diameter structure five and seven years after stand establishment

Element of structure	*5 year*						*7 year*					
	S_{6-7}			Pannonia			S_{6-7}			Pannonia		
	3 × 3 m	4.2×4.2m	6 × 6 m	3 × 3 m	4.2×4.2m	6 × 6 m	3 × 3 m	4.2×4.2m	6 × 6 m	3 × 3 m	4.2×4.2m	6 × 6 m
n	164	171	90	162	171	80	153	168	89	161	171	80
d_{min} [cm]	6	6	7	5	7	6	7	8	10	6	9	7
d_{max} [cm]	23	24	25	18	20	21	27	29	29	23	23	25
d_a [cm]	14.3	16.3	17.5	13.0	14.7	15.2	17.2	19.5	20.9	15.6	17.7	19.1
s_d [cm]	3.16	3.79	4.18	2.37	2.35	3.06	3.46	4.31	4.83	2.83	2.83	3.51
c_v [%]	22.0	23.2	23.9	18.2	16.0	20.1	20.2	22.1	23.2	18.1	16.0	18.4
a_3	-0.512	-0.462	-0.629	-0.767	-0.916	-0.594	-0.481	-0.612	-0.454	-0.341	-1.040	-0.660
a_4	3.115	2.570	2.640	4.169	4.588	3.335	3.514	2.877	2.347	4.206	4.412	3.625

Smaller stand density caused increase in absolute variability of tree basal areas already in 5th year in both clones, with absolute variability maintained at close values.

Clone Pannonia had smaller both absolute and relative variability of tree basal area structure in relation to clone S_{6-7}. In comparison with diameter structure the basal area structure had less expressed assimetry, and decreased leptocurtic distribution (table 7).

Numerical indicator of structure of diameter increment for period from 5th to 7th year showed equal absolute variability depending on stand density, which with the increased mean value at smaller densities led to decreased relative variability. At the same time clear differences between studied clones (table 8) were not observed. Smaller stand density caused decrease in positive assimetry, as well as decrease in coefficient of flatness. Clearly expressed leptocurtic distribution of diameter increment in moderate dense and dense stand turned into normal or platicurtic distribution in rare stand in both studied clones.

Table 7. Numerical indicators of structure of basal area five and seven years after stand establishment

Element of structure	*5 year*						*7 year*					
	S_{6-7}			Pannonia			S_{6-7}			Pannonia		
	3 × 3 m	4.2×4.2m	6 × 6 m	3 × 3 m	4.2×4.2m	6 × 6 m	3 × 3 m	4.2×4.2m	6 × 6 m	3 × 3 m	4.2×4.2m	6 × 6 m
n	164	171	90	162	171	80	153	168	89	161	171	80
g_{min} [cm^2]	28	28	38	20	38	28	38	50	79	28	64	38
g_{max} [cm^2]	415	452	491	254	314	346	573	661	661	415	415	491
g_a [cm^2]	169	220	254	136	174	188	241	314	360	198	253	295
s_d [cm^2]	68	93	107	45	51	69	90	125	151	68	73	100
c_v [%]	40.1	42.2	42.4	33.3	29.2	36.8	37.4	39.7	42.1	34.4	28.8	33.9
a_3	0.143	0.002	-0.159	-0.074	-0.241	-0.029	0.234	-0.104	-0.051	0.448	-0.469	-0.111
a_4	3.302	2.170	2.329	3.070	3.615	2.494	3.632	2.446	2.075	3.738	3.244	2.430

Similar trends were noticed in the structure of basal area increment, with greater relative variation in basal area increment in relation to diameters at breast height increment (table 8).

Table 8 – Numerical indicators of structure of diameter increment (i_d) and basal area increment (i_g) for period from 5th to 7th year

Element of structure	i_d [$cm \cdot year^{-1}$]						i_g [$cm^2 \cdot year^{-1}$]					
	S_{6-7}			Pannonia			S_{6-7}			Pannonia		
	3 × 3 m	4.2×4.2m	6 × 6 m	3 × 3 m	4.2×4.2m	6 × 6 m	3 × 3 m	4.2×4.2m	6 × 6 m	3 × 3 m	4.2×4.2m	6 × 6 m
n	153	168	89	161	171	80	153	168	89	161	171	80
i_{min}	0.5	0.5	0.5	0.5	0.5	0.5	5	6	11	4	7	5
i_{max}	3.5	4.5	3.0	3.5	3.5	3.5	102	140	123	96	96	102
$\bar{i}$	1.24	1.57	1.71	1.31	1.51	1.94	32	46	54	31	39	53
s_d	0.55	0.72	0.62	0.58	0.52	0.60	18	25	28	17	16	22
c_v [%]	44.6	45.9	36.1	44.1	34.4	30.6	55.6	54.3	51.5	56.3	41.8	40.5
a_3	0.809	1.287	0.348	1.101	0.654	-0.055	0.898	0.862	0.580	1.520	0.481	0.268
a_4	4.236	6.155	2.611	4.783	4.138	3.018	3.991	4.176	2.615	5.852	3.332	2.480

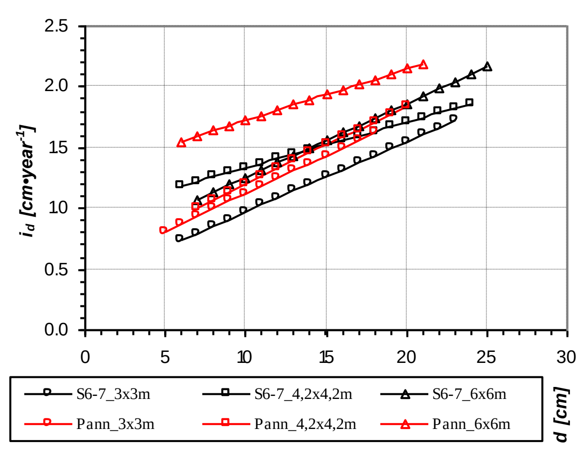
Linear dependence of diameter increment in the period from 5th to 7th year upon their diameters at breast height at all three stand densities in clone S_{6-7}, and dense (3 × 3 m) and moderate dense stand (4.25×4.25m) in clone Pannonia (table 9, graph 3) was determined.

In stand at rare spacing (6×6m) in clone Pannonia dependence of diameter increment on diameters at breast height was not determined, which revealed that in regard to this treatment all trees had close values of diameter increment due to unexpressed influence of density of the mentioned age.

Table 9. Parameters and estimation of model of dependence of diameter increment on their diameters at breast height

Clone	S_{6-7}			Pannonia		
Spacing	3×3m	4.2×4.2m	6×6m	3×3m	4.2×4.2m	6×6m
Model: $i_d = a \cdot d_{1.3} + b$						
a	0.05815	0.0378	0.06074	0.06207	0.06423	0.04246
b	0.38854	0.9558	0.64873	0.50293	0.56044	1.29888
R^2	0.0963	0.03916	0.17072	0.06138	0.0851	0.04754
s_e	0.52783	0.71093	0.56428	0.56184	0.49627	0.5847
s_a	0.0145	0.01453	0.01435	0.01925	0.0162	0.02152
n	153	168	89	161	171	80
t	4.011^{***}	2.601^{*}	4.232^{***}	3.224^{**}	3.965^{***}	1.973^{ns}

Graph 3. Dependence of diameter increment on their diameters at breast height



DISCUSSION AND CONCLUSIONS

Investigation results showed that clones S_{6-7} (*Populus deltoides* Bartr. ex Marsh.) and Pannonia (*Populus × euramericana* (Dode) Guinier) had different growth dynamics in the period from 5th to 7th year after stand establishment. Previous investigations showed that clone S_{6-7} had faster increment during the initial period in relation to clone Pannonia (Andrašev, 2003). Faster growth of clone S_{6-7} was the cause of pronounced significance of the differences in obtained mean values of diameters at breast height (d_g, $d_{g20\%}$) and basal areas (g_s) between dense (3×3m) and rare (6×6m) stands five years after establishment. Significant influence of different spacing for tree growth in clone Pannonia on mean element values was determined in 7th year after stand establishment due to slower growth. (table 1, 2).

Mutual differentiation among trees was caused by different diameter increment of individual trees in the stand, which was confirmed by numerical indicators of diameter structures and basal area. More expressed differentiation was determined in the 7th year, which was understandable since numerical structure indicators revealed the state of internal stand structure at certain age, as cumulative result of tree growth in the stand. Since clone S_{6-7} had more pronounced increment in relation to clone Pannonia in the initial period, the process of tree differentiation in this clone was more pronounced.

Increase in abosulte variability (standard deviation) of diameters at breast height and basal area with decreased stand density in both studied clones, and at the same time with retaining relative variability (correlation coefficient) at similar values was confirmed by the attitude of Koprivica (1980) that it was needed to observe both mentioned parameters in order to make proper quantification of effects of different growth in stands. Numerical indicators of diameter structure, and basal area structures (table 6, 7) pointed out to the fact that it was necessary to observe all other numerical indicators, such as assimetry coefficient and flatness coefficient in order to estimate the process of tree differentiation in the stand. Assimetry coefficient (α_3) pointed out to existence of trees with retarded growth of diameters at all stand densities, while flatness coefficient (α_4) revealed that retarded trees from denser stands were not close to the arithmetical mean in relation to less dense stands. This led to the conclusion that differentiation of trees was more expressed in denser stands. i.e. individual trees revealed retardation in diameter growth.

In the period from 5th to 7th year studied clones achieved similar values of current increments in diameter (i_{dg}, $i_{dg20\%}$) and basal area (i_g, I_G). Different stand densities were the cause of different mean values of current increments, but also of differences in their structure. Greater stand density, i.e. smaller spacing for tree growth during early development (from 5th to 7th year) was the cause of smaller current increments, their greater variability, expressed positive assimetry and leptokurtic distribution, and significant dependence of diameter increment on their diameters at breast height, which pointed out to the expressed differentiation of trees in the stand. Results obtained using non-parametric Kolmogorov-Smirnov test in the studied trial stands during early stages of development gave more precise estimation of differences between diameter and basal area structures, as well as the structure of current increment of diameters and basal areas in relation to the test of least significant difference when comparing mean values of growth elements. This confirmed that its application in differentiation of obtained growth elements in poplar stands was justified.

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STUDY OF HORNBEAM (*Carpinus betulus* L.) FRUCTIFICATION FROM THE ASPECT OF PLANNING THE RECONSTRUCTION OF DEGRADED COMMON OAK - HORNBEAM FORESTS

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Summary: *The results of the study of hornbeam (Carpinus betulus L.) fructification in the initial phase are presented in the aim of defining the biological-ecological base for the reconstruction of degraded common oak-hornbeam forests.*

The study was performed in the area of southwest Srem on permanent sample plots in juvenile, predominantly pure hornbeam stands under the effect of different silvicultural treatments. The stands represent a degradation form of the forest of common oak, hornbeam and narrow-leaved ash (Carpino-Fraxino-Quercetum roboris Jov. et Tom. 1979, subass. caricetosum remotae Jov. et Tom. 1978 - on meadow black soils to leached meadow black soils in the unflooded area).

In the phase of seed maturation, ocular assessment of the yield was based on the number of the inflorescences compared to the number of branches on the crown periphery. The yield was assessed on hornbeam trees of the dominant crown class, which were previously marked by random principle. In the stand which was thinned in early 2003 (OP-1), the yield was assessed in the period 2003 - 2008 on altogether 110 trees, and in the seed year 2008, the yield was assessed comparatively in the thinned (OP-2) and unthinned (OP-3) parts of the stand on altogether 88 trees.

On OP-1, in the period 2003 – 2008, the inflorescences were formed with different abundance in five seed years, i.e.: in 2003 on 76.4 % trees, in 2004 on 92.7 % trees in 2006 on 45.5 % trees and in 2007 on 92.7 % of trees and in 2008 on 94.6 % of trees. In the seed year 2008, in the thinned part of the stand, the inflorescences were formed on all study trees, and in the unthinned part of the stand, the inflorescences were formed on 94.2 % of the analysed trees. In the years with a greater number of trees with seed yield, the percentage of trees with abundant and medium abundant yield was also greater. In the seed year 2008, the percentage of trees with abundant and medium abundant yield in the thinned area (91.7 %) was greater than that in the unthinned area (76.9 %), so it can be concluded that the silvicultural treatment affected the abundance of the yield.

Based on periodicity and abundance of seed yield, it is concluded that the reconstruction of pure hornbeam stands already in the initial phase of fructification, depends on the hornbeam generative potential.

Key words: *Carpinus betulus L., generative potential, stand reconstruction, Srem.*

INTRODUCTION

Hornbeam has an important role in the process of common oak raising, but from the aspect of production of highly valuable technical roundwood, it is a secondary species, compared to common oak. The role of hornbeam depends on the development phases of common oak-hornbeam forest, which also conditions the different silvicultural strategy to this tree species. In the stage of stand regeneration, hornbeam is a highly expansive species, which soon dominates in the regeneration structure on the regeneration areas. When, in the stand regeneration stage, the percentage of hornbeam in the regeneration structure is too high, from the silvicultural aspect, it is defined as weed species (Bobinac *et al.*, 2004a). In such cases, natural renewal of common oak-hornbeam forests and the reconstruction of stands dominated by hornbeam, should primarily be based on hornbeam bio-ecology. In the subsequent development stages of common oak-hornbeam forests, hornbeam is a highly desirable species in the biological stand structure and it has a very important ecological role in the forest ecosystem. On the sites favourable for the production of common oak veneer logs, on which hornbeam is a tree of the first order (height above 30 *m*), the only limitation from the silvicultural aspect is the limited number of hornbeam trees in the dominant story.

The reconstruction of hornbeam dominated stands (degraded forms of common oak-hornbeam forests) in the area of Srem, is traditionally based on clear cutting and sowing of common oak acorns, and in the past, it was often included in forest-agricultural management (Bobinac, 2008a). Before the period of hornbeam fructification, the reconstruction of degraded stands depends on the reduction of coppice regeneration potential of the felled trees, and after hornbeam fructification, stand reconstruction depends on the reduction of coppice potential and generative regeneration potential of both hornbeam and other secondary species on regenerated areas. In this context, a significant biological property of hornbeam seeds is to preserve the germination capacity through a long period (up to 3-4 years, Наконечный, 1989).

In addition to providing the required quantity of acorns, the stand reconstruction management includes also the definition of seed yield elements of hornbeam trees in the regeneration area (Bobinac, 2008b; Bobinac & Rađević, 2005; Bobinac *et al.,* 2004a), the prevention of too frequent fructification (Bobinac *et al.,* 2004 b), definition of seed bank of woody and herbaceous weed species in the soil (Deiller et al., 2003; Vrbničanin & Janjić, 2004) and the management of the process of seedlings and regeneration development in the regeneration areas (Grime, 1979; Suzuki, 2000, Bobinac, 2004). Because of the early, frequent and abundant fructification, it is necessary, by a rationalised procedure, to collect the information on hornbeam seed yield and to form the system of seed yield forecasting of the tree species in the regeneration area. The system should be used in the planning of common oak-hornbeam forest natural regeneration and in the reconstruction of their degraded forms.

MATERIALS AND METHODS

Hornbeam seed yield abundance was assessed in three permanent sample plots (OP) in juvenile hornbeam stands, which are the degradation formations of the forest of common oak, hornbeam and ash (*Carpino-Fraxino-Quercetum roboris Jov. et Tom. 1979, subass. caricetosum remotae* Jov. et Tom. 1978 - on meadow to leached meadow black soils in the unflooded area, Jović *et al.*, 1989/1990). The study area is Southwest Srem (FMU "Vinična – Žeravinac-Puk, Compartments 13, 14 and 22). The altitude is 80 *m*, climate temperate continental; with mean annual temperature of 11.3 *°C* and mean annual precipitation 650 *mm*.

On OP-1, the abundance of seed yield was assessed in the period 2003-2008, on altogether 110 trees of dominant crown class and diameter at breast height ranging from 10 to 20 *cm*. In 2003, the stand was 30 years old, and the trees developed under a very dense canopy. The structural characteristics of hornbeam stand in the above ages were reported by Babić (2006); Bobinac *et al.* (2006). By the end of 2003, the stand was thinned for the first time which enabled the better light to the crowns of the remaining trees.

In seed year 2008, hornbeam seed yield was comparatively assessed in the stand thinned by the end of 2003 and 2007 (OP-2) and in the unthinned part of the stand (OP-3), on altogether 88 trees.

The trees on sample plots were permanently enumerated with paint and in the period 2003-2008 in late summer, the number of inflorescences was ocularly assessed compared to the number of branches in the crown periphery. Seed yield of individual trees was characterised by the following empirical category: 1= abundant seed yield, 2= medium abundant seed yield, 3= poor seed yield, 4= no seed yield. The data were processed by the program Excel.

RESULTS

Table 1 presents the characteristics of hornbeam seed yield on the permanent sample plot in the period 2003 - 2008. In the study period, the inflorescences were recorded in five seed years. In seed year 2003, inflorescences were formed by 76.4 % of trees, and in seed year 2004 by 92.7 % of trees. In 2005, no inflorescences were formed, so 2005 was a characteristically unfertile year in the wider area of Srem. In the following years, inflorescences were formed by 45.5% of trees in seed year 2006, by 91.8 % of trees in seed year 2007 and by 94.6 % of trees in seed year 2008.

In seed year 2003, abundant inflorescences were recorded on 17.3 of % trees, medium abundant - on 22.7 % trees, and individual - on 36.4 % trees. In seed year 2004, there was an increasing trend of hornbeam seed yield compared to 2003, expressed by the increase of the total number of trees with inflorescences and by the increase of the number of trees with abundant and medium-abundant inflorescences. After the infertile 2005, there was an increasing trend of seed yield in the successive years. In seed year 2006, the inflorescences were recorded on 45.5 % of trees, in seed year 2007 inflorescences were formed on 91.8 % of trees, and in seed year 2008 on 94.6% of trees.

Table 1. Characteristics of hornbeam yield in 2003 – 2008 on the area of 0.20 ha

Seed yield category	Year											
	2003		2004		2005		2006		2007		2008	
	N	%	N	%	N	%	N	%	N	%	N	%
1	19	17.3	45	40.9			6	5.5	28	25.5	45	40.9
2	25	22.7	28	25.5			23	20.9	38	34.5	46	41.9
3	40	36.4	29	26.4			21	19.1	35	31.8	13	11.8
4	26	23.6	8	7.3	110	100	60	54.5	9	8.2	6	5.4
Total	110	100	110	100	110	100	110	100	110	100	110	100

Table 2 presents growth elements of hornbeam trees and seed yield frequency in thinned (OP-2) and unthinned (OP-3) stands in seed year 2008. In seed year 2008, inflorescences were formed on all study trees in the thinned area, and they were absent on only 5.8 % of trees in the unthinned area. Also, the percentage of trees with abundant and medium-abundant seed yield was higher in thinned area (91.7 %), compared to unthinned area (76.9 %), so it can be concluded that silvicultural treatment affected the abundance of seed yield.

Table 2. Growth elements of hornbeam trees and seed yield frequency in thinned (OP-2) and unthinned (OP-3) part of stand in seed year 2008

Seed yield category	OP-2		OP-3	
	N	%	N	%
1	22	61.1	12	23.1
2	11	30.6	28	53.8
3	3	8.3	9	17.3
4			3	5.8
Total on OP	36	100	52	100
Total per *ha*	133		179	
d_s [cm]	17.4		16.3	
s_d [cm]	2.8		1.9	
c_v [%]	16.0		11.7	
d_{min} [cm]	12.8		12.7	
d_{max} [cm]	22.4		21.0	
h_s [m]	18.7		18.7	
s_d [m]	0.8		0.8	
c_v [%]	4.5		4.3	
h_{min} [m]	16.6		17.2	
h_{max} [m]	20.4		20.4	

DISCUSSION

In the area of Srem, the stands in which hornbeam is dominant are predominantly in the stage of fructification (Babić *et al.*, 2007), and their reconstruction is based on clear cutting and multiannual application of herbicides over the same area, in the form of total foliar treatment. To prevent the expected excessive hornbeam seed regeneration, the same areas are usually treated with herbicides in two years (Figures 1 and 2).

It is generally known that a lower number of woody and herbaceous species can develop in the stands with a good degree of closure, in which hornbeam is dominant in the ground layer, compared to less closed stands. Also, hornbeam has a slower development in the stands with a good degree of closure (Bobinac, 2004). If the vegetative regeneration of hornbeam and other tree species in the process of stand preparation for reconstruction is excluded a priori, the multiannual application of herbicides is primarily decided by the seed bank of hornbeam and other species in the soil. The frequency of hornbeam seed yield on the permanent sample plot through the period 2003 - 2008 with five seed years, in which there were three successive seed years, justifies the technological procedure of reconstruction based on clear cutting and multiannual application of herbicides over the same area in the form of total foliar treatment.

Figure 1. Clear cutting in hornbeam stand - autumn 2006 (FMU "Vinična –Žeravinac-Puk, compartment 13 a, Photo M.Bobinac)

Figure 2. Prepared area for sowing of common oak acorn - autumn 2008 (FMU "Vinična – Žeravinac-Puk, Compartment 13 a, Photo M.Bobinac)

However, the above procedure of stand reconstruction has some significant disadvantages. Forest soil is excluded from production during a two-year period, the regeneration area is treated several times with total herbicides and the surface soil layer is denuded and exposed to weather effects through a significant part of the growing season. Also, in the initial stage of reconstruction (during the clear cutting), the required quantity of the adequate acorn cannot be planned in real quantities, as it is impossible to forecast seed yield and because of the time limit of acorn storage. The impossible real planning of the required acorn quantity demands stand reconstruction on smaller areas, and the prepared areas in the years of poor seed yield are emphasised for the priority for sowing, compared to the stands under regeneration cutting.

The stand preparation for reconstruction by the above procedure is enabled under a high degree of repressive measures and the control of hornbeam regeneration process and other species in the initial stage. To minimise the use of herbicides in forest ecosystems (Bobinac & Šimunovački, 2000), stand reconstruction should be based more on the elements of biological-ecological nature, and because of the limited potentials of the above (classical) reconstruction procedure, the alternative silvicultural solutions should also be defined. According to Bobinac (2008b), the observed periodicity and abundance of hornbeam seed yield (in harmony with other regularities in the process of common oak-hornbeam stand establishment) indicates that it is possible to create an alternative silvicultural strategy of natural regeneration and reconstruction of stands in which hornbeam is dominant, and which should be based on a high degree of control of the process of common oak regeneration, which could mean the rationalisation of the procedure.

CONCLUSION

Based on the study of hornbeam fructification in the juvenile stands planned for reconstruction, it can be concluded as follows:

- In the period 2003 – 2008, there was only one characteristic infertile year (2005), and seed yield was recorded in five seed years, in which there were three successive seed years.
- In seed year 2008, the percentage of trees with abundant and medium-abundant seed yield was higher in the thinned area (91.7 %) than in the unthinned area (76.9 %).
- Under the a priori exclusion of vegetative regeneration of hornbeam and other accessory species in the regeneration area, the reconstruction of stands in which hornbeam is dominant, already in the initial stages of fructification, is basically decided by the seed bank in the soil.
- The reconstruction of stands in which hornbeam is the dominant species, in addition to the procedure based on clear cutting and multiannual application of herbicides over the same should be based on the biological-ecological elements, and due to the limited potentials of the applied (classical) procedure, the alternative silvicultural solutions should also be defined.

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FUNGICIDES IN CONTROLING POWDERY MILDEWS

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Abstract: *Powdery mildew is one of the most widespread and easily recognized plant diseases. There are many hosts; and although this disease is not considered fatal, plant damage can occur when the infestation is severe. Many plants have been developed to be resistant or tolerant to powdery mildew. Most mildews produce airborne spores and infect when temperatures are moderate (15 to 27 degrees C); the disease is not favored during the hottest days of the summer.*

As with all diseases, optimum plant health is the first line of defense. This begins with selection of healthy plants that are planted properly and in the proper location, giving attention to requirements for light, soil, and moisture. Space them so they are allowed to grow without being crowded and water thoroughly during establishment, and later during dry periods. Avoid overhead irrigation which raises the level of relative humidity within the plant canopy.

For fungicides to be effective, they must be applied as soon as symptoms are noticed. Product labels will provide information on how often to spray. When ranges are given, use the shorter interval during cool, damp weather. Be sure to cover both the upper and lower surfaces of the leaves.

On some plants, powdery mildews will occur in spite of good cultural control measures. Thus, fungicides must be used. For best results, spray programs must begin as soon as mildew symptoms are seen. Spray on a regular schedule, more often during cool, damp weather. Use a good spreader-sticker with the fungicides. Be sure to cover both surfaces of all leaves with the spray.

For chemical control are used fungicides based on a.i.- Azoxystrobin, chlorothalonil + fenarimol, Myclobutanil, Neem oil, Propiconazole, thiophanate-methyl, thiophanate-methyl + mancozeb, Triadimefon, Triflumizole, Triforine and Sulfur.

Apply fungicides at seven- to 14-day intervals to provide continuous protection throughout the growing season. Follow the instructions on the fungicide label for use on specific plant species, varieties, rates to be used, timing of applications, and waiting periods before harvest.

Key words: *powdery mildew, controlling, fungicides, resistance*

INTRODUCTION

Powdery mildews affect virtually all kinds of plants: cereals and grasses, vegetables, ornamentals, weeds, shrubs, fruit trees, and broad-leaved shade and forest trees. They are severe in warm, dry climates. Many plants have been developed to be resistant or tolerant to powdery mildew. Succulent tissue is more susceptible to infection. Once the disease is a problem, avoid late summer applications of nitrogen fertilizer. In most cases, prompt recognition and control actions can prevent severe damage to plants from the powdery mildews (Dreistadt, 1994).

The severity of the disease depends on many factors: variety of the host plant, age and condition of the plant, and weather conditions during the growing season.

Remember that each species of powdery mildew has a limited host range. Infection of one plant type does not necessarily mean that others are threatened.

CULTURAL CONTROL

The best method of control is prevention. Before using chemicals you should attempt to control powdery mildew by other means (Anonimus,1998). The following are good ways to manage the disease culturally:

- Buy varieties of plants resistant to powdery mildew.
- Avoid shade and overcrowding of susceptible hosts. Pruning may help. When establishing new landscape beds, mildew-susceptible plants should be widely spaced in open, sunny areas. Plant heavily shaded areas with disease-resistant cultivars.

- Water in the morning instead of the evening. Avoid overhead irrigation which raises the level of relative humidity within the plant canopy.
- For mildews attacking young foliage, cut down on nitrogen applications to decrease excessive growth in the late summer. Pruning overhanging trees and shrubs to improve air circulation and sunlight penetration will help slow the spread of disease. Maintaining a slow, even growth rate with light, frequent nitrogen applications will help suppress disease development. Avoid fall nitrogen fertilizer applications which stimulate new mildew-sensitive growth.
- On indoor plants try to reduce the relative humidity. In greenhouse settings, venting and heating at night is necessary to hold the relative humidity below levels needed for spore germination and fungal growth. Also, use fans to improve air circulation. If cultural practices alone do not maintain sufficient disease control, fungicides may be needed to protect susceptible plants from powdery mildew. Generally, good disease control can be obtained when fungicide applications are begun as soon as powdery mildew first appears on the foliage.
- In the fall, destroy or discard fungus-infested debris of annual flowers, deciduous shrubs, and trees. This will reduce the risk of carrying over the fungus to the next growing season. Do not compost infected plant debris.
- For outside plantings, begin fungicide applications on powdery-mildew-sensitive plants at or shortly before budbreak. Follow a one- to three-week spray schedule, depending on the fungicide chosen and the weather conditions, until conditions no longer favor the spread of disease.
- The addition of a spreader-sticker or liquid dish detergent is recommended for tank-mixes of wettable powder fungicides to ensure thorough coverage of the foliage. In greenhouse settings, use sulfur in addition to fungicide applications to control powdery mildew.
- Sulfur fumes may be generated by vaporizing sulfur on steam pipes or special hot plates. You may also apply wettable powder and flowable sulfur formulations directly to the foliage with standard spray equipment. Be careful when using sulfur because of its phytotoxicity to some floral and woody ornamental crops.

CHEMICAL CONTROL

On some plants, powdery mildews will occur in spite of good cultural control measures. Thus, fungicides must be used. For best results, spray programs must begin as soon as mildew symptoms are seen. Spray on a regular schedule, more often during cool, damp weather. Use a good spreader-sticker with the fungicides. Be sure to cover both surfaces of all leaves with the spray.

For fungicides to be effective, they must be applied as soon as symptoms are noticed. Product labels will provide information on how often to spray. When ranges are given, use the shorter interval during cool, damp weather. Be sure to cover both the upper and lower surfaces of the leaves (Matijević,1991).

When deciduous plants are infected, consider the season. Generally, foliar diseases occurring in late summer do little damage. The leaves have already produced food for the plant and are going to fall off soon anyway. Just be sure to rake and dispose of them as they fall.

As with any pesticide, read the label and heed all precautions. Sulfur, for example, can damage plants if applied when temperature and humidity are high.

Practices used to control powdery mildew diseases in the landscape, greenhouse, and nursery settings are similar. Cultural practices can often slow or, in some cases, prevent disease development. Powdery-mildew-resistant varieties are the best and least troublesome method of disease control. Chemicals are most effective when combined with cultural controls.

If cultural controls fail to prevent disease buildup or if the disease pressure is too great, an application of a fungicide may be necessary. These include pesticides in Table 1.

Codes and comments courtesy of FRAC. Chemical groupings follow classification as used in the Pesticide Manual 14th Edition.

In some situations, especially when growing roses, fungicides may be needed. Fungicides function as protectants, eradicants, or both. A protectant fungicide prevents new infections from occurring, whereas an eradicant can kill an existing infection. Apply protectant fungicides to highly susceptible plants before the disease appears. Use eradicants at the earliest signs of the disease. Once mildew growth is extensive, control with any fungicide becomes more difficult.

Table 1. Fungicides Recommended for Powdery Mildew Control - Listing by FRAC Code
Woody Trees, Shrubs, and Perennial Ground Covers

FRAC CODE	TARGET SITE OF ACTION	GROUP NAME	CHEMICAL GROUP	COMMON NAME	COMMENTS
1		MBC-fungicides (Methyl Benzimidazole Carbamates)	Thiophanate	Thiophanate-methyl	Resistance common in many fungal species. Several target site mutations, mostly E198A/G/K, F200Y Positive cross resistance between the group members. Negative cross resistance to N-Phenylcarbamates. High risk. Apply at first sign of disease (June) and continue sprays at 7- to 14-day intervals. Resistant varieties are available.
3	C14-demethylase in sterol biosynthesis	DMI-fungicides (DeMethylation Inhibitors) (SBI: Class I)	Triazole	Myclobutanil	There are great differences in the activity spectra of the different DMI fungicides. Resistance is known in various fungal species. Several resistance mechanisms are known incl. target site mutations, e.g. V136A, Y136F, I381V in cyp51 gene, ABC transporters and others. Generally wise to accept that cross resistance is present between DMI fungicides active against the same fungus. DMI fungicides are Sterol Biosynthesis Inhibitors (SBIs), but show no cross resistance to other SBI classes. Medium risk. Apply at first sign of disease and repeat at 10- to 14-day intervals. Add a non-ionic surfactant to the tank mixture of Eagle and Systhane. Systhane has been cleared only for greenhouse and nursery use.
	C14-demethylase in sterol biosynthesis	DMI-fungicides (DeMethylation Inhibitors) (SBI: Class I)	Triazole	Propiconazole	
	C14-demethylase in sterol biosynthesis	DMI-fungicides (DeMethylation Inhibitors) (SBI: Class I)	Piperazine	Triforine	

	C14-demethylase in sterol biosynthesis	DMI-fungicides (DeMethylation Inhibitors) (SBI: Class I)	Pyrimidine	Fenarimol	
	C14-demethylase in sterol biosynthesis	DMI-fungicides (DeMethylation Inhibitors) (SBI: Class I)	Triazole	Triadimefon	
	C14-demethylase in sterol biosynthesis	DMI-fungicides (DeMethylation Inhibitors) (SBI: Class I)	Imidazole	Triflumizole	
11	Complex III: cytochrome bc1(ubiquinol oxidase) at Qo site	QoI-fungicides (Quinone outside Inhibitors)	Strobilurin	Azoxystrobin	Single-site inhibitor. Inhibits fungal respiration at Qo site. Locally systemic. Resistance known in various fungal species. Target site mutations G143A, F129L and additional mechanisms. Cross resistance shown between all members of the QoI group. High risk. Apply at first sign of disease and repeat at 14- to 28-day intervals.
M2	Multi-site activity	Inorganics - suphur	Sulphur	Sulphur	Generally considered a low risk fungicide with no signs of resistance developing. No cross resistance between group members M1 to M9.
M5	Multi-site activity	Chloronitriles (phthalonitriles)	Phthalonitrile	Chlorothalonil	Multi-site inhibitor. Protectant, non-systemic. Generally considered a low risk fungicide. No cross resistance between group members M1 to M9. Apply at first sign of disease and repeat every 10 to 14 days. Use higher rate and shorter interval when disease pressure is heavy and conditions favor spread of disease.
NC	Unknown	Inorganics - carbonates	Inorganic	Potassium hydrogen carbonate	Resistance not known

DMI fungicides: do not inhibit spore germination, but attack the fungus during its early growth stage within the plant. Thus, they provide only limited residual or protective activity, but do provide significant post-infection activity. This probably explains why they were once so effective under extended spray intervals; that is, when new (unprotected) leaves developed after one spray and became infected, control was provided by the post-infection activity of the next spray. However, their activity is declining in many parts of the world, due to increasing levels of resistance developed by the PM fungus (Gubler,1992).

For this reason, very few growers still rely on the DMIs for PM control during the critical bloom through vegetation period. DMI fungicides have remained useful components in many control programs, and it is beneficial to consider how resistance to these materials develops and what you can do to minimize resistance in order to maintain their utility. It is important to recognize that fungicide rates are set by experimentation (usually on populations with little previous exposure to related materials), and that economic and regulatory pressures encourage labeling of the lowest rates that will provide full control of about 98% or 99% of the “baseline” population (Matijević,1990).

However, once such a fungicide (or group) has been introduced and applied repeatedly, the most sensitive members of the population get eliminated, leaving only those that require a “full” rate, +along with the original few that were only partially controlled by that rate. If the dose is then reduced — either intentionally or through poor spray coverage — many such individuals become capable of growing to variable extents. Although they may be at least partially controlled by each spray, they gradually build up to damaging levels. Eventually, the fungicide “just doesn’t work as well as it used to. ”

For powdery mildew, this “creeping” loss of control typically happens more quickly in regions where many generations or disease cycles occur every year (moderate summers) versus those with shorter periods of fungal activity (long, hot summers) (Petrović,1992).
Disease was rated before harvest, and 40 individual mildew colonies from each treatment were tested in the lab to determine their resistance status. In this way, we could calculate not only the total disease control, but also the control of the resistant portion of the population in each treatment.

The three theoretical anti-resistance principles actually work under real world conditions. In particular case, limiting DMI fungicides to three applications per season in rotation with sulfur, using the material before the disease was well established, and maintaining recommended rate provided the best total disease control and the least selection of resistant mildew individuals.

Strobilurin fungicides:
appear to be the most important new group of fungicides since introduction of the DMIs. Because the “strobies” are likely to become increasingly important in grape disease management, it’s worthwhile to understand how they work. These are excellent protectant fungicides, providing their best activity when present on the foliage or fruit before a spore lands and tries to infect. They also provide some post-infection control against PM, although it is less reliable and possibly more dangerous in terms of future resistance development.

Additionally, the strobies show significant “anti-sporulant” activity. That is, when applied after infection has occurred but before symptoms develop, they may allow lesions to form but inhibit the production of new spores from those lesions. This is a particularly significant attribute, since it limits the infectious agents responsible for continued disease spread.

Sulfur:
is a traditional material with two major positives: it’s cheap and effective. Furthermore, it’s been used to control PM around the world for nearly 150 years, with no development of resistance. However, because sulfur acts largely through the vapor phase, its activity is temperature-sensitive. Conventional wisdom says that sulfur is relatively inactive at cool temps, and can be phytotoxic at high temperatures. Although sulfur products have been used effectively under such “suboptimal” conditions, these potential limitations should be recognized.

Sulfur products have been used to manage powdery mildew for centuries but are only effective when applied before disease symptoms appear. The best sulfur products to use for powdery mildew control in gardens are wettable sulfurs that are specially formulated with surfactants similar to those in dishwashing detergent. However, sulfur can be damaging to some ornamental cultivars. To avoid injuring any plant, do not apply sulfur when temperature is near or over 30°C and do not apply it within 2 weeks of an oil spray. Other sulfur products, such as liquid lime sulfur or sulfur dust, are much more difficult to use, irritating to skin and eyes, and limited in terms of the plants they can safely be used on.

Alternative fungicides:

The PM fungus is different from all other fungal pathogens of grapes in that it grows primarily on the surface of infected tissues. Thus, it is vulnerable to topical applications of various materials that do not control other diseases, whose causal organisms are embedded within the tissues and not exposed to such treatments.

"Alternative" products that are labeled for PM control on grapes include various oils, potassium salts (monopotassium phosphate, potassium bicarbonate), and dilute solutions of hydrogen peroxide. In extensive tests with one such material — monopotassium phosphate — we've found that it provides virtually no protective or residual activity, i.e., no control when sprayed on plants that were inoculated with the PM fungus one to 10 days later. In contrast, we've found significant eradicative and anti-sporulant activity when it was thoroughly applied one to seven days after inoculating with the fungus.

Similarly, we've gotten much better activity in field trials when plants were sprayed every seven days (numerous post-infection "hits") compared to every 14 days with twice the rate (same amount of fungicide per season, but only half as many "hits").

I suspect that this scenario (a quick knockdown with little or no protection against subsequent infections) is applicable to some of the other "alternatives" (such as bicarbonates, oils, and hydrogen peroxide), and that they'll need to be applied on a more frequent basis than traditional fungicides. Of course, such activity as there is assumes complete coverage of the leaves and fruit, which often is problematical.

Several least-toxic fungicides are available, including horticultural oils, neem oil, jojoba oil, sulfur, potassium bicarbonate, bicarbonate of soda (baking soda), and the biological fungicides AQ10 and Serenade. With the exception of the oils, these materials are primarily preventive, although potassium bicarbonate has some eradicant activity. Oils work best as eradicants but also have some protectant activity.

Oils. To eradicate mild to moderate powdery mildew infections, use a horticultural oil such as JMS Stylet Oil, Saf-T-Side Spray Oil, Sunspray Ultra-Fine Spray Oil, or one of the plant-based oils such as neem oil or jojoba oil. *Be careful, however, to never apply an oil spray within 2 weeks of a sulfur spray or plants may be injured. Also, oils should never be applied when temperatures are above 30°C or to drought-stressed plants.* Some plants may be more sensitive than others, however, and the interval required between sulfur and oil sprays may be even longer; always consult the fungicide label for any special precautions. Of the horticultural oils, JMS Stylet Oil is the most highly refined and therefore the least likely to damage plants, but it may be more difficult to obtain than the others.

Bicarbonates. Also available is a fungicide containing potassium bicarbonate and a fungicide that can be made at home by combining 2-1/2 tablespoons of horticultural oil in a gallon of water and adding 4 teaspoons baking soda. This solution is sprayed on plants to prevent powdery mildew infections. Sprays of both potassium bicarbonate and baking soda can injure the plant, so use these materials with caution. Also, baking soda sprays can have deleterious effects on soil structure and should be used sparingly.

Biological Fungicides. Biological fungicides are commercially available beneficial microorganisms formulated into a product that, when sprayed on the plant, destroys fungal pathogens. AQ10 is a parasitic fungus, *Ampelomyces quisqualis,* that actively attacks and destroys the powdery mildew fungus. The active ingredient in Serenade is a bacterium, *Bacillus subtilis,* that helps prevent the powdery mildew from infecting the plant. These products have some effect in killing the powdery mildew organism but are not as effective as the oils or sulfur in controlling it.

Resistance warning

The risk of fungal pathogens developing resistance to the strobies appears to be high. To date, strobilurin resistance has followed the Benlate (all or nothing) model, i.e, most resistant isolates are virtually immune to the fungicides and multiply with impunity if they are not controlled by some other material, such as by tank-mixing with sulfur. Therefore, it will be critical to manage these materials carefully in order to maintain their effectiveness over time.

Minimize the number of annual applications (cost should encourage that!), use in strict rotation with other fungicide groups, and don't spray a strobie as an emergency "rescue" treatment if PM gets out of control. Minimize the number of annual applications (cost should encourage that!), use in strict rotation with other fungicide groups, and don't spray a strobie as an emergency "rescue" treatment if PM gets out ofcontrol (Rajković,1988),.

CONCLUSIONS

- The period of PM activity is determined by temperature. Extended periods greater than 30 °C will arrest its development, whereas growth is "explosive" at temps in the mid-60s to mid-80s.
- Although less important than temperature, high humidity promotes disease development and low humidity reduces it.
- Although DMI fungicides remain effective in many cultures, their efficacy has been compromised by resistance in many locations. Limited-use, conscientious choices of rates, and thorough application techniques should be implemented in order to maintain their usefulness.
- New strobilurin fungicides are extremely effective against PM, but their use should be limited for resistance management purposes. Two applications during the flowering and/or early post-flowering period have provided superior control of fruit infections while also providing supplementary control of Botrytis.
- PM is uniquely susceptible to topical applications of numerous alternative products (oils, potassium salts, hydrogen peroxide). However, these appear to act primarily as temporary eradicants, with little or no protective activity against new infections.

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THE FSC FOREST CERTIFICATION PROCESS OF THE PUBLIC ENTERPRISE „VOJVODINAŠUME“

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Abstract: *Public Enterprise „Vojvodinašume“ Petrovaradin in the course of 2006 decided to undertake and entered the certification process of the forests under its management. Accordingly, it expressed its strong and indisputable commitment to introduction and application of international standards, with the aim to obtain the official certificate of forest management in a cost/efficient, environmentally friendly and socially responsible manner which is granted by the independent international certification body. The process was initiated upon submitting the application for the project „Certification of Sustainable Forest Management According to the FSC Programme“ to the Forest Authority, that is, to the Ministry of Agriculture, Forestry and Water Management, which provided appropriate financial resources for the project implementation. In the selection process of the authorised certification body and the certification model for the sustainable forest management according to the FSC Programme (Forest Stewardship Council), on the basis of the public tender, the Enterprise awarded the contract for the forests certification according to the individual model to the company „SGS Beograd d.o.o.“ (Société Générale Surveillance) within SGS Qualifor Programme (FM/CoC – Forest Management /Chain of Custody).*
Key words: *sustainable development, FSC forest certification, responsible forestry*

INTRODUCTION

Public Enterprise (PE) „Vojvodinašume“ Petrovaradin was founded in 2002 by the Assembly of the Autonomous Province of Vojvodina. The enterprise comprises five divisions, of which there are four forest estates (FE), FE „Novi Sad“ Novi Sad, FE „Sremska Mitrovica“ Sremska Mitrovica, FE „Sombor“ Sombor and FE „Banat“ Pančevo and a specialised division of the enterprise for hunting and hunting tourism „Vojvodinašume-Lovoturs“ Petrovaradin. The main business activity of the enterprise is aimed at sustainable management of forests, hunting grounds and fishing waters, as well as management of protected natural areas.

The enterprise manages 115 thousand hectares of forests and forest grounds, 14 open and enclosed hunting grounds, manages 16 protected natural areas, and is also a beneficiary of forests and forest grounds within another 7 protected natural resources, by using the latest scientific achievements, proved practical experiences, modern technologies and support of scientific and educational institutions.

Despite the fact that Vojvodina represents the area with the lowest afforestation in Europe, with around 7.1%, the forests managed by the Public Enterprise „Vojvodinašume“ represent the most productive forests in Serbia, among which the pedunculate oak forests in the Sava iver basin and plantations of highly productive selections of poplar and willow trees are particularly prominent.

The society requirements for rational use of natural potentials and resources of forest ecosystems are becoming more significant, which requires special definition of goals and methods in the forest management. The forest certification is one of flexible tools developed in the fastest manner which may be used for adjusting the forestry to the contemporary understanding of „sustainable management“. What is the forest certification? The forest certification is the process in which the third party provides the written guarantee that a specific product, process and service are in compliance with particular requirements. The forest certification provides for the whole range of significant benefits, such as: the certificate of sustainable forest management in accordance with the principles of environmental protection; better communication with the environment; meeting the market demands; Chain-of-Custody certification “from the forest to the shop”; improving the quality of the management in all its aspects; strategic orientation in accordance with the forestry principle “from continuous to sustainable management” and others. In addition to the above mentioned benefits, there are also certain shortcomings including: dependence on the certification process; increase of costs (both direct and indirect); possibility of professional confrontation and greater influence of non-governmental organisations (NGOs).

At the global level, there are several forest certification systems. The most widespread certification systems in Europe are PEFC (Programme for the Endorsement of Forest Certification schemes) and FSC (Forest Stewardship Council). Forest Stewardship Council is an international body which accredits particular organisations to issue certificates, thereby guaranteeing the authenticity of their findings. The FSC programme is aimed at promotion of environmentally responsible, socially useful and economically sustainable forest management in the manner in which the generally-accepted standard is established which is to be observed by means of responsible forestry principle. The selection of the certification system according to the FSC standards imposed itself as the only possible solution for the forestry in Serbia at this moment due to the following reasons: the FSC standard proved to be better in the cases of one owner and greater forest area; the FSC standard applies generic standards, i.e. external standards; FCS standard does not require drafting of national standards which is a direct time saving benefit; drafting of national standards envisages establishment of a working group at the national level – registration of the working group and drafting of the statute are also desirable; the expenses arising from establishing of the working group and drafting of national standards exceed by far the costs of the FSC certification; the FSC certification is supported by the NGO sector; the wood processors' needs for the certified raw materials with a view to certifying their own products from so-called Chain-of-Custody and unrestricted access to the international market; the AP of Vojvodina is strategically committed to introduction of the quality standards.

Any producer may be certified by the FSC standard as well as wood and non-wood forest products, since this is a current and relevant issue for all actors in the Chain-of-Custody, starting from the sustainable forest ecosystems management, through raw materials, primary and final wood processing to the transport of goods to the market. FSC certificate proves that a product originating from the forest was not generated as a result of uncontrolled forest destruction. Aside from certifying forests and forest products (Forest Management – FM certificate), the Chain-of-Custody may also be certified (Chain of Custody – CoC certificate). CoC certificate is a certificate which is issued for the transport and processing of wood products, from the forest through the processing stage to the final product for the buyer, meaning that it covers the entire chain "from the forest to the shop".

According to the FSC on 16 September, 2008, the area of 48,076,616 hectares was certified in Europe (which accounts for 46.89% of totally certified areas) with 404 certificates. There is an area of 102,531,951 hectares that has been certified around the world with 940 certificates in 81 countries.

MATERIALS AND METHODS

The guarantee of sustainable forest management is provided by entering the forest certification process according to the one out of several existing models. PE "Vojvodinašume" Petrovaradin, owing to the lack of national standards, opted for the FSC model of forest certification in which an independent certification body provides a written guarantee that a product, method and service are in compliance with specific requirements of generic FSC standards, elaborated actions and procedures. The certificate is an assurance that forests under the custody of the competent authority are managed in a sustainable manner, observing specific principles and criteria. The standard comprises 10 principles and criteria. The principles include as follows:

1. Compliance with the law and FSC principles (6 criteria)
2. Rights and responsibilities of land tenure and use (3 criteria)
3. Rights of indigenous population (4 criteria)
4. Relations with the local community and rights of workers (5 criteria)
5. Forest-derived benefits (6 criteria)
6. Environmental impact (10 criteria)
7. Management plan (4 criteria)
8. Monitoring and assessment of the condition of the forest (5 criteria)
9. Maintenance of the High Conservation Value Forests (HCVF) (4 criteria)
10. Plantations (9 criteria).

The sustainable forest management certification process comprises the following phases: initial questions and decisions, proposals of certification bodies, pre-assessment, stakeholder consultations, preparation of the checklist, selection and checking of the expert team, main assessment, review, registration – certificate and annual supervision within the period of five years.

In the FSC certification process of sustainable forest management, in the phases of pre-assessment and annual supervision, the two assessors were involved (the main assessor from "SGS Slovakia spol. s.r.o." and the assessor from "SGS Beograd d.o.o."), whereas in the main assessment phase, the three assessors were involved (the main assessor from "SGS Slovakia spol. s.r.o.", the assessor from "SGS Beograd d.o.o." and the independent assessor).

RESULTS AND DISCUSSION

Public Enterprise "Vojvodinašume" Petrovaradin opted for the certification of forests under its management throughout 2006. Accordingly, it has expressed its indisputable commitment to introduction of standards into the forest management with the aim to obtain the official certificate of forest management in a cost-efficient, environmentally friendly and socially responsible manner, through permanent improvement of methods and modes of operation and development of responsibility for managing the forest resources. PE “Vojvodinašume” submitted its project “Certification of Sustainable Forest Management According to the FSC Programme” to the Ministry of Agriculture, Forestry and Water Management which invited the public call for tenders for allocation of funds aimed at protection and improvement of forests for 2006 on the basis of which the specific funds were granted for preparation and implementation of the certification process. Afterwards, the working group responsible for certification of the forests of the Public Enterprise was tasked to participate in activities and coordination of overall matters concerning the forest certification process of the PE “Vojvodinašume”, as a whole and its constituent parts. As a result, the activity of informing forest and other experts in the Enterprise about the main course of the entire certification process commenced, along with the trainings aimed at providing conditions required under certification standards and harmonisation of existing procedures and systems in accordance with their requirements.

In the process of public procurement of services – the Selection of the Certification Body and the Model for Certification of Sustainable Management of Forests of the PE «Vojvodinašume» according to the FSC programme – upon the announcement published in the Official Gazette of the Republic of Serbia no.79 of 22 September 2006, in the open procedure, the authorised Commission of the Enterprise drafted a complete tender documentation. The tender documentation had been bought out by the following entities: Lawrensilva, S. L. (Spain), EcoSylva Ltd. (Great Britain) and SGS Beograd d.o.o. In the process of opening the bids, it was established that only one bid submitted by SGS Beograd d.o.o., which is the member of SGS Group, arrived in due time and that there were not any untimely bids. The Commission subsequently requested from SGS Beograd d.o.o. to submit the Certificate of Business Entity Registration issued by the Agency for Registration of Business Entities of the Republic of Serbia and the Contract concluded between the branch offices of SGS Beograd d.o.o. and SGS Slovakia spol. s.r.o. as the Technical Assistance Office, which is accredited for certification of sustainable forest management and Chain-of-Custody. Regardless of the fact that only one bid had arrived, it was assessed according to the criterion of economically most favourable bid, which is based on the elements precisely set out in the tender documentation (price, references and deadline). Based on the aforementioned bid analysis, in the process of selection of the certification body and the model for certification of sustainable management of forests according to the FSC programme, PE “Vojvodinašume“ Petrovaradin awarded the contract to SGS Beograd d.o.o. with a view to certification of the forests under its management according to the most convenient **individual model** of certification of PE “Vojvodinašume“ forests. Individual forest certification model envisages certification of the entire PE “Vojvodinašume“ Petrovaradin, where a mistake made by any of participants in the system of the Enterprise functioning may result in loss of the certificate.

In order to effectively implement the entire certification procedure, throughout 2007 the intensive preparations and staff trainings were undertaken in the Enterprise head office and forest holdings as its constituent parts. At the beginning of preparation phase we were faced with expected oppositions within the system, such as:

- we don't need that,
- this is only draining our funds,
- we have been managing forests in this way for ages,
- why are we being checked out by some foreigners – let them show their forests if they dare,
- don’t tell me we will ask “The Greens” for their opinion,
- we are not giving our data to anyone.

Since foresters are conservative by nature, the changes have been mainly introduced by younger colleagues, since some senior colleagues felt threatened and did not take any initiatives. This brought about the situation in which, at first, so-called "general management" did not sufficiently support this process, which consequently resulted in a poor communication at vertical level – up to the very last forester on the ground.

However, in the preparation phase, the members of the management and working groups, the union representatives, safety officers, as well as forest, economic, legal and other experts in charge of the certification process implementation were actively involved. In this way, the process aimed at provision of necessary prerequisites for further development and preserving of the Enterprise's good standing started, by means of improving the methods, manners and technology of forest management and with a view to keeping the trust of present and future users of the forest products, as well as of many other stakeholders.

The pre-assessment procedure of sustainable forest management was carried out by the international assessors in the period from 24 to 27 April 2007 within which the documentation was reviewed in the Enterprise head office and head offices of its constituents, FE Novi Sad and FE Sremska Mitrovica. The grounds were visited in the FA (Forest Administrations) Kovilj and FA Bačka Palanka, within FE Novi Sad, and in FA Morović, FA Višnjićevo and FA Kupinovo, within FE Sremska Mitrovica. In the pre-assessment process the protected natural areas were also visited including: SNR (Special Nature Reserve) "Koviljsko-petrovaradinski rit (Kovilj-Petrovaradin Marshes)", SNR "Bagremara", SNR "Obedska bara", Natural Landmark "Artificial Stand of the Swamp Cypress", as well as the hunting grounds "Karakuša".

According to the prescribed procedure, the main assessment process of sustainable forest management in the Public Enterprise was conducted in the period from 5 November to 13 November 2007 within which the documentation was reviewed in the Enterprise head office and head offices of it other constituents, FE Novi Sad, FE Sombor and FE "Banat" Pančevo. The grounds were visited in FA Futog and FA Plavna, within FE Novi Sad, FA Apatin and FA Subotica, within FE Sombor, as well as in the Forest Management Unit of "Deliblatska Peščara Sands" in FA Deliblato and FA Bela Crkva, within FE "Banat" Pančevo. In the course of the main assessment process, the protected natural areas were also visited including the Special Nature Reserve "Gornje Podunavlje", SNR "Deliblatska Peščara Sands", Area of Outstanding Beauty "Subotičke šume" (Subotica Forests), Natural Park "Begečka jama", as well as the hunting grounds "Plavna". In the course of the main assessment process of the sustainable forest management, the international assessors also held working and consultative meetings with a number of stakeholders, such as: non-governmental and governmental organisations, local communities, wood processors, non-wood forest products' users, educational and scientific institutions etc.

Official acknowledgement of the certificate award was received on 7 August 2008, that is, PE "Vojvodinašume" Petrovaradin was awarded the FSC certificate of sustainable forest management by SGS Qualifor (SGS-FM/COC-005064), by which it is acknowledged and confirmed that the forests are managed and used in compliance with strict international standards. For the summary of the Report on the Forest Management Assessment for PE "Vojvodinašume" Petrovaradin, visit the SGS website www.forestry.sgs.com. FSC certificate is an international recognition, which confirms that the forest management method of PE "Vojvodinašume" Petrovaradin is in accordance with the strict generic principles and criteria.

Within the first regular supervision conducted between 3 November and 6 November 2008 the documentation was reviewed as well as the activities aimed at correction of certain incompatibilities, as a result of Minor Corrective Action Request by SGS, in the Enterprise head office and head offices of its constituents; FE Sremska Mitrovica, FE "Banat" Pančevo and FE Sombor. The grounds were visited in FA Kupinovo and FA Klenak, within FE Sremska Mitrovica and in FA Pančevo and FA Vršac, within FE "Banat" Pančevo. During the first regular supervision, the protected natural areas were also visited, including the Special Nature Reserve "Obedska bara" and the Area of Outstanding Beauty "Vršačke planine" (Vršac Mountains).

CONCLUSION

Having acquired FSC certificate of sustainable forest management according to the individual model, the Public Enterprise „Vojvodinašume" Petrovaradin is in a position to offer a whole assortment of wood and non-wood products from certified forests, ensuring at the same time a number of generally beneficial forest functions.

The certification system benefits are as follows:

- Possibility of better negotiating position in the process of selling products due to higher price and long-term planning;
- Demonstration of good planning in compliance with the international standards – improved enterprise management;
- Economically viable business operation;
- All kinds of quality management require well-trained labour force and partners.

Complete effects of initiated forest certification process may only be achieved providing that wood processing industry enters the certification process of the Chain-of-Custody and technological process of wood processing. Consequently, this ensures that certain forest assortments and wood products originate from the responsibly managed forests, which provides for unrestricted access to the international market. This is also in line with the strategic commitment of the Autonomous Province of Vojvodina to introduction of quality standards with regard to products and services.

Successful development and preservation of the Enterprise's good standing, in accordance with economic development priorities of the AP of Vojvodina, is an obligation of all employees and it can be achieved through joint efforts and permanent improvement of efficiency in accomplishing all work tasks. This is the best way to gain recognition and possibility for keeping the present and attracting the new beneficiaries of products and services of the PE "Vojvodinašume" Petrovaradin, along with meeting demands and expectations of buyers, beneficiaries, employees and society as a whole. As the first enterprise in Serbia and region which entered this process according to the individual model for certification of forests under its management, upon awarding of this certificate, it is going to be the only enterprise in Serbia which will be in a position to offer a full range of FSC certified wood assortments.

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ECOLOGICAL AND PRODUCTION CHARACTERISTICS OF THREE POPLAR CLONES IN THE ECOLOGICAL CONDITIONS OF THE DEFENDED PART OF THE ALLUVIAL PLAIN IN THE CENTRAL DANUBE BASIN

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Abstract: *The ecological conditions in the defended part (area protected from the floods after the construction of levees - dams) of the alluvial plain in the Central Danube Basin were researched on three soil type (humofluvisol, fluvisol - sandy and loamy forms). The study includes the analysis of ecological characteristics of the sites in Vojvodina climate region, as well as the growth of two varieties of eastern cottonwood and one euramerican poplar. Growth analysis was based on tree height, diameter and volume. Average growth traits of each variety were calculated then comparated among varieties growing on the same soil type, and within varieties on the diferent soil types.*

The unfavourable influence on plantation growth, i.e. on wood volume production, on humofluvisol was most probably the high content of silt fraction and the low air capacity. In loamy fluvisol, the unfavourable influence on production was probably the higher content of silt fraction, which acted unfavourably on water and air properties of this soil form. Sandy fluvisol had a low capacity of readily available water, which could cause a reduced production of wood volume. On humofluvisol, eastern cottonwood clones 618 and 450 attained greater heights and diameters than Euramerican black poplar cl. I-214.

Key words: poplars, eastern cottonwood, Danube, humofluvisol, fluvisol, poplar growth

INTRODUCTION

Poplars (*Populus spp.*) have been planted in Europe and Asia since very early times (Heilman, 1999). Poplars (*Populus*) is genus comprising 29 species of trees that are widely distributed over the northern hemisphere and planted in other parts of the world as well (Ceulemans et al. 1999). Various classifications have been suggested, the most recent 29 species that are grouped under six separate sections (Eckenwalder 1996). Poplar trees are becoming extremely important as fast growing species due to the increasing need for wood. Many countries with limited natural forests use poplar from plantations as an important source of timber. Primary forestry products based on poplar include sawn timber, veneer, panels and wood chips for pulpwood, and fuel (Bradshaw et all.2000, Williams et all. 2006). In Serbia, as in most other European countries, poplar (*Populus spp.*) has mainly been grown for the plywood and veneer industry (Terrasson and Valadon 1995; Laureysens et all., 2004). Potential for intensive cultural practices for increasing wood production was researched by Toth (1958), Ek et all. (1976), Frison (1991), Ceulemans (1999), Heilman (1999), Stanturf et all. (2001), Puri et all. (2002), Fuhrer et all. (2003), Tufekcioglu et all. (2005), Williams (2006), Safar (1963), Zivanov (1977), Markovic (1979), Markovic (1982), Herpka (1986), Ivanisevic (1991), Orlovic (1996), Galic (2000), Klasnja et all. (2005). Many improvments have been made in yield or productivity by using carefully selected interspecific hybrids (Ceulemans et all. 1999). It is the reason that in Serbia, as in other European countries different varieties of *Populus spp.* (poplars) have been planted - cultivation of poplars offers advantages over other broadleaved species.

Rapid growth is the hallmark of poplars (Bradshaw et all. 2000). Interest in growing poplars has fluctuated, and objectivies have shifted between producing sawlogs, pulpwood, or more densely spaced «woodgrass» or biofuels (Stanturf et al. 2001). In Vojvodina (part of Serbia) is the most interest in sawlog production. Poplar trees are becoming extremely important as fast growing species due to the increasing need for wood in Vojvodina. Poplar plantation culture depends on tree things: planting the best quality stock on high-quality sites and providing timely and appropiate cultural treatments (Stanturf et all.2001).

Poplar plantations provide a source of wood for nonforested areas of the world, and also can be important in supplying wood in areas where natural forests have been the primary source (Heilman, 1999). The total forest area in Vojvodina (Autonoumus Province in Serbia) is about 130 000 ha or 6 % of total area. In Vojvodina, there are some 30 000 ha of poplar plantations. Poplars are widely cultivated on the lowland River Danube, Tisa, Sava and Tamis. More than 90% of the poplar plantations in the Vojvodina are constituted by the Euroamerican clones *P*opulus x *euramericana* cl. I-214. Clone *Populus* x *euramericana* cl. I-214 became susceptible to canker disease: therefore, tree health is of primary concern in selecting poplar clones for the region.

Riparian poplar forests in Vojvodina are ecologicaly and hydrologicaly important features of the arid landscape, like in USA (Gazal et all. 2006). The Central Danube Basin and the entire Vojvodina are situated in the zone of temperate continental climate, which mostly predetermine the occurrence of the type of vegetation. The economically valuable forest stands and plantations of semiarid to arid climate are grown only in the inundations (alluvial plain) of lowland rivers. An especially important issue for lowland afforestation is the water balance in these artificially established plantations. The selection of favourable sites for poplar cultivation, in consuction with the application of adequate agro-engineering, is limited by the quantity of available water in the climate conditions of Vojvodina.

All regions of the world experienced an extreme accelertion of dam-building activity (Petts et all. 2005). River regulation have impact on the hydrology of the forest sites (Zsuffa et al. 1995). Similar observations are reported from Austria (Shume et all. 2004), and from United States (Rood et al. 1990). Depending on the severity of the alteration of lowering the water table and reducing annual amplitude, hybrid poplar stands have exhibited reduced productivity. For this reason, special attention should be focused to the defended part of the alluvial plain, because of the absence of flooding. Defended areas in Central Danube Basin were flooded until 1928. After the construction of levees (dams) to protect from the Danube floods, these areas receive additional moisture only by groundwater. The consequence is the changed direction of the pedogenetic process compared to the part of the alluvial plain affected by flooding, which causes the changes of site conditions. The differences are seen in the vegetation type compared to the part of the alluvial plain influenced by additional moisture and flood and underground waters.

This paper presents the study of ecological conditions in the defended part of the Central Danube Basin alluvial plain, as the precondition for the cultivation of some varieties of black poplars.

MATERIAL AND METHODS

The study sample plots are situated at the localities “Vojno Dobro” and “Topolik” near by city Novi Sad. They were established in 1978 by “deep planting”, planting space 5 x 5 metres with several varieties of black poplar, of which the following varieties were analysed: *Populus x euramericana* (Dode) Guiner cl. I-214, *Populus deltoides* Bartr. cl. 618 and *Populus deltoides* Bartr. cl. 450.

The data on mean monthly temperature and precipitation during the period 1978 - 1997 are based on the measurements of the weather station at Rimski Sancevi.

The systematic soil units are classified based on the valid Soil Classification of Yugoslavia (Skoric et al. 1985). The areas on which the plantations were established were flooded until 1928. After the construction of levees (dams) to protect from the Danube floods, these areas receive additional moisture only by ground water. The elevation of the study plantations is between 75 and 76 metres a.s.l. The plantation on humofluvisol is at the lowest elevation (75.20 m).

Particle size composition (%) was determined by the international B-pipette method with the preparation in sodium pyrophosphate (Bosnjak et all. 1997). Soil particle classification in the particle size composition was based on Atterberg’s classification.

Growth and development of 3 poplar varieties (*Populus* x *euramericana* cl. I-214, *Populus deltoides* 618 and *Populus deltoides* 450) were evaluated using average of height (m), diameter (cm) and volume (m^3 ha^{-1}) for each variety with a soil type. Comparasion were made among varieties a soil type and also within a variety across different soil types.

RESULTS WITH DISCUSSIONS

Climate characteristics

Generally during the vegetation growth period the prevailing climate is semiarid as indicated by the climatic data. However, the aridity is not well reflected by these average values. In addition, extremly cold days occur during the winter months, and more cloudy or rainy days occur during the summer months.

Exceptional aridity occurs during the months with the highest capacity for poplar growth. During the growth period number of days with temperature above 30˚C was averagly 29. In July and August, the monthly percipitation average is achieved in two to three days, and the periods without rainfall can be longer than two months. In May, June and July in average was 6 rainy days, and in August 5-6 rainy days. Average percipitation per day in May was 6,2 mm, in June 7,5 mm, in July 6,5 mm and in August 7,5 mm.

The lack of precipitation of hydrologically conditioned tree species, such as poplar, limits their cultivation to the flooded part of the alluvial plain where water is avalaible from floods and ground water. However, in the defended part of alluvial plain the additional moisture is provided only by ground water.

Soil Properties

The results of laboratory analysis presented in Table 1. indicate that the dominant fraction in humofluvisol (profile 1) is silt, ranging between 43.4 and 58.0 %, average 51.9 %. The most significant fraction silt+clay ranges from 51.2 to 79.4 %, average 69.4 %.

Table 1. Particle size composition of the soil in the study systematic soil units

Horizon	Depth	Particle size composition %						Textural class
		Coarse sand	Fine sand	Silt	Col. Clay	Total sand	Total clay	
		> 0.2	0.2 – 0.02	0.02 - 0.002	< 0.002	> 0.02	< 0.02	
	Cm	mm	mm	mm	mm	mm	mm	
1	2	3	4	5	6	7	8	9
Sample plot “Vojno Dobro” – Humofluvisol- Profile no. 1								
A_a	0-45	3.3	17.3	58.0	21.4	20.6	79.4	silty loam
C	45-85	0.4	29.0	54.4	16.2	29.4	70.6	silty loam
G_{so}	85-150	11.1	30.7	43.4	14.8	41.8	51.2	Loam
Average	0-150	4.9	25.7	51.9	17.5	30.6	69.4	
Sample plot nursery building “Topolik” – Sandy fluvisol – Profile no. 2								
A_{mo}	0-30	3.1	62.8	30.7	3.4	65.9	34.1	sandy loam
I	30-80	0.8	82.0	16.0	1.2	82.8	17.2	loamy sand
II G_{so}	80-140	1.3	72.2	19.5	7.0	73.5	26.5	sandy loam
III G_{so}	140-225	1.8	90.8	1.2	6.2	92.6	7.4	Sand
Average	0-225	1.8	77.0	16.9	4.3	78.8	21.2	
Sample plot nursery building “Topolik” – Loamy fluvisol - Profile no. 3								
A_{mo}	0-40	2.8	32.8	47.3	17.1	35.6	64.4	Loam
I G_{so}	40-75	0.4	57.1	28.8	13.7	57.5	42.5	Sandy loam
II G_{so}	75-180	1.2	86.2	9.3	3.3	87.4	12.6	Loamy sand
Average	0-180	1.5	58.7	28.5	11.3	60.2	39.8	

In the group of fluvisol profiles (profiles 2 and 3), the dominant fraction is fine sand. Average content of this fraction in the sandy form (profile 2) is 77.0 %, and in the loamy form (profile 3) it is 58.7%. The content of fine sand fraction increases regularly with depth in the loamy form. This shows that the process of fluvial sedimentation here is very similar to that in humofluvisol.

Regarding silt + clay fraction, fluvisols differ significantly at the level of the forms. The sandy form of fluvisol contains on the average 21.2%, and the loamy form contains 39.8% (Table 1). The Table shows that loamy fluvisol has a high content of silt fraction in the surface layer. This property could have an adverse effect on water and air properties of loamy fluvisol. Taking into account the relationship of individual fractions, it is clear that fluvisols have a discontinuous distribution, which points to the dominant processes of fluvisol formation. The high variability of individual fractions in the study forms of fluvisol results in the variability of its water and air and chemical properties.

Poplar growth

The average stem volume at age 19 of all clones ranged from 179.34 on the sandy fluvisol to 223.97 m^3 ha^{-1} on humofluvisol. The eastern cottonwood clone 618 had the maximum average stem volume of 245,02 m^3 ha^{-1}. Both of the eastern cottonwood clone 618 and 450 have a greater stem volume than I-214 on the humofluvisol and sandy form of fluvisol.

Table 2. Statifical significance of volume at age 19

Clone	Soil type			Average
	Humofluvisol	Fluvisol		
		Sandy form	Loamy form	
I-214	133.11	168.86	229.69	*177.22 c*
618	298.23	190.84.	245.98	*245.02 a*
450	227.62	178.31	196.25	*200.73 b*
Average	*219.65 a*	*179.34 b*	*223.97 a*	
Interaction clone x soil *** LSD 0.5= 0.61229				

On the study soil systematic units, the cultivar 618 showed the best results. The clone 450 showed the highest ecological adaptability, i.e. the lowest variation regarding growth traits (Table 2).

Two categories of soils were singled out in relation to ecological and productivity characteristics: humofluvisol and loamy fluvisol are classified in one category, and sandy fluvisol is another category. The most favourable categories were determined for each study clones based on multivariate analysis (Figure 5).

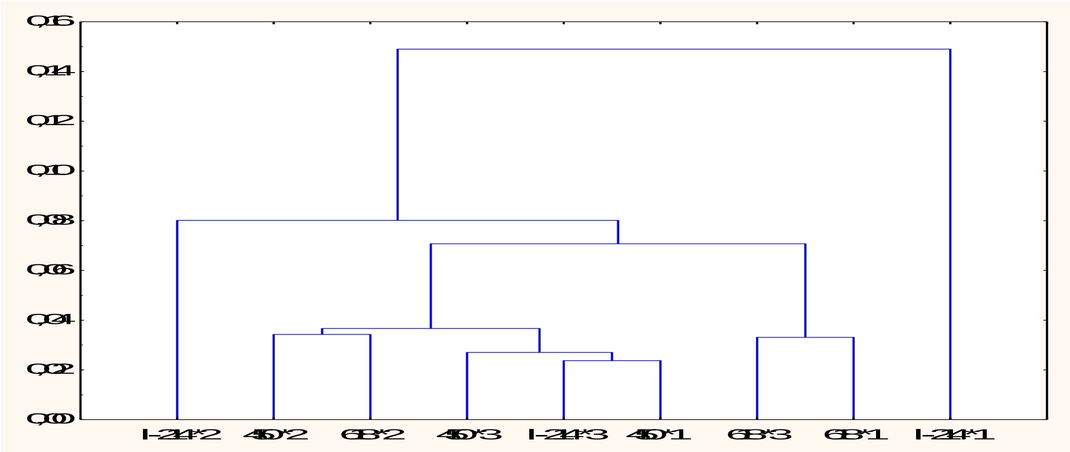

Figure 1. Cluster analysis dendrogram of growth traits of the clones on a different soil types
1* humofluvisol 2* sandy fluvisol 3* loamy fluvisol

Populus x *euramericana* cl. I-214 on the sandy fluvisol and humofluvisol is independent from the others. The clones *Populus deltoides* 618 and 450 are more similar in performance as shown by the dendrogram. Dendrogram showed three groups of growth traits: clone 618 on humofluvisol and loamy fluvisol were in the first group, clone 450 on humofluvisol and loamy fluvisol, as well as cultivar I-214 were in the second group, and clones 618 and 450 on sandy fluvisol were in the third group.

Effect of fraction silt+clay on volume of the clone are shown in Figure 2.

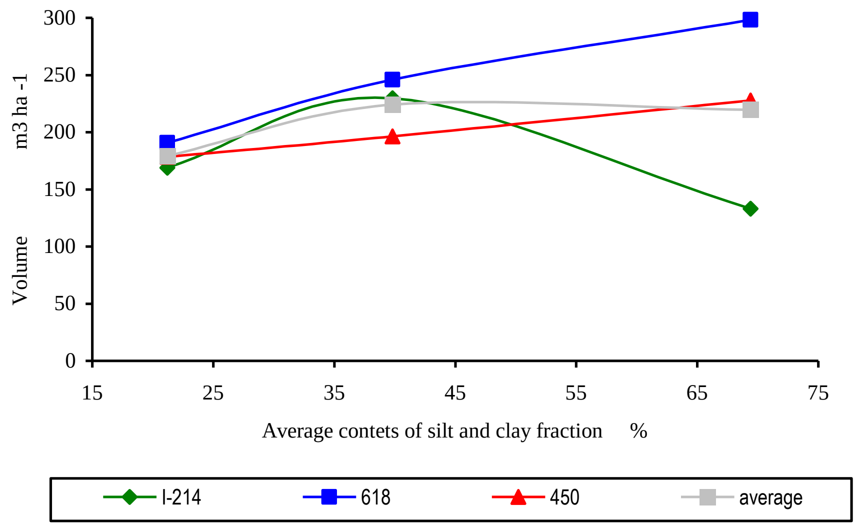

Figure 2. Effect of fraction silt+clay on volume of the clone

Clones 618 and 450 (eastern cottonwood clones) had greater height with an increaser silt + clay fraction, characterized by the humofluvisol. The study results indicate that the clone I-214 attained the maximum volume when silt + clay ranged from 30 to 50% per profile depth, as the maximum volume was measured under the average content of 39.8%, which proves the conclusion on the effect of this fraction on volume of the clone I-214. Clone I-214 attained maximum volume on the soils of 'lighter' textural class. Tufekcioglu (2005) reported that poplar grows best in soils having 35% or less clay content. Our results indicate that clone I-214 prefers soils with content of silt+clay ranged from 30 to 50%. In contrast clones 618 and 450 prefers soils containing increaser content of silt+clay. This soil have high water-holding capacities but inadequate aeration (Troeh and Thomson, 1993).

CONCLUSION

The study was done in the defended part of the alluvial plain in the Central Danube Basin where the climatic conditions are mainly semiarid. A marked arid climate occurs during July, August and September, the period of the most intensive poplar growth.

The results of the study of particle size composition of the study soils point to the differences of these properties in the study soils. The unfavourable influence on plantation growth, i.e. on wood volume production, on humofluvisol was most probably the high content of silt fraction and the low air capacity. In loamy fluvisol, the unfavourable influence on production was probably the higher content of silt fraction, which acted unfavourably on water and air properties of this soil form. Sandy fluvisol had a low capacity of readily available water, which could cause a reduced production of wood volume. On humofluvisol, eastern cottonwood clones 618 and 450 attained greater heights and diameters than euramerican poplar cl. I-214. This fact is explained by eastern cottonwood origin: It which grows naturally in North America on the soils with a higher percentage of silt and clay fraction, in the conditions of increased moisture, as opposed to the requirements of euramerican clone I-214, which demands lighter loamy soils and favourable moisture conditions per profile depth.

The above data lead to a conclusion that the clones of eastern cottonwood prefer the cultivation on clay-loamy soils with increased moisture, while Euramerican black poplar cl. I-214 prefers sandy and loamy soils with moderate moisture. These conclusions confirm the need of further research of the reaction of selected black poplar clones from different geographical regions to different soil systematic units in the Danube alluvial plain.

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CONTRIBUTION TO THE STUDY OF PARASITIC AND SAPROPHYTIC MYCOFLORA ON WILD CHERRY (*Prunus avium* L.)

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Summary: *Results relating to study of saprophytic and parasitic mycoflora on wild sweet cherry (Prunus avium L.) in Serbia are presented in this paper. Dendro-material with damages and disease symptoms was collected during investigation and placed into the herbarium, and then approach to the identification of fungi followed.*
In previous studies on wild cherry 21 fungi were found, from which Polistigma rubrum (Persoon) Saint-Amons and Daedaleopsis confragosa (Bolt.: Fr.) J. Schroet were of the greatest significance. Both species cause great economic damages, and lead to plant deterioration.
Key words: *wild sweet cherry, Prunus avium, fungus, Serbia*

INTRODUCTION

Prunus L. is a large family consisting of 200 mostly broadleaf (coniferous are rare) species naturally spread across the northern hemisphere.

Wild cherry is very significant due to its ecologic adaptability – it appears from along the river streams of lowland forests to the areas of high mountain beech at the forest vegetation edge. It appears most often at the forest edge and on the sites where birds, the main seed distributors stay. According to its biological traits relating to increase and yield, wild cherry belongs to the group of fast growing species, with rotation period of some 40-60 years.

The significance of wild cherry is very important. Its fruits are used for human, bird and animal consumption. Seedlings produced from wild cherry, smooth bark, tiny, black and bitter fruits, late maturing, are used as the basis for grafting. Due to early and abundant flowering it is an excellent source for bee feeding. Tea made of cherry stems is the excellent diuretic and blood pressure regulator. Cherries with black fruits containing antocians have dieto-profilactic, and dieto-therapeutic action in sight healing. It is suitable for establishment of tree-lines, and field protection belts, because it smells nice, gives honey, it is healing, edible, and besides that it gives a valuable wood mass. Studies on the terrain, and collection of the materials were done in natural stands in which wild cherry is a mixed species, in seed stands and in nurseries across the territory of Serbia. (Fruška gora, Zlatibor, Golija, Rtanj, Juhor, Bor, Crni vrh, Zlot and nurseries: Batočina, Naupare, Milentija, Institute of lowland forestry and environment Novi Sad).

MATERIAL AND METHODS

Dendro-material with visible damages and necrotic barks, tumors and similar changes was collected, placed into the herbarium and brought to a laboratory for identification and isolation of fungi on nutritive media.

Temporal microscopic preparations were made prior to fungi identification, and then determination of species was done on the basis of appearance of fruiting bodies, spore-bearing organs, and reproductive organs.

Identification of fungi was carried out based on the keys given by Dennis (1978), Grove (1935 and 1937), Booth (1971 and 1977), Lanier (1978), and Ellis (1975). In all these cases where only disease symptoms were found, and not the fruiting bodies causing it, nutritive media was used for the isolation of the causing agent. Isolation process encompassed the following stages:

- preparation of nutritive media PDA (potato-dextrose-agar) and MEA (malt extract agar) according to Booth (1971);
- cutting fragments from diseased tissue usually 4 x 4 mm, and surface sterilization in 25% ethanol solution;
- washing fragments in sterile distilled water;
- transferring to the surface of nutritive medium in Petri dishes 9 cm in diameter, at 30 (22^{o}C);

- development of the first culture as soon as 8 days upon sterilization;

Determination of fungi was done on the basis of obtained and developed pure cultures (appearance of the mycelia).

RESULTS OF INVESTIGATION

In previous investigation 21 fungi were found on wild cherry. From that number 10 species were found on bark, 5 on leaves, and 6 decay causing fungi on trees. Determined fungi were divided into 3 groups depending on the significance. Fungi developing as parasites which can cause serious damages to host plant were placed into the first group. Among these the most significant were *Polistigma rubrum* (Persoon) Saint-Amons and *Daedaleopsis confragosa* (Bolt.: Fr.) J. Schroet.

Fungi found on wild cherry are presented in table 1.

Table 1. Fungi determined on wild cherry

№	Nome of the fungus	Colonized part of the plant	Significance
1	*Cytospora prunorum* Sacc. Syd.	bark	+ +
2	*Fusarium lateritium* Nees.	bark	+ +
3	*Diaporthe pernicicosa* Marchal	bark	+ +
4	*Eutypella prunastri* (Persson ex Fries) Saccardo	bark	+ +
5	*Fusarium equiseti* (Corda) Sacc.	bark	+ +
6	*Scherophoma pythiophila* (Sda) Höhn	bark	+
7	*Fusarium poae* (Peck) Wollenw. in Lewis	bark	+
8	*Phomopsis* sp.	bark	+ +
9	*Phoma* sp.	bark	+
10	*Alternaria* sp.	bark	+
11	*Polystigma rubrum* (Persson) Saint - Amons	leaf	+ + +
12	*Botrytis cinerea* Pers.	leaf	+
13	*Penicillium* sp.	leaf	+
14	*Cladosporium* sp.	leaf	+
15	*Ceuthospora lauri* (Grev.) Grev.	leaf	+ +
16	*Fomes fomentarius* (L. ex Fr.) Fr.	Tree-decay causing	+ +
17	*Trametes cinabarinus* (Jacq.) Fr.	Tree-decay causing	+
18	*Trametes versicolor* (L. ex Fr.) Pilat	Tree-decay causing	+ +
19	*Daedaleopsis confragosa* (Bolt. ex Fr.) J. Schröt	Tree-decay causing	+ + +
20	*Lenzites tricolor* (Bull.) Fr.	Tree-decay causing	+ +
21	*Irpex lacteus* (Fr. ex FR.) Fr.	Tree-decay causing	+

+ + + - - - - - fungi which appear often and can cause serious damages to the host plant,

+ + - - - - - fungi which appear rarely and can cause damages only in very exceptional situations,

+ - - - - - fungi which appear rarely, developed mostly as saprophytes and have no practical significance

DISCUSION

In his capital work „Sylloge Fungorum“ Sacardo (1898) was the first to describe the great number of fungi on wild cherry – on dendro material collected across the globe he determined 33 fungal species.

Masson (1949) described 15 fungal species on ***Prunus*** family, from which 2 were found on wild cherry *Taphrina cerasi* (Fck.) Sad and *Venturia cerasi* Aderh.

Sutton (1980) described 13 fungal species on ***Prunus*** in detail, mentioning their global dissemination. From this number only *Foveostroma drupacearum* (Lèv.) di Cosmo was found on wild cherry.

Ellis and Ellis (1985) described 45 parasitic fungi on ***Prunus*** family in Great Britain, 11 on leaves, 3 on flower and fruits, and 31 on bark and tree. From the mentioned fungi found on wild cherry 4 species were registered (on bark and tree *Dermea cerasi* (Pers.) fr., *Amphisphaeria vibratilis* (Fuckel) E. Müller and *Calosphaeria pulchella* (Pers.) Schöter, and on leaves *Taphrina wiesneri* (Rathay) Mix).

In our previous investigations we found 21 fungi. From that number 10 on bark, 5 on leaves, and 6 decay causing fungi on tree. If we compare results obtained from our previous investigations with those from the literature we can conclude that we have found significant number of fungi on wild cherry, and that we can hope for even more significant results in the future work.

CONCLUSION

On the basis of implemented investigations the following conclusions can be made:

- in previous investigations total of 21 fungi was found on wild cherry (10 species on bark, 5 on leaves, and 6 decay causing fungi on tree)
- all fungi were divided into three groups according to their significance, with the first group (*Polistigma rubrum* (Persoon) Saint-Amons and *Daedaleopsis confragosa* (Bolt.: Fr.) J. Schroet) being the most significant
- it is necessary to apply protection measures to control these fungi which cause enormous economic damages and decay of wild cherry.

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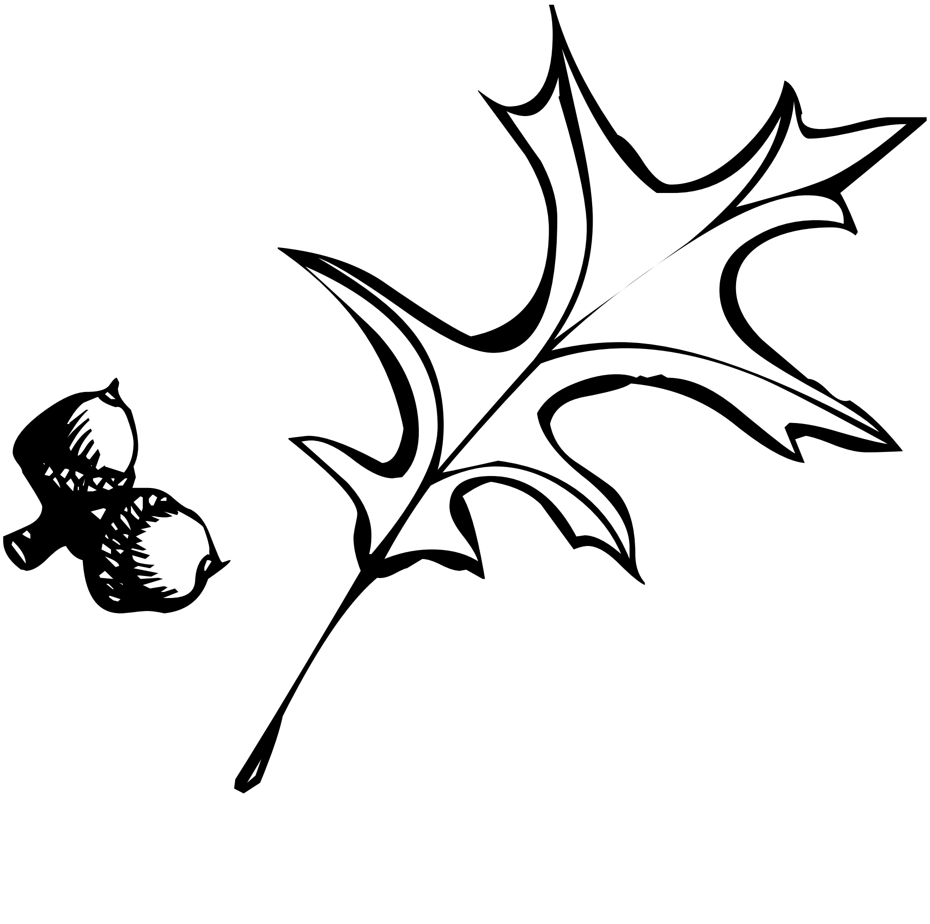

BIOTECHNOLOGY AND CONIFERS REGENERATION *IN VITRO*

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Abstract: *The progress in the development of the technologies of plant tissue and cell culture in forestry has been made. Since the first report in 1950, several coniferous species have been induced to produce adventitious or axillary buds or somatic embryos. Tertiary relics and endemics Bosnian pine Pinus heldreichii Christ. and Macedonian pine Pinus peuce Gris. are among the most significant species of the Balkan Penninsula. The limited natural regeneration, late reproductive maturity, periodicity of good seed yield, seed dormancy, but also the adverse anthropogenic effect, point to the necessary study of these species and to the significance of finding out the methods of their conservation and regeneration at their natural sites. We studied the factors that control the development of ovules and mature zygotic embryos of Bosnian pine and Macedonian pine in vitro.*
Keywords: *Adventitious bud, axillary bud, somatic embryogenesis, plant regeneration*

INTRODUCTION

Bosnian pine *Pinus heldreichii* (Christ.) and Macedonian pine *Pinus peuce* (Gris.) are Tertiary relics and endemics of the Balkan Penninsula. These highmountain pines are among the most significant species of the Balkan dendroflora. They usually grows in the mountain influenced by mediterranean climate, i.e. on Prokletije, Koritnik, Šarplanina, Ostrovica, Kodža-Balkan, Pelister and others. It spreads up to 2000 m often even beyond that altitude (Janković, 1991). Bosnian pine grows on steep and dry limestone slopes, most often in pure stands, while Macedonian pine grows on slopes on siliceous soils, rarely on carbonate soils (Vidaković, 1982).

Both coniferous species are adapted to dry and hot mountain summer with very intensive solar radiation. *Pinus heldreichii* is tolerant of drought and saline conditions. *Pinus peuce* is tolerant of winter cold and also of wind exposure. Ecological tolerance and frost resistance make them very valuable for forest tree breeding (Mitchell, 1996). From the ecological point of view, both species are recommended as a melioration trees suitable for planting on degraded and devastated soils. They are also a popular ornamental trees in parks and large gardens. Becouse of its limited area of natural distribution, both species requires special attention and implementation of measures for its conversation (Janković, 1991).

Most gymnosperms are propagated sexually through seeds. Sexual reproduction preserve some of the mean traits of selected families. On the other hand, vegetative propagation is a procedure with potential to maintain the best characteristics of selected individuals, and therefore is the most using method of propagation.

P. heldreichii and *P. peuce* are usually propagated by seeds but full seed production is unpredictable and delayed by the time required for a tree to reach its full reproductive capacity. Vegetative propagation of these species has been only partially successful. Therefore, the tissue culture can be used as a significant contribution to conventional methods of vegetative propagation of both coniferous species.

In vitro techniques offer possibility for growth of tissue or organ segments on suitable media that stimulate plant regeneration along several pathways. Using suitable hormonal composition of the growth media induction of axillary or adventitious bud could be obtained. The ability of buds to elongate into shoots and ability of shoots to form roots depends of species. Somatic embryogenesis is the process by which asexual or somatic cells are induced to form embryos in culture. Plant regeneration by the somatic embryogenesis occurs through a sequence of defined steps which include induction and proliferation of embryogenic tissue, embryo maturation and germination.

However, specific conditions for plant regenetation via axillary or adventitious bud induction also for embryogenic tissue initiation as well as for embryo maturation should be defined for every species. These techniques offer possibility to supply plants to forest nurseries but further improvement of methods for early field test before large scale planting is necessary.

MATERIALS AND METHODS

Cones of *Pinus heldreichii* were harvested from open-pollinated trees on Lovćen Mountain (Montenegro) during October 2004. Cones of *P. peuce* were harvested from open-pollinated trees located on Mučanj Mountain during October 2003 and 2004. Seeds were extracted from the cones and kept at 4 °C. After surface-sterilization in 20% sodium hypohlorite for 30 minutes, seeds were rinsed three times with sterile distilled water, and then seed coats were removed. The embryos were aseptically isolated from megagametophyte and placed horizontally on the surface of the GD (Gresshoff and Doy, 1972) medium as modified by Sommer et al. (1975).

For axillary bud induction mature embryos were vertically placed into test tubes on 1/4 MS (Murashige and Skoog, 1962) culture medium supplemented with 2% sucrose. Four weeks after embryo germination hypocotylary explant (radicle were cut off) were treated with solution containing BA (seven different concentations) and 0.1% dimetyl sulfoxide for 1 h. After this pulse-treatment, explants were transferred on the GD medium supplemented with 3% sucrose, 0.5% activated charcoal and 0.7% agar. Axillary shoots formed on explants were then isolated and cultured on the half strength GD medium. Cultures were maintained at temperature of 25 ± 2 °C, a 16-h photoperiod.

For adventitious bud induction 30 embryos per treatment were placed on GD medium supplemented with 0.7% agar, 3% sucrose and BA (0.5 or 1.0 mg/l). Also, the effect of kinetin, 2iP and combination of BA + kinetin on bud induction was tested. Explants were kept on induction medium for 4 weeks, and then transferred to the same basal medium but at half-strength, growth regulator-free, containing 5 g/l activated charcoal to promote bud elongation. For rooting shoots were pulse treated with a solution of 1mM NAA or IBA for 2 or 5 h. After puls-treatment, shoots were transferred to 1/2 GD supplemented with 2% sucrose. Cultures were maintained at temperature of 25 ± 2 °C, a 16-h photoperiod.

For somatic embryogenesis induction cultures were grown for 5 days on GD medium, GDa medium with nitrogen salts reduced to one half, or GDb medium with nitrogen salts totally reduced, supplemented with: 2,4-D 2 mg/l and BA 0.5 mg/l or NAA 2 mg/l and BA 0.5 mg/l. Culture medium GD without growth regulators or GD with 12 μM ABA was used as medium for somatic embryos maturation. All media were supplemented with 3% sucrose and 0.7% agar (Torlak, Belgrade). The pH of the media was adjusted to 5.7 prior to autoclaving for 25 minutes at 115 °C. Cultures were maintained at 25 ± 2 °C in darkness during induction and proliferation stages, and at 16 h photoperiod during maturation phase.

RESULTS AND DISCUSSION

Pinus heldreichii and *Pinus peuce* regeneration was achieved by the induction of axillary buds *in vitro*. Axillary buds on hypocotylary explants were obtained after pulse treatment with benzyladenine (BA) water solution. The number of buds formed on explants increased with the BA concentration which was applied in pulse treatment. The maximal number of buds was obtained on explants after treatment with 50.0 mg/l BA, and the further increase of BA concentration did not increase the number of formed buds (Table 1, 2). Succsesful axillary bud formation using pulse-treatment has been described for some other conifer species (Austin Burns et al., 1991, Goldfarb et al., 1991, Stojičić and Budimir, 2004).

Axillary buds were not formed on the control explants, which were treated only with water solution. With the increase of BA concentration in pulse treatment the average axillary bud length also increased. Maximal bud length was achieved on explants grown on 50 mg/l concentration.

Eight weeks after the pulse treatment, axillary buds were separated from explants and placed on the media for rooting, because they were already sufficiently elongated on the maternal explant. Axillary bud rooting on GD half-strength medium with activated charcoal added, or the application of NAA, did not show the expected results. The successful induction of roots was achieved by infecting the axillary shoot basal area Bosnian pine and Macedonian pine with bacterium *Agrobacterium rhizogenes.*

The regeneration of *Pinus heldreichii* and *Pinus peuce* was achieved by the induction of adventitious buds *in vitro*. The organogenesis capacity of embryo explants of the genus *Pinus* is affected by several factors, among which the culture conditions have an important role. In conifers, adventitious buds are formed on the embriogenic tissue when it is grown on the medium with exogenously supplemented cytokinins. Taking into account that the seeds for this research were collected from the trees with free pollination, it is possible that a part of the variability of the number of buds formed on Bosnian pine and Macedonian pine explants after identical treatments, is the consequence of the genetic differences.

Table 1. Effect of BA on *Pinus heldreichii* axillary bud induction. Explants were grown on GD for 4 weeks and then transferred to the bud elongation medium for another 4 weeks

BA (mg/l)	Explants with axillary buds	Average no. of buds per explant x ± SE	Average shoot length (mm) x ± SE
0.0	-	-	-
1.0	20.0	0.20 ± 0.09^{ab}	4.75 ± 0.41^{a}
2.5	38.9	0.78 ± 0.25^{ab}	**7.57 ± 0.87^{b}**
10.0	76.9	1.25 ± 0.35^{b}	**7.70 ± 0.92^{b}**
25.0	75.0	3.25 ± 0.66^{c}	**7.97 ± 0.49^{b}**
50.0	**85.7**	**4.57 ± 0.60^{d}**	**8.09 ± 0.36^{b}**
100.0	75.0	2.78 ± 0.58^{c}	**7.96 ± 0.87^{b}**

Means in the column followed by different letters are different according to Duncans' Multiple Range Test ($p \leq 0.05$). SE = standard error.

Table 2. Effect of BA on *Pinus peuce* axillary bud induction. Explants were grown on GD for 4 weeks and then transferred to the bud elongation medium for another 4 weeks

BA (mg/l)	Explants with axillary buds	Average no. of buds per explant x ± SE	Average shoot length (mm) x ± SE
0.0	-	-	-
1.0	21.7	1.23 ± 0.39^{a}	4.00 ± 0.32^{a}
2.5	28.3	1.30 ± 0.38^{a}	4.37 ± 0.45^{a}
10.0	55.0	1.88 ± 0.42^{a}	7.31 ± 0.82^{b}
25.0	**80.0**	**4.65 ± 0.60^{c}**	**8.82 ± 0.56^{c}**
50.0	**80.0**	**4.96 ± 0.62^{c}**	**9.23 ± 0.66^{c}**
100.0	70.0	2.05 ± 0.31^{b}	7.87 ± 0.80^{b}

Means in the column followed by different letters are different according to Duncans' Multiple Range Test ($p \leq 0.05$). SE = standard error.

The success of adventitious bud induction depends on the selection of the primary explant, then on the explant age, hormonal treatment and nutritive medium. The culture medium used in the induction of adventitious buds on Bosnian pine and Macedonian pine explants was GD and cytokinin benzyladenine in two different concentrations. The highest average number of buds on Bosnian pine explants was formed under the effect of 1.0 mg/l BA, and on Macedonian pine explants under the effect of 0.5 mg/l BA. The buds formed on explants treated with 0.5 and 1.0 mg/l BA respectively had the highest average lengths.

In addition to benzyladenine, we also tested the effect of other cytokinins and their combinations on the formation of adventitious buds on Bosnian pine and Macedonian pine explants. The study results show that the most efficacious results in the number of formed adventitious buds were achieved by both BA concentrations, then the combination of BA and kinetin, then both concentrations of kinetin, and the poorest effect was attained by 2iP (only for Bosnian pine explants). BA was also the most effective cytokinin for adventitious bud induction in *Pinus sylvestris* (Sul and Korban, 1998), *P. pinaster* (Rancillac, 1991) and *P. heldreichii* (Stojičić et al., 1999).

A greater number of elongated buds were obtained when the explants, with each successive transfer to the fresh medium, were divided into smaller pieces. In this way, the explants had the possibility of greater absorption from the media, but also the density of the surrounding buds was reduced.

The rooting of adventitious buds of conifer species obtained *in vitro*, is difficult and with many problems. Only a small number of conifer species root spontaneously. After treatment with 1mM indole-3-butyric acid isolated shoots of Bosnian pine and Macedonian pine were rooted. After rooting, the plants were successfully acclimatized.

Table 3. Effect of cytokinins on *Pinus heldreichii* bud induction. Explants were grown on GD for 4 weeks and then transferred to the bud elongation medium for another 4 weeks

Cytokinins (mg/l)	**Explants with buds after 8 weeks in culture (%)**	**Average no. of buds per explant x ± SE**
BA 1.0	100.0	**16.30 ± 1.51**^{a}
BA 0.5	100.0	**11.70 ± 1.25**^{a}
kinetin 0.5 i BA 0.5	100.0	6.94 ±□ 0.83^{b}
kinetin 0.25 i BA 0.25	100.0	8.42 ± 0.85^{bc}
kinetin 1.0	96.0	6.60 ± 0.86^{bc}
kinetin 0.5	100.0	5.20 ± 0.65^{c}
2iP 1.0	17.2	1.00 ± 0.55^{d}
2iP 0.5	15.0	1.50 ± 0.62^{d}

Means in the column followed by different letters are different according to Duncans' Multiple Range Test ($p \leq 0.05$). SE = standard error.

Table 4. Effect of cytokinins on *Pinus peuce* bud induction. Explants were grown on GD for 4 weeks and then transferred to the bud elongation medium for another 4 weeks

Cytokinins (mg/l)	**Explants with buds after 8 weeks in culture (%)**	**Average no. of buds per explant x ± SE**
BA 1.0	93.3	12.95 ± 1.03^{b}
BA 0.5	100.0	**15.12 ± 0.99**^{c}
kinetin 0.5 + BA 0.5	98.3	5.33 ± 0.83^{a}
kinetin 0.25 + BA 0.25	95.0	5.12 ± 0.85^{a}
kinetin 1.0	100.0	5.46 ± 0.86^{a}
kinetin 0.5	100.0	5.32 ± 0.65^{a}

Means in the column followed by different letters are different according to Duncans' Multiple Range Test ($p \leq 0.05$). SE = standard error.

The induction of somatic embryogenesis was achieved in the culture of immature ovules of both conifer species. The correct choice of explants is the first step in the successful induction of somatic embryogenesis. The genotype which is to be propagated is the genotype of the mature plant; therefore the plant material isolated from the mature plants should be introduced to the culture *in vitro*. However, the mature plant tissue is at the higher degree of differentiation, so the process of somatic embryogenesis induction is considerably more difficult. For this reason, the juvenile tissues are the most often used explants not only in the *Pinus*, species, but also in the majority of gymnosperms.

The immature seeds of Bosnian pine and Macedonian pine had soft testa and ovule of transparent white colour, soft to touch. The isolated ovules were placed on culture media for the induction of somatic embryogenesis: GD, GD with half-strength nitrogen compounds, and GD without nitrogenous compounds.

The induction of embriogenic callus on Bosnian pine explants was most suited by GD medium and GD with half-strength nitrogen compounds. The medium without any nitrogen compounds suited the induction of embriogenic callus on Macedonian pine explants (Table 5, 6).

Culture media for the somatic embryogenesis induction contained auxin (2,4-D or NAA) and cytokinin BA. Growth regulators are mainly necessary during the induction, growth and propagation of embryogenic tissue. The highest somatic embryogenesis initiation frequency in *Pinus heldrichii* and *P. peuce* was 10%, similar results were obtained for *P. banksiana* (3.9%) (Park et al. 2006), *P. nigra* (8%) (Salajova et al., 1995), *P. elliottii* (10%) (Liao and Amerson, 1995) and *Pinus heldreichii* (6.7%) (Stojičić et al., 2007).

After the transfer of the ovules to the medium with the reduced concentration of growth regulators, the embryogenic callus formed on the micropyle pole of the ovule. The proliferation of the embryogenic tissue was intensive on the culture medium with the same growth regulators as in the inductive treatment, but the concentrations were five to ten times lower.

The embryogenic cultures of *P. heldreichii* and *P. peuce* on the solid agar medium were transparent whitish in colour, covered by mucilaginous gel which gives this tissue a characteristic lustre. The light microscope shows that this tissue consists of a high number of densely interwoven embryos, so this tissue is called embriogenic tissue. In all stages of development, the embryos of *Pinus* species are organised as bipolar structures with meristematic cells on the distal end and with a suspensor with a long vacuolised cells on the proximal end. The meristematic cells are isodiametric, densely compacted, with a dense cytoplasm, small vacuoles and a centrally positioned nucleus. The suspensor consists of elongated cells of different lengths, with a large vacuole which is centrally positioned and with a thin layer of cytoplasm along the cell wall.

The maturation of somatic embryos of different *Pinus* species mainly requires the presence of ABA in the medium. On the Macedonian pine embriogenic tissue which is transferred to the medium with ABA, there were some changes which pointed to the initial phases of embryo maturation.

Table 5. Induction of embryogenic tissue on Bosnian pine ovules. After the inductive treatment for 5 days, explants were transferred to media of the same mineral composition with a five times lower concentration of growth regulators

Culture medium	Growth regulators (mg/l)	Explants with embryogenic tissue (%)
GD	2 mg/l 2.4-D+0,5 mg/l BA	6.67
	2 mg/l NAA+0,5 mg/l BA	3.33
GDa	2 mg/l 2.4-D+0,5 mg/l BA	**10.00**
	2 mg/l NAA+0,5 mg/l BA	6.67
GDb	2 mg/l 2.4-D+0,5 mg/l BA	3.33
	2 mg/l NAA+0,5 mg/l BA	3.33

Table 6. Induction of embryogenic tissue on Macedonian pine ovules. After the inductive treatment for 5 days, explants were transferred to media of the same mineral composition with a five times lower concentration of growth regulators

Culture medium	Growth regulators (mg/l)	Explants with embryogenic tissue (%)
GD	2 mg/l 2.4-D+0,5 mg/l BA	3.33
	2 mg/l NAA+0,5 mg/l BA	6.67
GDa	2 mg/l 2.4-D+0,5 mg/l BA	-
	2 mg/l NAA+0,5 mg/l BA	3.33
GDb	2 mg/l 2.4-D+0,5 mg/l BA	**10.00**
	2 mg/l NAA+0,5 mg/l BA	3.33

According to obtained results, micropropagation and somatic embryogenesis have a potential use in the propagation of *P. heldreichii* and *P. peuce* but further improvement of the methods is required.

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FEEDING PREFERENCE OF *Phyllodecta vitellinae* (Coleoptera: Chrysomelidae) IN BLACK POPLAR CLONES

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Abstract: *One of the most prevalent pests in the nurseries and plantations of black poplars in Serbia is Phyllodecta vitellinae L. It is necessary to manage this defoliator in order to prevent loss in productivity of poplars. One way to cope with this problem is to identify low susceptible clones of poplar and then use them for the establishment of new plantations. In order to evaluate the feeding preference of insect among 15 black poplar (section Aigeiros Duby) clones pest damage assessment were carried out on 22nd Sept. 2006 and 21st Sept. 2007 in the Institute of Lowland Forestry and Environment Experimental Station gene pool. Feeding preference was assessed on the basis of percentage of leaves with more than 20% of damaged area. For every genotype leaves of two shoots on four plants were assessed. The data were transformed by arcsine – transformation to meet normal distribution. Average plant values were data for further statistics: analysis of variance and Duncan test. Genotypes were classified by Ahmed-method.*

Results showed significant differences among examined clones. The examined factors (genotype and year) as well as interaction genotype × year significantly affected variation of examined character. This emphasizes the significance of multiannual research on this matter, as well as the possibility of changes in the population structure of the pest. Seven clones could be considered as resistent to the attack of P. vitellinae, while the others appeared to be susceptible. Registered and widely used clones Pannonia and S1-8 appeared to be moderately susceptible, which corresponded to usual pest attack in recent years. According to the prevalence of P. vitellinae clones P. nigra, 40/94, and moderately resistant clones 478 and 4/94, could be proposed for utilization in wood production and breeding process.

Key words: *pest resistance, variability, breeding.*

INTRODUCTION

Leaf – feeding chrysomelid beetle *P. vitellinae* is a widely spread and significant pest in poplar stands and nurseries in Serbia. In order to prevent demages caused by its enormous multiplication chemical control measures are applied. One of the alternatives over the application of chemical control measures is the selection and introduction of poplar clones into production, for which the insect shows lower degree of preference.

From economic and ecological point of view this is certainly an acceptable way of preventing damages in relation to insecticide application. It has been known from previous investigation that there were significant differences among poplar clones in relation to degree of preferences that harmful insects showed (Ahmad, 1993; Augustin et al., 1993; Poljaković – Pajnik, et *al.*, 1999; Singh, 2000; Drekić et *al.*, 2006; Kovačević, et *al.*, 2007).

Results of investigation of poplar clone resistance to *P. vitelliae* attack can be found in the articles of Finet & Gregoire (1981, 1982) and Finet et *al.* (1982, 1983). Our preliminary investigations pointed out to the fact that there were differences among black poplar trees relating to degree of feeding preference of *P. vitellinae* (Drekić et *al.*, 2007*).*

A review of a two-year investigation of poplar clonal feeding preferences of *P. vitellinae* is given in this paper. In this way it was made possible to identify varieties among clones in the breeding process, for which the insect shows low degree of preference. Usage of these varieties in production or further breeding process would diminish damages caused to poplar production by this insect.

MATERIALS AND METHODS

In order to evaluate the feeding preference of insect among 15 black poplar clones pest damage assessment were carried out on 22nd Sept. 2006 and 21st Sept. 2007 in the Institute of Lowland Forestry and Environment Experimental Station gene pool. Estimation was carried out in the autumn when major damages caused by feeding of larvae and insect imago could efficiently be observed. All clones except Pannonia were developed as the result of black poplar improvement carried out over several decades in the Institute of lowland forestry and environment.

Table 1. Clone taxonomy, country of origin and status of examined clones

Clone	Clone Taxonomy	Country of origin	Status
16/94	*P. x euramericana*	Serbia	Experimental phase
19/94	*P. x euramericana*	Serbia	Experimental phase
34/94	*P. x euramericana*	Serbia	Experimental phase
5/94	*P. x euramericana*	Serbia	Experimental phase
4/94	*P. x euramericana*	Serbia	Experimental phase
41/94	*P. x euramericana*	Serbia	Experimental phase
478	*P. deltoides*	Serbia	Registered
15/94	*P. x euramericana*	Serbia	Experimental phase
S 1-8	*P. x deltoides*	Serbia	Registered
40/94	*P. x euramericana*	Serbia	Experimental phase
3/94	*P. x euramericana*	Serbia	Experimental phase
11/94	*P. x euramericana*	Serbia	Experimental phase
Panonnia	*P. x euramericana*	Hungary	Registered
42/94	*P. x euramericana*	Serbia	Experimental phase
P. nigra	*P. nigra*	Serbia	Experimental phase

Feeding preference was assessed based on the percentage of leaves with more than 20% of damaged area. Damages less than 20% were not taken into account in order to avoid confusions relating to smaller damages, that could be the consequence of attack of other defoliators and because they affected the leaf activity in a less extent.

For every genotype leaves of two shoots on four plants were assessed. Percentage of leaves with more than 20% of damaged area was derived for every shoot. It was later transformed by arcsine – transformation to meet normal distribution. Average plant values were data for further statistics: analysis of variance and Duncan test. Genotypes were grouped by Ahmed-method.

RESULTS AND DISCUSSION

Review of leaf mass damage degree in the form of percentage of participation of leaves with damages greater than 20% of leaf surface in total number of leaves is given in Table 2. The least degree of preferences was determined toward *P. nigra* clone, in which participation of leaves with damages above 20% of leaf surface was found in only 2.52% of assessed leaves. The greatest participation of leaves with this scale of damages was determined in clone 5/94. Results of Finet and Gregoire (1981, 1982) and Finet et *al.* (1982, 1983) pointed out to the resistance of some clones which belong taxonomically to *Populus nigra* species. They mentioned that *Populus nigra* Ghoy 1 clone was highly resistant, and Urban (2006) found out that some black poplar clones were highly resistant. Results of Duncan test showed that the most severely attacked clone 5/94, and the least attacked clones of *Populus nigra* and 40/94 differed significantly from the part of tested clones. Statistically significant difference was found for *P. nigra* clone in relation to all other clones. The examined factors (genotype and year) as well as interaction genotype × year significantly affected variation of examined character. This emphasizes the significance of multi annual research on this matter, as well as the possibility of changes in the population structure of the pest.

Grouping of studied poplar clones according to Ahmad's method is given in Table 3. According to this author clones were grouped into 6 categories due to their susceptibility i.e. resistance to poplar leaf beetle attack. Seven clones exerted different degree of resistance to this attack, while the rest of the clones were susceptible. The greatest degree of susceptibility to *P. vitellinae* attack was found in clones 3/94, 5/94, 34/94. Grouping of clones using this method revealed that Pannonia clone which is widely used today in establishing new stands belonged to the group of clones with moderate susceptibility. This was in accordance with the situation in nurseries and stands where greater number of cases of over multiplication on clone Pannonia was noticed in recent years.

Table 2. Duncan test and % of leaves with more than 20% of damaged area

Clone	**% of leaves with more than 20% of damaged area**	**Duncan test**
5/94	59.26	a
34/94	58.62	a b
3/94	53.26	a b
16/94	51.83	a b
S 1-8	51.77	a b
Panonnia	50.34	a b
19/94	45.81	a b c
15/94	42.53	a b c
11/94	41.00	a b c
41/94	39.56	a b c
42/94	37.08	b c
4/94	34.76	b c
478	34.37	b c
40/94	28.09	c
P. nigra	2.52	d
F-value= 7.901 ; p=0.000		

Table 3. Clones grouped by Ahmad method

R1 = Most resistant =	$\bar{x}(A) < (\bar{X} - SD)$
P. nigra, 40/94,	
R2 = Moderately resistant =	$(\bar{X} - SD) < \bar{x}(A) < (\bar{X} - SD/2)$
478, 4/94,	
R3 = Marginally resistant =	$(\bar{X} - SD/2) < \bar{x}(A) < (\bar{X})$
41/94, 11/94, 42/94	
S1 = Marginally susceptible=	$(\bar{X} + SD) > \bar{x}(A) > (\bar{X})$
15/94, 19/94	
S2 = Moderately susceptible =	$(\bar{X} + SD) > \bar{x}(A) > (\bar{X} + SD/2)$
S 1-8, 16/94, Panonnia	
S3 = Most susceptible =	$\bar{x}(A) > (\bar{X} + SD)$
3/94, 5/94, 34/94	

The greatest degree of resistance to the attack was determined in clones *P. nigra* and 40/94, while clones 478 and 4/94 belonged to group of moderate resistant clones.

CONCLUSIONS

Studies revealed that leaf – feeding chrysomelid beetle *P. vitellinae* exerted different degree of feeding preference toward black poplar clones.

According to the prevalence of *P. vitellinae* clones *P. nigra*, 40/94, and moderately resistant clones 478 and 4/94 could be proposed for utilization in wood production and breeding process.

Studies of *P. vitellinae* feeding preferences could be used as a breeding criteria in finding less endangered clones. Due to that these studies should be included in the regular procedure in black poplar selection.

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POSSIBILITIES IN PRODUCTION OF BLACK POPLAR CLONES – ROOTED CUTINGS (SECTION *AIGEIROS* DUBY) ON MARSH GLEIC SOIL

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Abstract: *Results presented in this paper revealed the fact that studied clones had equal rooted cutting survival rates on marsh-gleic soil ranging from 63.7-69.4% (which was pretty low) and similar mean diameters at root neck height ranging from 12-13 mm. Significant difference was determined in mean height of rooted cuttings, where the mean heights of clones S3-1 (197cm), and (194cm) were significantly higher than the rest of the clones (157-168cm).Difference in participation of number of rooted cuttings higher than 2m in the total number of obtained rooted cuttings was also determined. Clones were grouped into two groups: one group consisted of clones 155/81 (7.4%), 260/81 (11%), and* S_{6-7} *(13.4%), and another one of clones* S_{1-3} *(46.2%) and B-17 (53.9%).*
Obtained mean heights and height of rooted cuttings structure were not satisfying from the production aspect. Group of clones with favorable height structure (B-17 and S_{1-3}*) pointed out to the fact that with greater planting distances, and application of other technological measures during stand establishing and taking care of the stand, it would be possible to produce satisfying quantity of black poplar planting material even on marsh-gleic soil, with no irrigation used as one of the care measures.*
Key Words: *black poplar, nursary production, marsh-gleic soil*

INTRODUCTION

Production of quality poplar planting material is significant prerequisite for obtaining quality poplar stands. Nursery plants of different age 1/1, 1/2, 2/2 and even 2/3 were produced vegetatively in poplar nurseries (Rončević et *al.,* 2002) depending on the needs and technology that would be used in establishment of future stands. In order to produce needed quantities of nursary plants aged 1/1 which correspond to the planting norms it is necesary to apply care measures during establishment and production process of nursary plants. Since poplars naturally appear as higrophylic species on fluvisol. i.e. its different varieties, and on humofluvisol these soils are the most suitable, and the most often used in production of nursery plants (Živanov & Ivanišević*,* 1986). These soils have favorable textural composition and water+airy regime to be considered suitable for nursary production.

Although the mentioned soils are the most suitable for establishment of nursary production, this paper describes an attempt to produce rooted cuttings of black poplars aged 1/1 on soils belonging to the gleic class, i.e. marsh-gleic in order to reveal possibilities of usage of this type of soil in production of repromaterial and poplar nursary plants with no irrigation applied.

Since marsh-gleic soils have expressed supply of underground water for cultivation of black poplar nursary plants, irrigation of such soils can be omitted. The possibility is offered on hypo-gleic soils, due to good wetting with underground water. Considering hydrological conditions of marsh-gleic soils it is necessary to test black poplar nursary plants in order to choose clones which are the most tolerant to such habitat conditions, and which offer possibilties of obtaining quality planting material with no irrigation applied.

OBJECT OF INVESTIGATION AND METHODS

A trial with five black poplar clones was established on marsh-gleic soil during 2007 in the locality of the protected area of Central Danube basin on the trial property of the Institute of lowland forestry and environment. The following clones were used in the trial: S_{6-7} (*P. deltoides* Bartr ex Marsh), S_{1-3} (*P. deltoides* Bartr ex Marsh), B-17 (*P. deltoides* Bartr ex Marsh), 260/81 (*P. deltoides* Bartr ex Marsh) and 155/81 (*P.* × *euramericana* (Dode) Guinier). Four replications x 35 plants in a block random design were used in the trial.

Planting distance was 0.80 x 0.12, i.e. 0.096m^2 per one rooted cutting, or 104.167 rooted cuttings per hectare.

Pedological profile with morphological description was opened on the trial area. Soils samples for laboratory analyses were taken, and physical and chemical soil properties were determined in the laboratory using the following methods:

- Mechanical soil composition according to B-pipette method with preparation in sodium pyrophosphate, Group of authors (1977),
- Total porosity (P%) by calculation from specific and volumetric soil mass,
- moisture retention at pressure of 0,33 bars, using porous plate Group of authors (1977),
- moisture retention at pressure of 6,25 bars, using Pressure mambrane, Group of authors (1977),
- moisture content in the soil up to the depth of 60 cm, by thermo-gravimetric method, on each 15th day, Group of authors (1977),
- soil humus content using Tjurin method modified by Simakov,
- $CaCO_3$ content in the soil, volumetrically using Scheibler's calcimeter
- Soil chemical reaction, pH in the water electrometrically with glass electrode,
- Nitrogen according to Kjedahl's method,
- Easily available phosphorus and potassium using Al-method, Egner-Riehm –Dominigo,
- Level of underground water was measured using piezometer

At the end of vegetation period heights of rooted cuttings and neck root diameters were measured. Data were processed using standard methods of statistical analysis for mean value comparison (ANOVA, test NZR at 0.05 risk level). Transformation *arcsin (% of survival)$^{1/2}$* was used for determination of survival percentage. Programs EXCELL and STATISTICA 6.0 were used for data processing.

RESULTS AND DISCUSSION

Soil characteristics

Marsh-gleic soil (eugley) represents the hydromorphic order, which belongs to the class of gleic soils of A-G structure. It is characterized by dark grey horizon (Aa), of smaller thickness (50 cm) mostly loamy to clay textural composition. Humus was formed by decomposition of hydropholic vegetation under anaerobic conditions. According to analysis of textural composition of studied marsh-gleic soil (Table 1), a transitional horizon AaGso and horizon of secondary oxidation (Gso) were separated due to different texturall composition. Humus horizon (Aa,p) and transitional horizon (AaGso) had greater percentage of total clay and silt content than the total sand content. Textural class of these layers was silty loam and loam. Layer of secondary oxidation (Gso) was composed of reverse proportion of total sand and total clay and sand, so according to textural class this layer was loamy sand. Considering the textural composition of the profile it was evident that the participation of total sand increased with depth, while that of clay and silt dicreased. Although stratification of this soil, which pointed out to genesis caused by fluvial sedimentation was evident, according to the depth of underground water it ecologycally belonged to marsh-gleic soil, i.e. sub type hypogleic with the depth of gleic horizon below 90 cm. Considering depth of gleic horizon the studied marsh-gleic soil belonged to γ-gleic according to Wilde (1962), cited by Antić et *al.* (1984), where depth of Gr horizon was at the depth of 80-120cm. Production traits of these soils were dependent on gleic horizon depth, and on the way of wetting.

Table 1. Mechanical soil composition

Horizon	Depth (cm)	Silt 0.02-0.002%	Clay › 0.002%	Total clay + silt › 0.02%	Textural class
Aa,p	0-30	55.60	20.12	75.72	Silty loam
AaGso	30-60	46.76	18.40	65.16	Loam
Gso	60-90	7.12	4.80	11.92	Loamy sand
Average	0-90	36.49	14.44	50.93	-

Results of physical properties with total porosity of this soil ranging from 50.17 to 57.75 %vol. are given in table 2. Upper limit Rvk. 0.33b ranged from 48.14 %vol. in Aa,p horizon to 22.92 %vol. in Gso layer. For Rvk value of 6.25b upper limits ranged from 23.39

%vol., in Aap horizon to 6.82 % vol. in Gso layer. Values of cappilary rise ranged from 14.9 cm in humus horizon to 27.2cm in IGso layer.

Table 2. Water-air soil properties

Horizon	Depth (cm)	Total porosity (% vol.)	Water retention (% vol.)		Capillary rise (cm)
			0.33b	6.25b	
Aa,p	0-30	57.59	48.14	23.39	14,9
AaGso	30-60	57.75	41.85	19.62	27,2
Gso	60-90	50.17	22.92	6.82	24,9

In this soil (Table 3) a somewhat greater participation of carbonates was noticeable in transitional AaGso horizon in relation to humus horizon, and Gso layer, and according to classification it was rendered very limy. Soil alkalinity rises with increasing soil depth, and on average it was considered of low alkalinity. The humus content was greatest (2.59%) in humus horizon Aa,p, and it steadily decreased with depth. According to average humus value this soil was low-humuous soil. According to Voltman's classification these soils were poor in total nitrogen, but were considered as insufficiently supplied with easily available potassium according to the criteria of AL methods.

Table 3. Chemical soil composition

Horizon	Depth (cm)	$CaCO_3$ %	pH in H_2O	Humus %	Total N %	P_2O_5 mg/100g	K_2O mg/100g
Aa,p	0-30	17.9	7.40	2.59	0.08	5.4	17.3
AaGso	30-60	23.3	7.80	1.21	0.04	2.8	8.2
Gso	60-90	15.4	7.82	0.96	0.02	2.9	4.0
Average	0-90	18.9	7.67	1.59	0.05	3.7	9.83

Absolute soil moisture at the depth of 0-60 cm was in accordance to fluctuation of underground water, and percentage of moisture in Aa,p horizon (Graph 1) ranged from 63.54% to 54,77% of total porosity, and absolute soil moisture ranged on average between Rvk 0.33b and Rvk 6.25b values. In AaGso layer (graph 2) absolute soil moisture ranged from 77.90 to 56.35 % of total porosity. It can be seen from the above mentioned that water was available to plants all the time, and that in AaGso horizon plants were surviving in the period from 30.6 to 10.7 due to which non-capillary pores were only partially filled with water. Increased values of absolute soil water in AaGso layer in relation to Aa,p horizon were the result of greater wetting of soil with underground water and capillary rise in that layer.

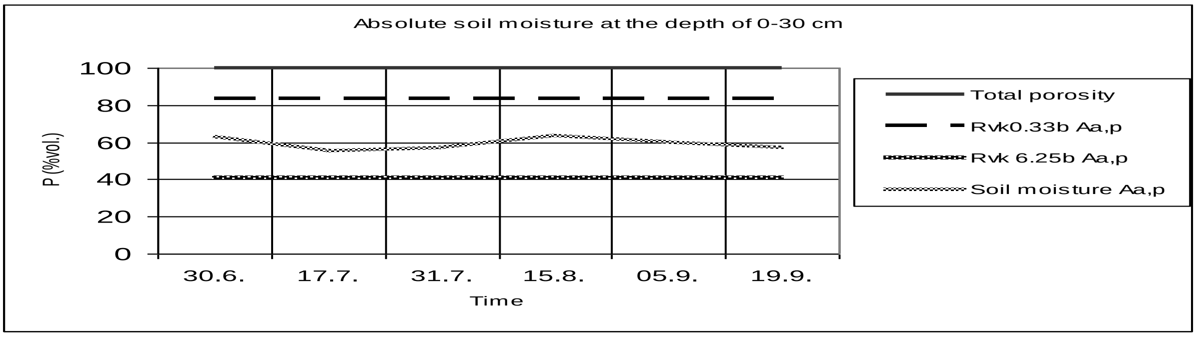

Figure 1. Dynamics of absolute soil water in Aa,p horizon

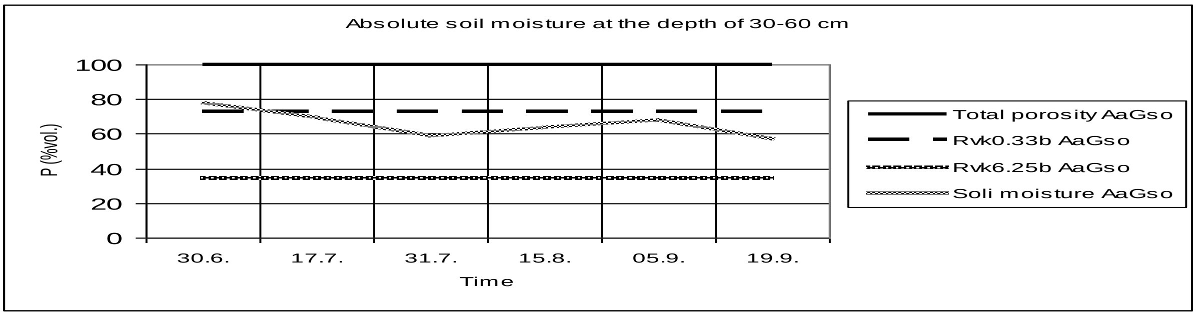

Figure 2. Dynamics of absolute soil water in AaGso horizon

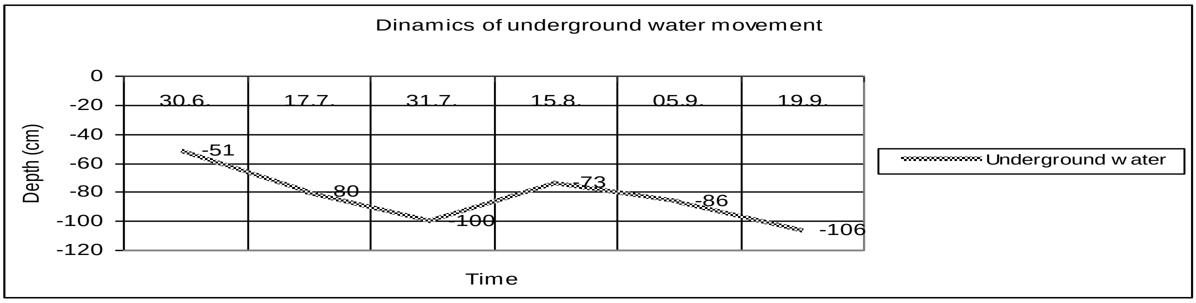

Figure 3. Dynamics of underground water movement

Depending on the way of water availability this soil was considered hypogleic (moisturized exclusively with underground water) according to classification of Škorić et *al.* (1985). These soils were more suitable for cultivation of willows than poplars due to over-moisturizing of this type of soil, and to heavier textural composition.

Characteristics of rooted cuttings

Rooted cuttings of studied black poplar clones (section *Aigeiros* Duby) had equal survival rate ranging from 63.7-69.4% at the end of vegetation period on marsh-gleic soil (Table 4). This survival rate can not be considered satisfactory from the aspect of successful production of black poplar planting material, and Kovačević et *al.,* (2008a, 2008b) confirmed the clear influence of differences among genotypes and soil properties on survival and growth of black poplar rooted cuttings.

It is well known that black poplar clones do not root easily (*P. deltoides* Bartr ex Marsh). Marković, et *al.*, (1981). The best survival of American black poplar rooted cuttings (450, 618, 457, 725 i 55/65) was achieved with supply of water in the soil, close to field water capacity (Živanov et *al.*, 1985). Depth of underground water (graph 3), as well as the content of absolute soil water in the rhizosphere zone supllied relatively favorable conditions for rooting and growth of the studied black poplar cuttings during vegetation period (graph 1, 2) only in the zone at 30-90cm, while the water in the surface zone was not easily available.

Table 4. Analysis of variance and the least significant difference at a risk level of 5%

Clone	**Measured elements**		
	Survival	**h**	**d_0**
	[%]	**[cm]**	**[mm]**
S_{1-3}	66.5 *a*	196.6 *a*	13.5 *a*
B-17	63.7 *a*	193.9 *a*	12.8 *ab*
260/81	65.8 *a*	168.0 *b*	12.5 *ab*
S_{6-7}	69.4 *a*	165.4 *b*	12.1 *b*
155/81	68.8 *a*	156.9 *b*	12.3 *ab*
F - ratio	0.18^{ns}	7.658^{**}	1.503^{ns}
p – value	0.9451	0.0014	0.2511

Andrašev et *al.*, (2003) mentioned significant influence of cuttings planting distance on survival rate of rooted cuttings of Euroamerican black poplar (I-214, Pannonia) and

American black poplar (S_{6-7}, PE 19/66, B-81, B-229) at dense planting distance, ranging from 50.000 do 150.000 cuttings per hectare, which was the case of the studied trial. Up to 30% lower survival rate was noticed in dense stand of 150.000 cuttings per hectare for all six clones on the average, and 15% lower survival rate was noticed at density of 100.000 in relation to 50.000 cuttings per hectare.

Applied planting dansity of over 100.000 cuttings per hectare was not appropriate for production of planting material, and was recomended for production of poplar reproductive material.

At the end of vegetation period significant differences in mean rooted cuttings heights were obtained, while equal neck diameters were obtained for studied clones (table 4). Test of least significant difference at the risk level of 5% divided two groups of clones according to mean heights: clones S_{1-3} and B-17 with 194-197 cm and clones S_{6-7}, 260/81 and 155/81 with 157-168 cm.

Mean heights of rooted cuttings depended on many factors such as: clone, soil, distance between planting, type of planting material, technological procedures at stand establishment, ferilization, irrigation, etc. (Marković & Rončević, 1986; Živanov et *al.*, 1985; Ivanišević, 1991; Rončević et *al.*, 2002; Andrašev et *al*, 2002, 2003, 2007).

Marković & Rončević (1986) mentioned that clone I-214 in two calendar year (1963 and 1964) achieved mean height of rooted cuttings of 185-196 cm at density of 83.333 cuttings per hectare, and at lower density of 33.333 of cuttings per hectare the mean heights of cuttings increased to 220 cm.

Andrašev et *al.*, (2003) mentioned that mean heights of rooted cuttings obtained on the same form of fluvisol soil at stand density of 102.500 cuttings per hectare, which corresponded to the given results, ranged from 166 cm (Pannonia) to 197 cm (PE 19/66). According to the same authors the mean height went from 222 cm (clone I-214) to 263 cm (clone PE 19/66) when density decresed to 50.000 cuttings per hectare. Orlović et *al.,* (2003) mentioned heights of 2.08m for clone S6-7 at the end of vegetation period on the soil with textural composition corresponding to loamy sand and sandy loam.

It can be seen from the previous investigations that mean heights of clones S_{1-3} and B-17 were obtained in accordance with the previous investigations. Applied planting density of cuttings proved to be the limiting factor for obtaining higher mean heights, and it was only realistic to assume that with decreased planting density from 25.000 to 30.000 cuttings/ha the higher mean heights of cuttings would be provided.

Different participation of poplar rooted cuttings over 2m in height was realized: 46-54% of cuttings over 2 m in height was obtained for clones S_{1-3} and B-17, which was in contrast to other clones with cutting participation ranging from 7-13% (graph 4).

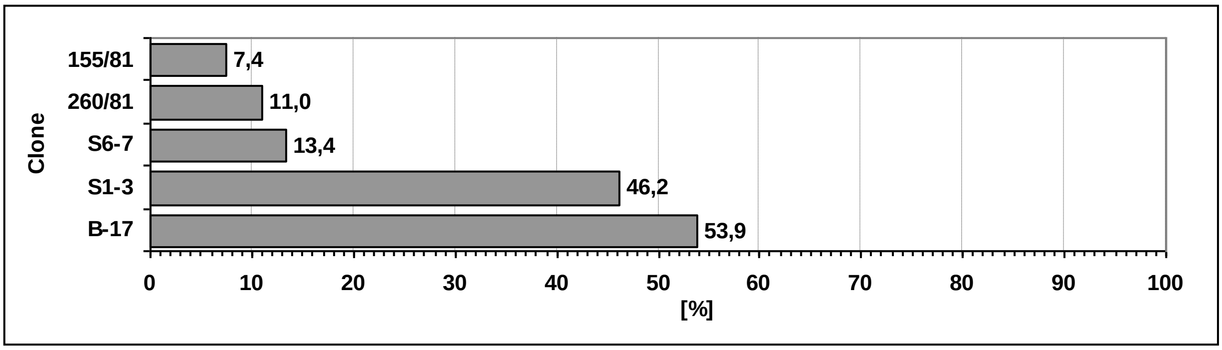

Figure 4. Percentage of rooted cuttings over 2 m in height

Andrašev et *al.*, (2003) mentioned that participation of cuttings over 2 m ranged from 35% (clone Pannonia) to 50% (clone B-229) on loamy fluvisol at densisty of 102.500 cuttings per hectare. At decreased density to 50.000 cuttings/ha participation of rooted cutting over 2 m went to 60-75% (Andrašev et *al.*, 2003).Participation of rooted cuttings over 2m ranged from 85-90% on sandy-loamy fluvisol at density of 25.600 cuttings per hectare (Andrašev et *al.*, 2002). Obntained results revealed the fact that great density of rooted cuttings was the limiting factor for cutting dimensions, which was in accordance with previous studies carried out on soils suitable for poplar nursary production.

Group of clones with greater mean heights, and favorable height structure of rooted cuttings (S_{1-3}, B-17) in studied dense planting distance, showed insignificant deviation from realized heights and height structures of rooted cuttings on soils suitable for poplar nursary production. Investigations relating to possibilities in production of poplar planting material on these soils should be continued in the future, with density from 25.000-30.000 cutings/ha, and even more so under different hydrological conditions during vegetation period - there is a real chance for these condition to be experienced on marsh-gleic soil.

CONCLUSION

Marsh-gleic soil, subtype hypogleic studied in this paper was of Aa,p - AaGso – Gso – Gr morphological structure. According to textural class this soil is of heavier mechanical composition in rhizosphere zone ranging from silty loam, loam to loamy sand. At 60 cm depth content of silt + clay fraction was increased.

Chemical properties of this soil pointed out to the fact that it was highly carbonative, slightly alkaline to alkaline, low in humus, and low in nutrient supply, i.e. poorly supplied with nitrogen and easily available phosphorous and potassium.

Absolute soil water movement in Aa,p and AaGso horizon was in accordance with underground water fluctuations. In horizon Aa,p water was hard to access, while in AaGso layer absolute soil water was between Rvk 0.33b and Rvk 6.25b limits, i.e. it was more accessible to plants.

Considering the above mentioned phisical and chemical soil properties and applied planting distances of 0.80 x 0.12m, or density of 104.167 rooted cuttings/ha, the percentage of plant surviving depended on clone and it ranged from 63.7-69.4%, i.e. all clones had pretty low surviving rate. It could be influenced by hardly accessible moving water, as well as evapotranspiration from the surface of the soil. Unfavorable traits of this soil accompanied by high planting density negatively influenced the survival rate, and the mean height of rooted cuttings.

Two groups of clones were separated according to mean heights: clones S_{1-3} and B-17 with 194-197 cm and clones S_{6-7}, 260/81 and 155/81 with157-168 cm. Group of clones with greater mean heights, and more favorable height structure of rooted cuttings (S_{1-3}, B-17) in studied dense stand insignificantly deviated from achieved heights and height structure of rooted cuttings on soils suitable for polar nursary production. This revealed different relation of individual varieties to soil hydrology regime.

Obtained mean heights and height structure of rooted cuttings can not be considered satisfactory from the aspect of nursery plant production, but in regard to the size of vegetation space per plant they can be considered satisfactory from the aspect of repromaterial production. However group of clones with favorable height structure (B-17 and S_{1-3}) pointed out to possibilities of production of satisfactory quantity of black poplar planting material even on marsh-gleic soil if greater planting distances, and application of other agrotechnical measures in nursary production are provided. These results also pointed out to the fact that irrigation (as a care measure) of these soils can be omitted, and in that way the costs of poplar nursary production can be decreased.

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THRIPS (Thysanoptera) ON POPLARS

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Abstract: *This paper presents the thrips community on poplar leaves. Three thrips species from suborder Terebrantia were recorded. These are Mycterothrips salicis (O.M. Reuter), Aeolothrips intermedius Bagnall and Frankliniella intonsa Trybom. The most abundant species was Mycterothrips salicis (O.M.Reuter). It is particularly important to note the complete lack of species Lispothrips crassipes Jablonowski, which is exclusive for poplars and recorded in literature as a pest on poplars in neighboring regions.*
During the health check of poplar Populus x euramericana cl. Pannonia seedlings at the nursery Crvenka II (N 44°51'58.67", E 20°24'15.22"), Forest Estate Beograd, Forest Unit Rit, we have recorded presence of thrips (Thysanoptera). The detailed analysis has shown presence of three species of thrips from suborder Terebrantia. These are Mycterothrips salicis (O.M. Reuter), Aeolothrips intermedius Bagnall and Frankliniella intonsa Trybom.
Key words: *poplars, Thrips (Thysanoptera)*

Figure 1. Location of polar nursery Crvenka II near Belgrade (Google Earth image)
44°51'51.77"N
20°24'19.37"E

The most abundant species was *Mycterothrips salicis* (O.M.Reuter 1879). This species used to be known as *Physothrips salicis* (O.M.Reuter 1879).

According to the description provided by Dyadechko (1977), head and thorax of the female are yellowish brown, abdomen dark brown, legs yellow, femora and tibiae gray in center, wings yellowish. Male has smaller proportions and lighter color.

This is a foliicolous species, inhabiting leaves and rarely flowers of woody plants, primarily *Populus* sp. and *Salix* sp., and sometimes also *Alnus* sp. and *Tilia* sp. (zur Strassen 2003).

This species hibernates beneath peeling bark, in scales of bark, various galls, plant litter, and turf (Dyadechko 1977). According to the available literature, feeding activities by thrips caused small necrotic spots on *P. alba* leaves (Haghighian F., Sadeghi S. E. 2003).

This species is widely distributed in Palearctic.

This is the second record of species *Mycterothrips salicis* in Serbia. The first record for our country was recorded in the paper by zur Strassen from 1984.

According to the official Check-list for countries of former Yugoslavia (Andjus 1997), it was also recorded in Croatia (zur Strassen 1984) and Macedonia (Jenser and Andjus 1984).

During this study we have also recorded presence of species *Aeolothrips intermedius* and *Frankliniella intonsa* but with smaller abundance.

The species *Aeolothrips intermedius* (family Aeolothripidae) is the commonest species of this genus and one of the commonest species of thrips in general. It usually appears in large populations. It shows a pronounced polyphagy, appearing on a large number of plant species, both in spontaneous flora and on cultivated plants. It is floricolous and foliicolous – inhabiting flowers and leaves of plants. As a zoophagous species it feeds on phytophagous thrips and aphids. It has a Palearctic distribution.

The species *Frankliniella intonsa* (family Thripidae) is a very common polyphagous thrips species on many plants where it feeds and breeds. It is floricolous – associated with flowers. According to some authors it may damage fruit trees, alfalfa and clover (Dyadechko 1977). It is widely distributed in Europe and also recorded in the Eastern Palearctic, in Middle East, the Nearctic and the Oriental Region.

It is very important to note that we have not recorded presence of the expected species *Lispothrips crassipes* Jablonowski, which is characteristic of poplar and recorded in literature as poplar pest in neighboring regions. In Italy, *Lispothrips crassipes* was reported in 1985 as a new pest of poplars (Lapietra G., Allegro G. 1985). First signs of dieback of poplar were observed in Croatia in 1986, and the cause was identified in 1989 – it was the thrips *Lispothrips crassipes* attack (Hrašovec B., Lovas O. 1996).

This species belongs to the suborder Tubulifera, family Phlaeothripidae.

Body color black, antennae and legs black (only fore tarsi light brown), wings absent.

This is an arboreal herbivore (Kettunen J.; Kobro S.; Martikainen P., 2005).

It lives under the peeling bark on aspen and poplar; females hibernate under peeling bark (Dyadechko 1977).

This is a ramicolous species of European distribution (zur Strassen 1984).

In the former Yugoslavia it was so far recorded only in Slovenia (Andjus 1997; Trdan et al 2003).

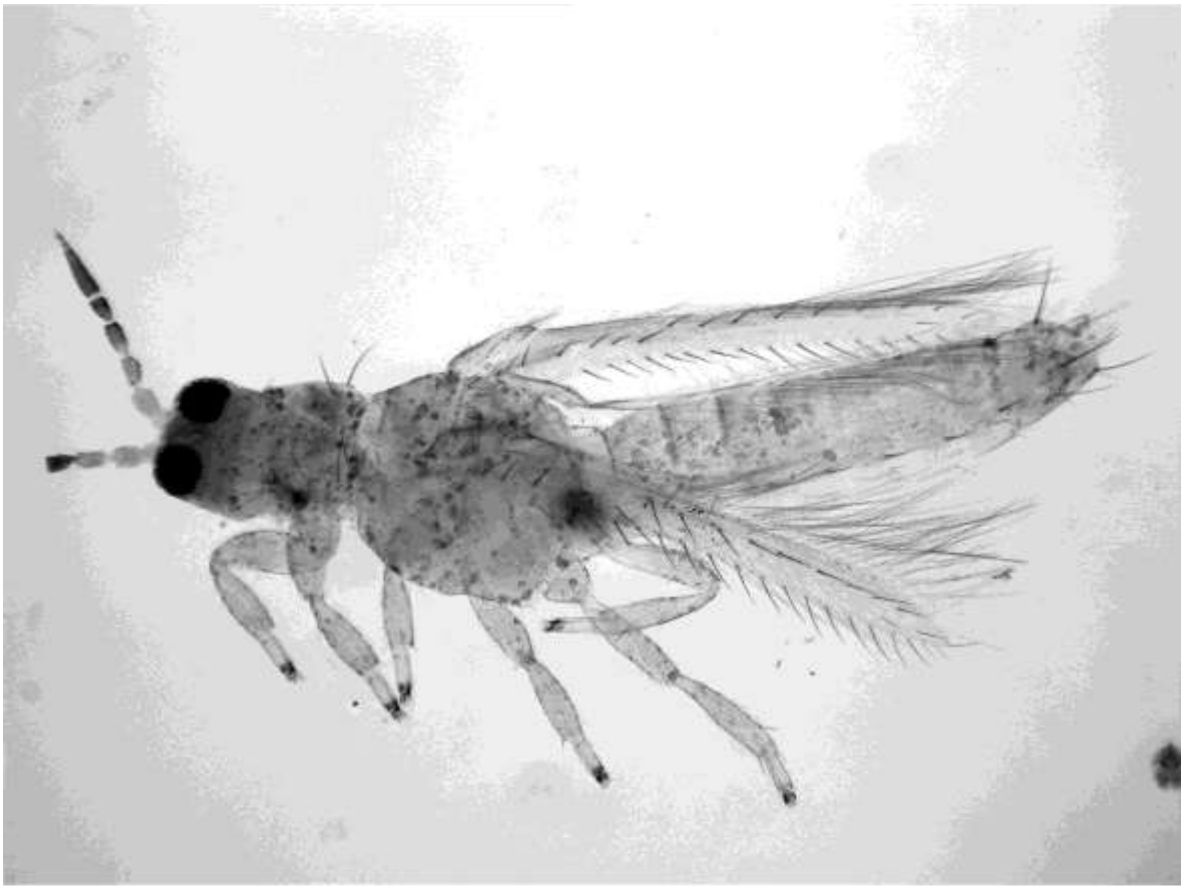

Photo 1. Male

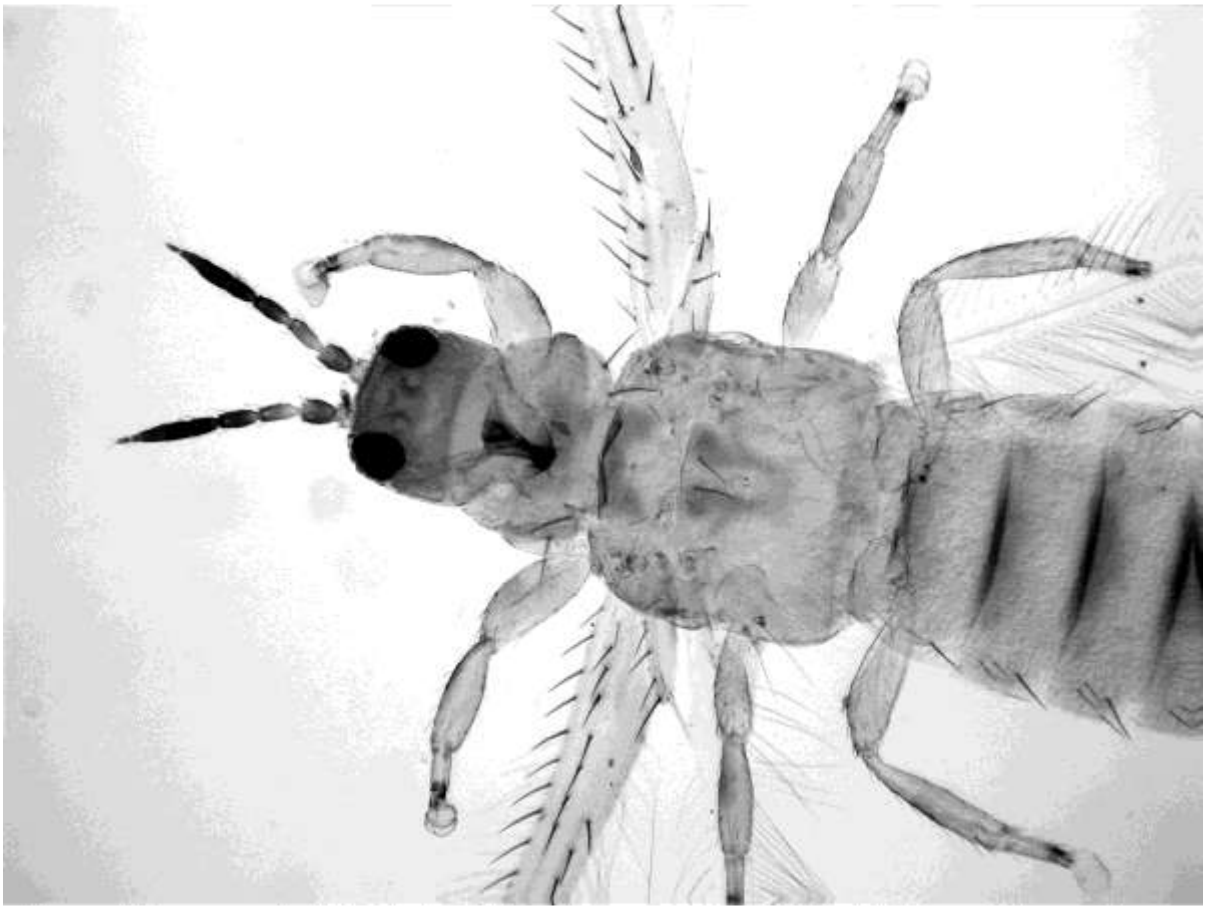

Photo 2. Female

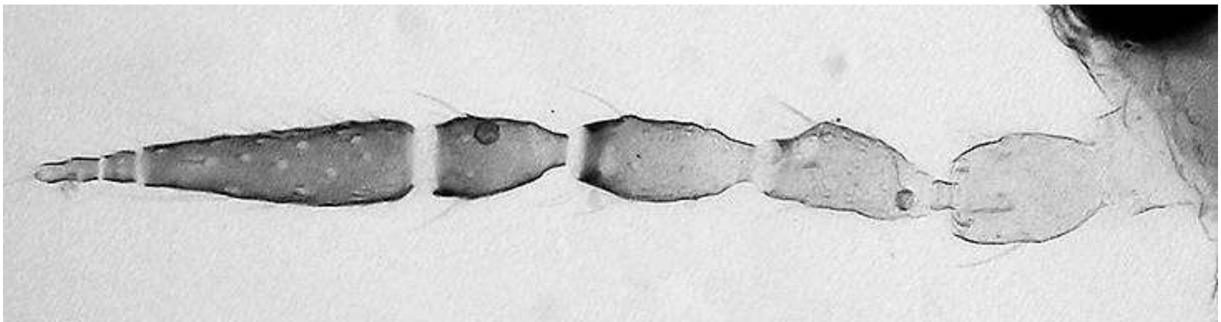

Photo 3. Male antenna

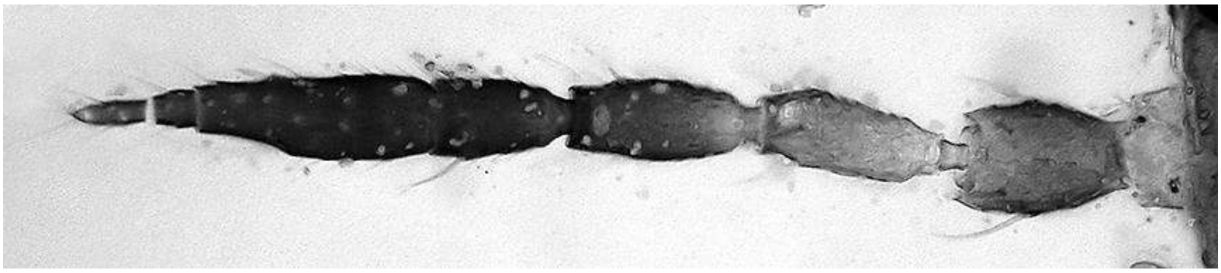

Photo 4. Female antenna

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CHARACTERISTICS OF BLACK POPLAR NATURAL HABITATS (Section *AIGEIROS* Duby) ON ALLUVIAL – HYGROPHILIC FORESTS IN VOJVODINA

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Abstract: *Results of several years of investigations of black poplar (Section Aigeiros) natural habitats on alluvial – hygrophilic forests in Vojvodina are published in this paper. These results revealed that black poplars naturally appear in ecological units on hydromorphic soils either as a dominant or accompanying tree species in the riparian zone of Danube, Tisa, Sava and Tamiš rivers, following wet and dry habitats, in ecological line: Rubeto–Salicetum albae → Salici-Populetum nigrae → Populo-Fraxinetum angustifoliae → Populetum nigrae → Populetum nigro-albae → Populetum albae → Populeto nigrae-Quercetum roboris → Populeto albae-Quercetum roboris. Number of members in ecological line of black poplar depends on the form of relief, composition of texture, i.e. flooding regime, and draining of physiologically active layer of soil (topographic-hydrological position).*

Results of this study revealed that ecological units, in which black poplars are dominant tree species, only young river terraces are naturally populated, where the following types of soils are formed:

- *fluvisol, morphological structure: (A) ili A - I - II - III - ... nG, i.e. A - I - II - Ab - ... nG,.*
- *humofluvisol, morphological structure: A - C - G,*
- *humogley , morphological structure: Aa - Gso - Gr i*
- *eugley, morphological structure: Aa - Gr.*

Growth and development of black poplars depend on two dominant factors of total habitat potential: edaphic (soil properties) and hydrological (flooding regime, and draining of physiologically active layer of soil – topographic-hydrological positions). Edaphic factor determines general growing model, while hydrological factor, besides influencing the general growing model, also causes fluctuations of annual increment.

Keywords: *black poplar, ecological units, habitat, soil*

INTRODUCTION

Contemporary utilization of selected cultivars of black poplars demands the knowledge of numerous bioecological factors on the relation: **cultivar → site → technology**. Poplars of the section *Aigeiros* (black poplars) are species dependent on hidrological conditions. They need large quantities of available water for their development (Ivanišević, 1993, Ivanišević, et al. 1997, 2003). For this demand, black poplars inhabit inundations of rivers (reparian zones, in narrow sense), were they can use, beside atmospheric precipitation, flooding water and ground water. Thus, natural habitats of black poplars are found in inundations of our rivers, forming with other hidrologically dependent species alluvial-hygrophilic forest complexes (Jenik, 1990, Jović, et al. 1991, Tomić, 2004, Ivanišević & Knežević, 2008). In inundations of rivers there could be found: young (recent) alluvial deposits, old deposits (lesoaluvium) and partially eolic deposits from pleistocen (loess), material (substrate) that was the basis for soil formation. The alluvial planes of the above rivers have significantly different terrain configuration, mineralogical and mechanical composition, hydrological regime and forms of natural plant communities - ecological units (Slavnić, 1952, Jovanović, 1965, Herpka, 1979, Madera et al. 2008).

Natural forests of black poplars have almost vanished or are presented just in fragments in the complex of aluvial – hygrophylic forests of Vojvodina nowadays.

On these habitats are now established stands with selected, highly-productive cultivars of poplars from section *Aigeiros* Duby.

MATERIAL AND METHOD

Data that is presented in this work is the result of long-term investigation of black poplar natural habitats on alluvial-hygrophilic forests in Vojvodina. Many pedological profiles were examined in every ecological unit (phytocenoses) of black poplars. Several characters were analyzed. From typical profiles (representatives) samples for laboratory analysis were taken.

Following parameters of soil properties were analyzed: humus content, content of the fraction of silt+clay and potential water supply in physiologically active profile.

Quantities of humus, silt+clay (t/ha) were calculated by the formula Q = 100 x H x D x X, were X is the content of silt+clay or humus content (%), H is soil layer thickness (m) and D is bulk density.

Potential supply of growth water (PGW, mm/m^2) were calculated by the formula PGW = 100 x H x X, where H is soil layer thickness (m) and X is the content of growth water (%,vol).

In morphological structure of soil A stands for humus-accumulative horizon, I, II, III are layers, C for substrate, G_{so} gley horizon of secondary oxidation and G_r for gley horizon of reduction.

RESULTS AND DISCUSSION

Characteristics of alluvial plane

The dominant process of floodplain genesis is the process of fluvial sedimentation which is functionally related to transitional power of a river. Depending on a transitional power of a river, three genetic parts of floodplains can be distinguished: along the bank, central and terrace (Viljams, 1950, Michal 1994), (Figure 1). Each has its evolution - genetic series of soil, specific hydrological regime and characteristic forms of plant communities (Herpka, 1979, Dister, 1990, Ivanišević, 1993, Ivanišević, et al. 1997, Letić, et al. 2006). According to these parameters, from a production-ecological aspect, floodplains are classified into several topographic-hydrological positions (Herpka, 1979).

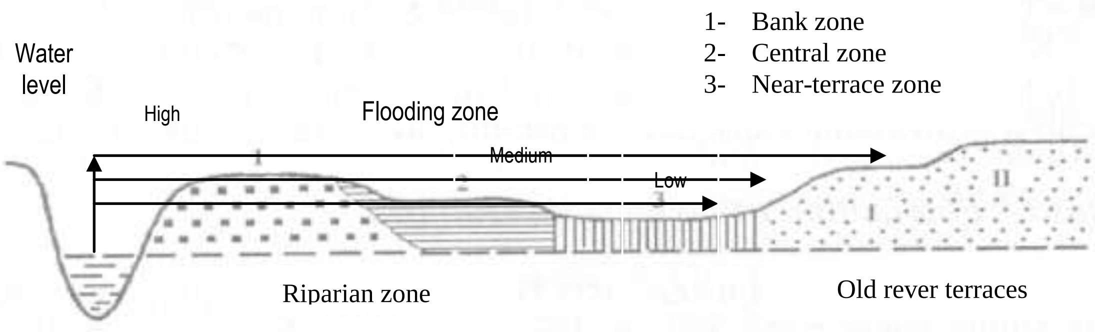
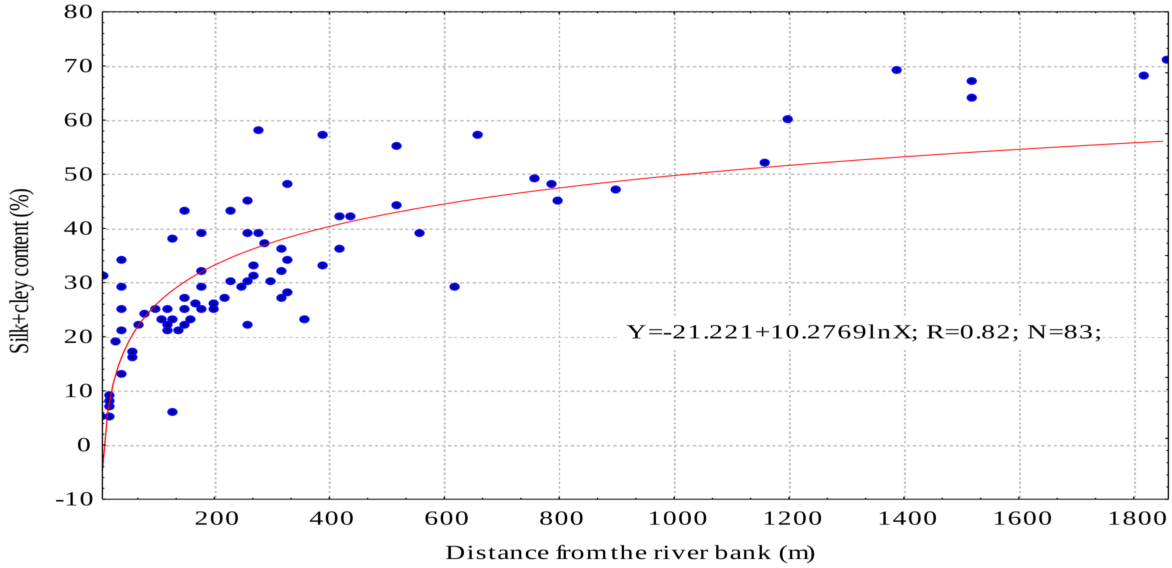


Figure 1. Cross-section of alluvial plain

On Graph 1 we can see that sedimentation of silt+clay fraction in river floodplain has a shape of a logarithmic curve (Ivanišević, 1993). Applied function indicates that the silt+clay fraction has the largest variability in the first 400 m from the river bank, which is related to the changes of the transitional power.

All indicators of soil fertility in reparian zone depend on the content of silt+clay fraction (Živanov, 1980, Ivanišević, 1993).



Graphic 1. Dust-clay fraction sedimentation on a cross-section of river Danube plain (data from Ivanišević, 1993).

Distribution model for forest phytocenoses on cross-section of alluvial plain

Long-term investigations of black poplar forests (Section *Aigeiros*) revealed that black poplars naturally appear in ecological units, either as a dominant or accompanying tree species, on hydromorphic soils in the riparian zone of the Danube, Tisza, Sava and Tamis rivers, following wet and dry habitats, in ecological line: *Rubeto–Salicetum albae* → *Salici-Populetum nigrae* → *Populo-Fraxinetum angustifoliae* → *Populetum nigrae* → *Populetum nigro-albae* → *Populetum albae* → *Populeto nigrae-Quercetum roboris* → *Populeto albae-Quercetum roboris* (Figure 2).

In the costal part of the riparian zone of our rivers, arrangement of ecological units populated with black poplars, most often make the ecological line: *Rubeto–Salicetum albae* → *Salici-Populetum nigrae* → *Populetum nigrae* → *Populetum nigro-albae* → *Populetum albae*, on the soils in the line of eugley → fluvisol (Figure 2).

In the central genetical part of the riparian zone of our rivers, ecological line of black poplar is made (due to small variability of soil traits and hydrological regime) of members with the following ecological units: *Salici-Populetum nigrae* → *Populetum nigrae* → *Populetum nigro-albae* → *Populeto nigrae-Quercetum roboris* → *Populeto albae-Quercetum roboris* → *Populetum albae*, on the soils in the line of eugley → humogley → humofluvisol.

At the contact of central and pre-terrace part of the riparian zone black poplars are often encountered in ecological units belonging to the line: *Fraxinetum angustifoliae* → *Fraxino-Quercetum roboris* → *Salici-Populetum nigrae* → *Populo-Fraxinetum angustifoliae* → *Populeto nigrae-Quercetum roboris* → *Populeto albae-Quercetum roboris*, on the soils in the line of eugley → humogley → humofluvisol.

On the pre-terrace area of the riparian zone of our rivers, due to increased sufficit wetting, black poplars form mixed forests which line is made of the following ecological units: *Salici-Populetum nigrae* → *Populo-Fraxinetum angustifoliae* → *Fraxino-Quercetum roboris*, on the soils in the line of eugley → humogley.

On the old river terraces where black poplars are mixed tree species, the line is made of the following ecological units: *Fraxinetum angustifoliae* → *Fraxino-Quercetum roboris* → *Populeto nigrae-Quercetum roboris* → *Populeto albae-Quercetum roboris* → *Carpino-Fraxino-Quercetum roboris*, on the soils in the line of eugley → humogley → humofluvisol → cambisol → chernozem.

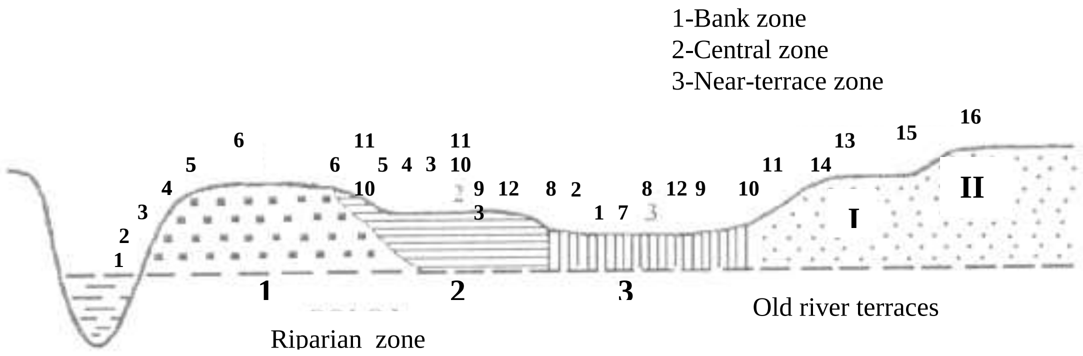


Figure 2. Distribution model for forest phytocenoses on cross-section of alluvial plain

Legend:

1. Salicetum albae Issl. 1926
2.Rubeto-Salicetum albae Soo 1940
3.Salici-Populetum nigrae Tüx. 1931
4. Populetum nigrae Knapp. 1948
5. Populetum nigro-albae Slav. 1952
6.Populetum albae Knapp. 1952
7. Salicetum cinereae Zol. 1931
8. Fraxinetum angustifoliae Glav. 1959
9. Populo-Fraxinetum angustifoliae Herpka 1965
10. Populeto nigrae-Quercetum roboris Jov et. Tom. 1979
11. Populeto albae-Quercetum roboris Jov. et Tom. 1979
12. Fraxino-Quercetum roboris Jov. 1951
13. Genisto elatae-Quercetum roboris Horv. 1938
14.Carpino-Fraxino-Quercetum roboris Miš. et Broz 1962
15. Carpino-Quercetum roboris Anić 1959
16. Carpino-Quercetum robori-cerris Jov. 1967

Characteristics of soils on riparian zone

Ecological units, in which black poplars are dominant tree species, only young river terraces are naturally populated, where the following types of soils are formed (Figure 3):

- fluvisol, morphological structure: (A) ili A-I-II-III- ... nG, i.e. A-I-II-Ab- ... nG,.
- humofluvisol, morphological structure: A-C-G,
- humogley , morphological structure: Aa-Gso-Gr i
- eugley, morphological structure: Aa-Gr.

In the riparian zone and inundation parts of rivers the most often flooded areas, in ecological units populated with black poplars, the most significant property (trait) is the content of dust-clay fraction (particle < 0.02 mm) in physiologically active layer of soil (Živanov, 1980, Ivanišević, et al. 1997). This trait is the most significant component of the **total habitat potential** of black poplar, which is marked as **edaphic potential of habitat**, on which all fertility parameters depend functionally.

The second component of total black poplar habitat potential is the prevailing hydrological regime of our rivers (Letić, et al. 2006), the variable value marked as **hydrological potential of habitat**. According to habitat hydrological potential the soils are differentiated to different flooding regime and draining of physiologically active layer of soil (topographic-hydrological position) from one side, to different categories of water supply accessible physiologically active (useful)) during vegetation period from another side.

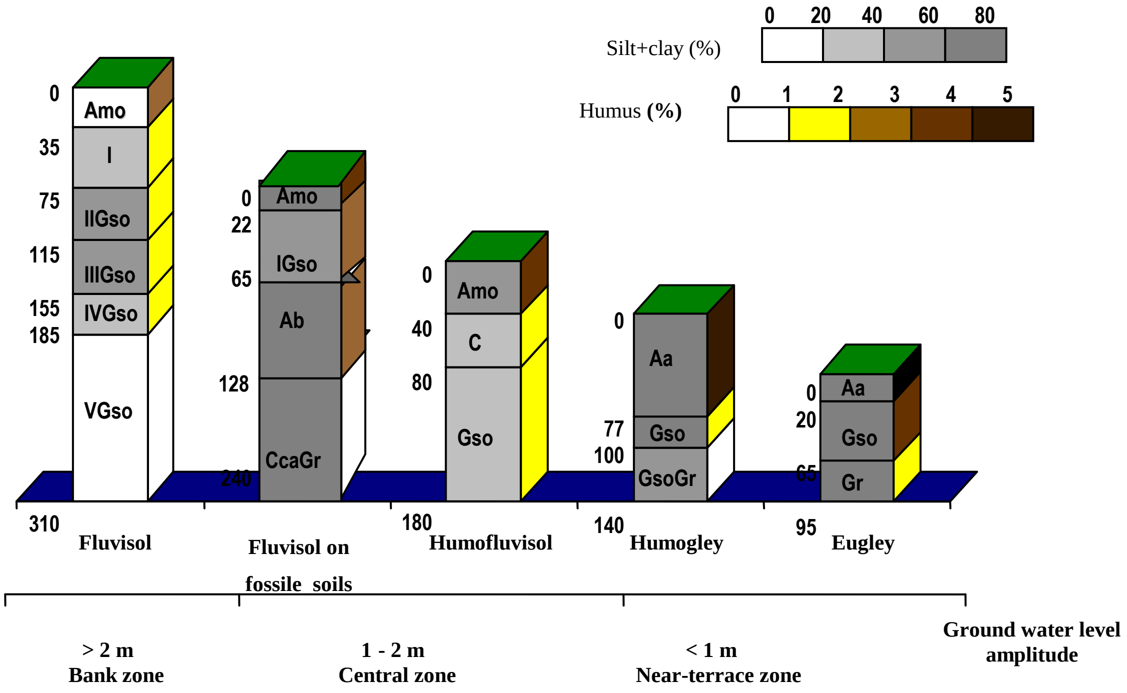
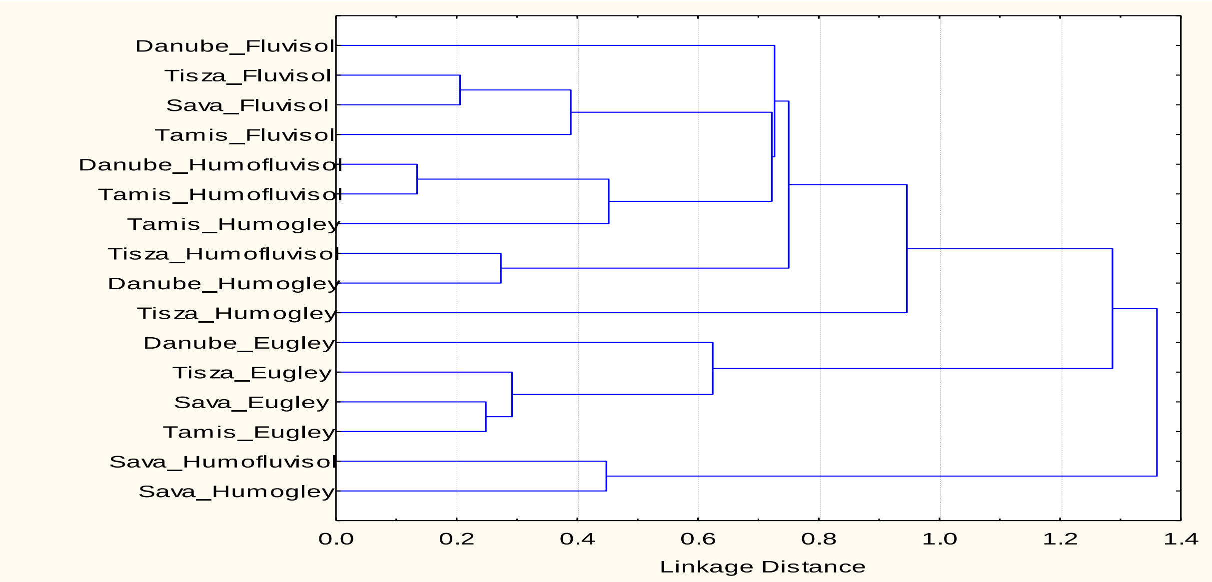


Figure 3. Soil distribution in riparian zone

Table 1. Analytical parameters of fertility indicators

River	Type of soils			
	Fluvisol	**Humofluvisol**	**Humogley**	**Eugley**
Supply of humus (t/ha)				
Danube	50-635 (235)	280-502 (326)	320-450 (401)	64-250 (130)
Tisza	142-385 (362)	323-479 (421)	398-536 (511)	94-198 (156)
Sava	180-412 (347)	350-635 (587)	427-650 (602)	120-211 (172)
Tamis	96-450 (334)	259-386 (321)	324-458 (378)	76-230 (188)
Supply of silt+clay (t/ha)				
Danube	900-18162 (5880)	7950-13450 (9210)	10540-15600 (12478)	1896-2232 (2087)
Tisza	3850-8447 (6458)	9542-15650 (12450)	11245-18552 (14589)	2437-2822 (3621)
Sava	2360-7125 (5836)	7890-11242 (9885)	9956-14895 (10223)	2165-4128 (3127)
Tamis	1240-16832 (5352)	6918-9120 (8730)	7902-11260 (9845)	2152-3012 (2804)
Potential supply of growth water (PGW, mm/m²)				
Danube	56-680 (271)	270-450 (326)	311-432 (362)	40-130 (89)
Tisza	156-386 (302)	298-412 (385)	342-514 (423)	125-226 (174)
Sava	189-423 (297)	285-412 (362)	326-487 (404)	135-214 (151)
Tamis	78-530 (262)	215-420 (326)	297-414 (345)	94-188 (131)
Category of water supply	56-680 very poor to very good	215-450 medium to very good	297-514 good to very good	40-226 very poor to medium

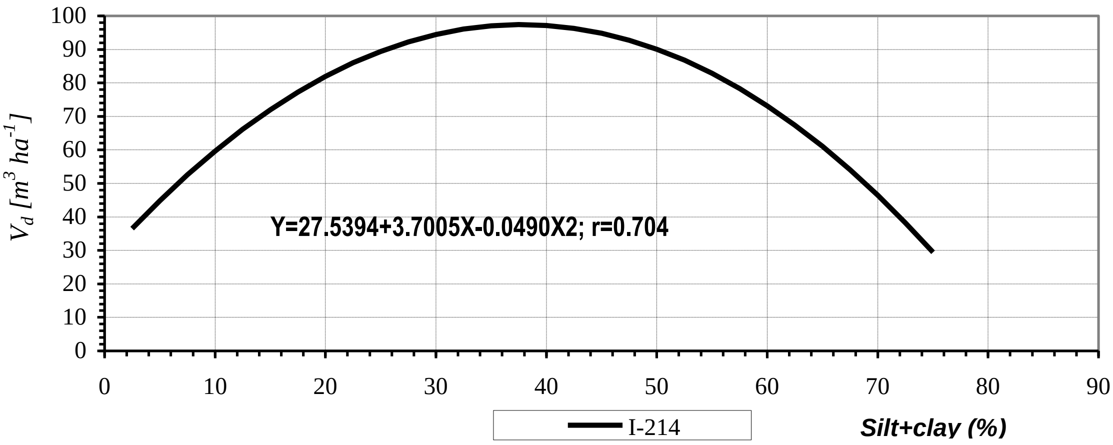
On Graph 1 the relationship among examined soil types in riparian zones of rivers in Vojvodina is presented, based on the most important fertility indicators:



Graphic 1. Cluster analysis for soil types and reparian zones of Vojvodina based on distribution of humus, silt+clay and available water content

This cluster analysis shows grouping of soil types according to examined characteristics. Fluvisol soils did not defer among reparian zones of examined rivers, in spite considerable variability in fertility indicators. Also, other types of soil: humofluvisol, humogley and eugley did not defer among reparian zones of examined rivers, except in the reparian zone of river Sava. That deviation is caused by the way of sedimentation and the age of the substrate.

As the autochthonous forests of black poplars disappeared, the stands of selected clones of black poplars could be found on that site nowadays. The influence of the average silt+clay content in the profile of reparian zone, on the growth of the most abundant clone (I-214) in the age of seven (the average time needed for the development of maximal assimilation area) is presented on the graph 2.



Graphic 2. Dependance of wood volume on the content of the silt+clay fraction for the clone I-214 after seven years of growth (data from Živanov, 1980)

This graph shows that the highest yield of wood volume on the soils with 40% of average content of silt+clay fraction in the physiologically active profile. Those are humofluvisol soils and deep and very deep varieties or sandy loam of loam forms of fluvisol soil type.

CONCLUSION

According to obtained results of multiannual research, phytocenoses of black poplars belongs to the complex of aluvial-higrophilic forests of Vojvodina.

These results revealed that black poplars naturally appear in ecological units on hydromorphic soils either as a dominant or accompanying tree species in the riparian zone of Danube, Tisa, Sava and Tamiš rivers, following wet and dry habitats, in ecological line: *Rubeto–Salicetum albae → Salici-Populetum nigrae → Populo-Fraxinetum angustifoliae → Populetum nigrae → Populetum nigro-albae → Populetum albae → Populeto nigrae-Quercetum roboris → Populeto albae-Quercetum roboris*. Number of members in ecological line of black poplar depends on the form of relief, composition of texture, i.e. flooding regime, and draining of physiologically active layer of soil (topographic-hydrological position).

Results of this study revealed that ecological units, in which black poplars are dominant tree species, only young river terraces are naturally populated, where the following types of soils are formed:

- fluvisol, morphological structure: (A) ili A - I - II - III - … nG, i.e. A - I - II - Ab - … nG,.
- humofluvisol, morphological structure: A - C - G,
- humogley , morphological structure: Aa - Gso - Gr i
- eugley, morphological structure: Aa - Gr.

Growth and development of black poplars depend on two dominant factors of total habitat potential: edaphic (soil properties) and hydrological (flooding regime, and draining of physiologically active layer of soil – topographic-hydrological positions).

Edaphic factor determines general growing model, while hydrological factor, besides influencing the general growing model, also causes fluctuations of annual increment.

It can be generalized that the production potential of the natural habitats depends on the nature of the relief, soil mechanical composition and naturally prevailing water-air regime.

In the poplar plantation establishment, the choice of the cultivar and planting technology depends on the soil properties, flooding regime, and draining of physiologically active layer of soil – topographic-hydrological positions) and ground water level.

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TECHNOLOGY OF INTENDED PRODUCTION OF POPLAR AND WILLOW BIOMASS FOR ENERGETIC PURPOSES

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Abstract: *Intended production of poplar and willow trees for energetic purposes should be considered as a new form of tree production which still contains number of unknown and unresolved parameters.*
Cross section results obtained on the basis of multiannual research work and the experience gained in experimental intended stands, i.e. one point of view relating to total technology of intended poplar and willow production for energetic purposes are presented in this paper.
The fact that smaller tree dimensions are used for biomass production presents the basic factor determining elements of technology of intended tree production for energetic purposes.
Optimal usage of genetic potential of poplar and willow in biomass production is conditioned by choice of clone, and determination of optimal stand density under certain habitat conditions.
Biomass production can be organized in stands of different densities ranging from 1.000 to 80.000 trees per hectare. Duration of production cycle can range from 1 to 10 years depending on density.
As starting material for intended stand formation the following were used: cuttings 0.2 m in length; cuttings 0.5m in length; root 0/1; nursery plant 1/0; nursery plants 2/0; nursery plants 1/1; nursery plants 1/2.
Production of biomass for energy purposes was performed in several cycles, and stand renovation was performed vegetatively by using regeneration force from tree stumps. In this type of stands it was possible to produce up to 70m^3 of biomass annually in appropriate habitat.
Key words: *poplar, intended stand, biomass*

INTRODUCTION

Intended production of poplar tree in short rotation periods presents the most intensive form of forest tree cultivation, with the sole purpose to produce the greatest possible amounts of usable biomass in the shortest period of time. This form of soil usage assumes that the parameters important for this production are known, checked, and scientifically synchronized, and that great investments in this type of ventures are accompanied by an average risk of this intensive plant production.

Maximum usage of genetic potential of fast poplar growing requires proper choice of variety/clone, optimal stand density (proper distance between the trees) and application of appropriate procedures for establishment and care of stands in suitable habitats for poplar cultivation.

In scientific circles this problem has attracted the attention of researchers, which has been confirmed by numerous papers: Herpka, (1982); Marković, (1985); Rončević et al. (1998). Poplars and willows, as the most productive forest trees in our climate present the greatest potential for short-rotations tree production. Choice of clones with favorable characteristics for cultivation in very dense short-rotation stands could open up possibilities for production of great amounts of biomass (by chopping whole trees together with branches, bark, and even roots at the end of the last production cycle), and no production of large dimension trees is necessary, for this technology uses chopped tree.

Intended tree production can be organized in stands of different densities with or without spacing. If the aim is to produce biomass for energetic purposes the stands are denser, and no spacing is needed. In this type of stand the length of production cycle is determined by stand density and it ranges mostly from one to ten years.

There are many problems remain to be solved in the process of poplar tree production technology. Realization of the mentioned goals depends on the solution of the above-mentioned problems, which have special influence on investments costs regarding establishment, cultivation and protection of stand. These questions are related to habitat choice, soil preparation, choice of planting material production technology, choice of planting method, choice of renovation method, stand care and protection measures.

The clone of euroamerican poplar (*Populus x euramericana*) I-214 was the most often used clone in the 60s of the last century, in the time when these studies began in former Yugoslavia, so the initial studies were conducted using this clone.

Massive introduction of American black poplar clones started in the 70s (*Populus deltoides*), so it was only natural for these clones to be included in further studies.

Production of maximum amounts of poplar biomass under optimal investment terms for certain form of stand cultivation requires well thought out, well synchronized and continuous research team work of great number of different specialists.

This paper presents a part of the results pertaining to investigation of biomass production, which are the part of investigation program for solution of biological-technological problems regarding the intended tree production, which has been realized during last 40 years of the Institute for lowland forestry and environment.

MATERIALS AND METHODS

Studies relating to problems of biomass production in intended stands were carried out in a series of multi-factorial trial stands.

Stand densities ranged from 400 to 80.000 plants/ha with different combinations of planting distances.

Data consisted of basic elements giving insight into amounts and characteristics of produced wooden mass with the aim to recognize the types of stand for intended biomass production, and duration of production cycle (rotation period).

The results present only a part of detailed analysis of each trial stand, which cannot be given as a whole in this paper due to its scope.

Trial series

The series of trial stands was formed with the aim to investigate the possibilities regarding poplar biomass production in one-year, two-year, three-year, four-year, and five-year rotation periods in stands formed depending on density, cuttings, roots and nursery plants, and the stand renovation was done using the regeneration force of tree stump. The whole series of trial stands was established using *Populus x euramericana* I-214 clone with planting distances ranging from 1,0 x 0,25 m, and 40.000 trees/ha to 2,0 x 2,0 m, i.e. 2.500 trees/ha. All trial stands from this series were established on fluvisol and humofluvisol soils of Central Danube Basin (Živanov, Ivanišević, 1986).

Trial No. 2

The trial was established on the trail estate of the Institute by placing the roots (0/1) of *Populus x euramericana* I-214 clone into 0.3 m deep holes. The soil was fluvisol with alternating thicker and thinner layers of loam and loamy sand. Underground water was at the depth of 2-3 m. Regular care and protection measures were undertaken. Trial treatments consisted of 4 planting distances with densities ranging from 6.944 to 13.888 roots per hectare. Clear-felling was done at the end of the third year of trial stand development. The stand was renovated using regenerating force of the tree stumps.

Trial No. 3

The trial was established in Central Danube basin on alluvial soil type protected from flooding. The soil was fluvisol loamy form (Škorić, et al., 1985).

The trial was set up in three replications and included eight poplar clones: four clones of American black poplar *Populus deltoides* (618, 457, 55/65 and 450), and four Euramericana poplar clones - *Populus x euramericana* (Ostia, 45/51, I-214 and 543).

The trial was planted with four types of planting material: a) nursery plants 2/0; nursery plants 1/1; nursery plants 1/0 produced in multiannual mother stand, and nursery plants 1/0 produced in rooting nursery and pruned down to ground level after planting. Nursery plants 1/1 were planted into 80 cm deep holes using “normal” planting method, and nursery plants 1/0 and 2/0 using “deep” planting at 2.8 m. Planting was done at the distance of 2,82 x 2,82 m, with 1257 trees/ha.

Production process consisted of two production cycles of 9 years in length and lasted for 18 years.

Stand was renovated after first production cycle using regenerating force of tree stumps. After first year of the second cycle number of shoots was reduced, and two best developed shoots were left on each surviving tree stump.

Trial No. 4

The trial was established in Central Danube basin on fluvisol sandy form protected from flooding. Trial treatments were: I planting distance a) 1,80x1,00 m – density 5.555 roots/ha ; b) 1,80x1,50 m – density 3.704 roots/ha, and II American black poplar clones – *Populus deltoides* (cl.457 and 450). As planting material for establishing a trial stand the roots (0/1) planted at depth of 30 cm were used. During the trial stand development an inter-row cultivation and loosening of the soil around the nursery plants were done on the regular basis. At the end of the fourth vegetation period detailed measurements and analysis of stand development were done. As provided by the trial plan the observation of trial stand development was continued till the end of the eight vegetation period. On this occasion we will present the research results regarding biomass production over a four-year and eight-year production cycles.

RESULTS AND DISCUSSION

Poplars and willows are extremely fast growing species in the juvenile development phase, and have the possibility of regrowth that may easily occur from the stump shoots, which make them the most suitable tree species for short-rotation biomass production intended for energy production in our climate.

Possibility for production of great amounts of biomass (by chopping whole trees together with branches, bark, and even tree roots at the end of production cycle) suitable for production of different energy forms (heating – direct combustion, combined heating and electrical, ethanol as biofuels etc.) can occur by solving biological-technological problems, proper choice of clones with favorable characteristics for growing in very dense short-rotation stands.

Usage of biomass as renewable resource which can be produced in very short production cycles, with no emission of sulphur or nitrogen as in classical energy sources (which is of great significance for environmental protection) can be of great advantage in obtaining this form of energy.

The possibility of biomass production in one-year, two-year, three-year, four-year, and five-year rotations in stands established with poplar cuttings, roots and nursery plants, the renovation of which was done using emergence power of tree stumps after stand felling, was studied in the series of stands established with *Populus x euramericana* cl. I-214, which was the bearer of our poplar production in that time.

In this way from 327m^3/ha to 485 m^3/ha or annual average from 41,0 to 53,9 m^3/ha. (Table 1) was produced in the production process lasting from 8 to 10 years, with two to nine rotations lasting from one to five years.

Respecting the fact that the greatest production was achieved in trial stands with annual rotation in production process of nine years and having in mind that establishment and care of stand, tree felling, manipulation and preparation for usage was far simpler and cheaper in relation to biomass from other production forms, it can be concluded that this form of poplar stand was the most profitable for production of biomass intended for energy production (Marković, Rončević and Pudar 1996).

In trial No. 2 (Table 2) small planting distances i.e. great densities ranging from 6.944 to 13.888 trees/ha were treated. Planting distances were basically adapted to production of nursery plants 2/3, and prolonged cultivation enabled testing of possible production of small wood dimensions (Rončević, Andrašev and Marković). During cultivation period of three years tree diameters ranged from 5 cm (13.888 trees/ha) to 7 cm (6.944 to 9.260 trees/ha). Heights ranged from 9-11 cm in third year of stand development (Table 2).
Total produced biomass ranged from 140 do 166 m^3/ha at the end of the third year. Average annual production in this type of stand ranged from 47 do 52 m^3/ha. Yield of absolute dry mass produced in this way ranged from 49 do 54,5 t/ha. This type of stand supplied significant amounts of small dimensions biomass in production cycle of three years.

Marković et al. (1986) mentioned that greater susceptibility of clone I-214 to bark (*Dothichiza populea*) and leaf disease (*Melampsora* sp. and *Marssonina brunnea*) caused significant decline of its productivity, and when mentioned diseases prevailed possibility of stand establishment with this clone was out of the question. Work on development and introduction of new poplar clones into production was intensified due to above mentioned reason. A network of comparative stand trials with the newest selections was established. Results of these investigations have contributed to acknowledgment of the first series of American black poplar (*Populus deltoides*), which was approved in 1980, and the second one was approved in 1987.

Table 1 - Biomass production of the Clone I-214 depending on density, rotation length, and duration of production cycle.

Planting distance	Taxation parameters	Production rotation									Total
		I	II	III	IV	V	VI	VII	VIII	IX	M^3/ha
1,0x0,25 Rotation 1st year	N–piece/ha d_s - cm h_s - m V/rotation–m^3	40.000 2,0 3,0 15,0	80.000 2,5 3,5 50,0	100.000 2,8 3,5 80,0	100.000 2,8 3,5 80,0	100.000 2,8 3,5 80,0	80.000 2,8 3,5 60,0	60.000 2,8 3,5 50,0	50.000 2,8 3,5 40,0	40.000 2,8 3,5 30,0	I_{VP}- 53,9 485,00
1,2x0,50 Rotation 2nd year	N–piece/ha d_s - cm h_s - m V/rotation.–m^3	16.667 4,2 5,8 48,0	48.000 4,0 5,0 114,0	48.000 4,0 5,0 114,0	18.000 4,3 5,9 51,0						I_{VP}- 41,0 327,00
1,8x0,80 Rotation 3rd year	N–piece/ha d_s - cm h_s - m V/rotation–m^3	6.944 7,4 11,0 156,0	13.400 6,2 9,6 167,0	6.500 7,0 9,0 116,0							I_{VP}- 49,0 439,00
2,0x2,0 Rotation 4th year	N–piece/ha d_s - cm h_s - m V/rotation–m^3	2.500 12,8 14,4 192,0	4.850 8,6 11,7 189,0								I_{VP}- 47,6 381,00
1,7x1,40 Rotation 5th year	N–piece/ha d_s - cm h_s - m V/rotation–m^3	4.170 10,3 14,0 236,0	7.506 8,7 12,3 246,0								I_{VP}- 48,2 482,00

Table 2 – Influence of stand density on biomass production of clone I – 214 in a three-year cycle

Planting distance	Number of nursery plants	Taxation parameters				
		N	d_s	h_s	V	I_{vp}
M	trees/ha	trees/ha	cm	M	m^3/ha	m^3/ha
1,8x0,4	13.888	13.888	5	9	156	52
1,8x0,6	9.260	9.231	7	10	140	47
1,2x1,0	8.333	8.312	7	10	140	47
1,8x0,8	6.944	6.938	7	11	150	50

Herpka (1985), Živanov and Ivanišević (1985), Rončević and Belčić (1987), Herpka and Guzina (1987) pointed out to developmental characteristics of stands established with new clones (clones from I and II series) aged from 5 to 10 years. All authors mentioned significant advantages of American black poplar clones over clone I-214 due to its susceptibility to pathogens. Authors mentioned that fast growing trait, especially in clones from the II approved series, was expressed in the first developmental years "juvenile phase", and in clone I-214 in later developmental stage so called "adult phase". This conclusion was of the utmost importance regarding the choice of clones used for biomass production for energetic purposes in short rotations.

For trial No. 3 (Table 3) results regarding biomass production during eight-year developmental period of trail stand in two rotations lasting nine years were shown.

Diameters and heights ranged from 12,8 to 18,8 cm, i.e. 17,0 to 21,9 m in most productive clones at the end of the first cycle. Number of survived nursery plants at the end of the first rotation per clones ranged from 41-94%, i.e. 526 to 1194 per hectare, from 1275 planted trees per hectare. These data have revealed that the choice of clones was of special importance for dense poplar stand establishment. Diameters at breast height ranged from 17,9 to 18,9 cm in *P. Deltoides* clones (618, 55/65, 457 and 450), while in clones 543 and I-214 (most often used for stand establishment up to now) breast diameters were below 15 cm. Great differentiation among clones regarding survival of planted nursery plants was revealed. It is important to mention that clones with greater tree dimensions also had the greatest percentage of nursery plants survival ranging from 86 to 94%. On the other hand, mentioned clones with smallest tree dimensions had significantly smaller percentage of survived nursery plants ranging from 400 to 80%. Compared values of current and average wood mass growth in 9 years old stand revealed that current growth was significantly higher than the average growth. This fact led to a conclusion that clear-felling nine years after stand trial development was premature, for in most productive clones, maximum yield could be expected between 11 and 13 years old trial stand. The most productive clones under these developmental conditions provide annual production of 30 to 35 m^3/ha of wood mass, which according to its characteristic satisfy production of biomass for energetic purposes. Yield of clones from *P. Deltoides* group ranged from 120 to 165 t/ha per area unit, expressed as absolute dry mass. Results regarding investigation of possibilities of poplar stand renovation by regrowth from the cut stumps after clear-felling have revealed that under studied conditions renovation was realistic and possible. Number of samples, which during nine years after clear-felling of the previous stand developed into normal trees ranged in all studied clones from 59 to 89%. From the number of renovated stumps some 14 to 68% had two trunks on one tree stump. Average number of trunks on each tree stump ranged from 1,14 – 1,68 depending on clone. These data unambiguously revealed that regeneration by regrowth from the cut stumps after clear-felling is the genetic trait. There is possibility to produce poplar trees (using this poplar trait) intended for bioenergy production in stands based on regenerating trees by regrowth from the cut stamps with greater number of cycles.

Table 3 – Biomass production per clones in two nine-year cycles

Taxation parameters	Clone							
	618	55/65	457	450	45/51	Ostia	I-214	543
d_I-cm	18,8	18,7	18,6	17,9	16,3	16,3	14,5	12,8
d_{II}-cm	13,9	15,2	14,8	13,8	13,9	13,8	12,0	11,1
h_I-m	21,9	20,7	21,0	20,8	19,6	17,1	18,9	17,0
h_{II}-m	14,0	15,2	15,0	14,0	16,2	15,3	15,4	14,5
N_I-kom/ha	1194	1153	1083	1044	1030	1017	1004	526
N_{II}-kom/ha	1347	1547	1073	1287	815	1170	679	453
I_{VPI}-m^3/ha	32,0	31,0	30,0	25,0	20,0	18,0	15,0	6,0
I_{VPII}-m^3/ha	16,0	28,0	16,0	16,0	9,0	11,0	5,0	3,0
V_I – m^3/ha	287,9	280,0	267,2	228,4	178,0	159,5	139,0	50,9
V_{II} –m^3/ha	146,2	222,8	148,4	139,7	80,7	101,3	47,1	25,6
V_{I+II}-m^3/ha	433,2	502,8	415,6	368,1	258,7	260,8	186,1	76,5

d_I, h_I, N_I, V_I, I_{VPI} – Taxation elements for the first nine-year production cycle
d_{II}, h_{II}, N_{II}, V_{II}, I_{VPII} - Taxation elements for the second nine-year production cycle

One of the basic assumptions for rational and economic biomass production in several production cycles is the possibility to regenerate the intended poplar stands by using natural forces of stumps regeneration. Process of initiation, duration and termination of vegetation is a complex physiological process of all plant species, and question regarding time of clear-feeling from the view of optimal stand regeneration process by using stump shoots is of great significance for general technology used in this type of regeneration of intended poplar stands for biomass production. Percentage of formed shoots, and their growth are directly dependent on time of felling (January-April). When felling iss done in January, at the latest, and February, and especially in April the number of coppice shoots produced from stumps is greater (Herpka, Marković 1980). Investigation results regarding total biomass produced during the first and the second cycles revealed that in these stands the most productive clones could produce biomass ranging from 600 to 750 m^3/ha during period from 24 to 25 years. Yield in absolute dry mass ranged from 240 to 300 t/ha.

It can be expected that natural forces of stump regeneration, after felling of new clones from the second cycle would provide possibility for new regeneration using stump shoots, and biomass production in the third cycle during the period from 10 to 15 years. Somewhat lesser biomass production can be expected in the third cycle in relation to the first and the second one. Taxation elements for two lengths of production cycle, one with four, and another one with eight years of rotation period are shown in table 4.

At the end of four-year developmental period diameters of mean trees ranged from 6,1 to 7,0 cm, and heights from 9,7 to 10,6 meters.

During this period of stand development differences between diameters and heights among trail stand treatments were not statistically significant.
In relation to planting distances biomass of both clones was greater in denser stands (1,8x1,0 m) clone 457 registered biomass of 72,88 m^3/ha, and clone with the record number 450 biomass of 80.67 m^3/ha. In less dense stands (1,8x1,5 m) at the end of fourth vegetation period the clone 457 registered biomass of 60,72m^3/ha, while at the same time clone 450 registered 53,29 m^3/ha in.

Table 4 – Basic indicators of stand trial development with different production cycle lengths.

Taxation elements	Planting distance			
	1,80 x 1,10 m		1,80 x 1,50 m	
	Clone			
	457	450	457	450
d_I - cm	6,1	6,6	7,0	6,7
d_{II} – cm	9,6	9,6	10,2	10,6
h_I - m	9,7	10,6	10,5	9,8
h_{II} – m	15,9	15,3	15,8	16,4
N/ha	4.444	4.314	3.036	2.912
V_I – m^3/ha	72,88	80,67	60,72	53,29
V_{II} – m^3/ha	237,31	232,52	187,02	193,65
I_{VPI} - m^3/ha	18,22	20,17	15,18	13,32
I_{VPII} - m^3/ha	29,66	29,07	23,38	24,21

d_I, h_I, V_I, I_{VPI} – Taxation elements of four-year production cycle
d_{II}, h_{II}, V_{II}, I_{VPII} - Taxation elements of eight-year production cycle

In this period of stand development the maximum average growth ranged from 20,17 m^3/ha in dense plantation to 15,8 m^3/ha in less dense plantation (table 4).

CONCLUSION

It can be concluded on the basis of data from stand trials marking basic stand production characteristics in previous period that technology of dense planting can be realistic possible solution for producing poplar biomass intended for energetic purposes in short rotations.

The greatest production was achieved in series of stand trials established with clone *Populus euramericana* I-214 with annual rotations (production process of 9 years). Since establishment and care measures, clear-felling, manipulation and preparation for usage were far simpler and a good deal cheaper in relation to biomass from other source of production it can be concluded that this form of poplar stands was the most profitable for biomass production intended for energy production. In nine production cycles of one year in length, the amount of obtained biomass ranged from 485m^3/ha, or on average 53,9m^3/ha annually.

Fast growing trait especially in clones from II approval series was pronounced during first years of development in so called “juvenile phase”, and in clone I-214 during later stage of development in so called “adult stage” when we managed to provide protection against pathogen attack .

This trait was of crucial significance in regard to choice of clones for biomass production intended for energetic purposes in short rotations.

Usage of biomass as renewable resource which can be produced in very short production cycles, with no emission of sulphur or nitrogen as in classical energy sources (which is of great significance for environmental protection) can be of great advantage in obtaining this form of energy.

Results of these investigations confirmrealistic possibility for regeneration of poplar dense stands by using regeneration source of tree stumps after clear-felling. If we wish to regenerate energetic stands using vegetative means the stands have to be felled during end of vegetation period. The best results are obtained when trees are felled during January – February in comparison to felling done in March or April.

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MODELS FOR STAND FORM FACTOR AND FORM HEIGHT OF BEECH HIGH STANDS IN SERBIA

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Abstract: *The dependence of stand form factor and form height on site and stand factors in beech high stands was defined by regression models. The basic study material consisted of the data collected in eleven all-aged beech stands, by using the simple systematic sample. 241 circular sample plots of 500 m² were established and all trees above 10 cm in diameter were measured. The data were processed by several dendrometric and statistical methods. The values of stand form factor and form height of high beech stands were estimated by the derived regression models. The gross effects of the site and stand factors were assessed and the most significant are: Lorey's mean stand height, the stand quadratic mean diameter, and tariff series (site class). It was concluded that the derived results could be applied in practice.*
Keywords: *beech, stand, model, stand form factor, form height*

INTRODUCTION

Stand volume can be calculated by numerous methods based on the complete or partial measurement of the trees above the taxation level. Some methods perform also a complete evaluation of stand volume. All methods are characterised by a level of accuracy and economicity, i.e. efficacy. The basic formula for the calculation of stand volume is V=G H F, where V - stand volume, G - stand basal area , H - Lorey's mean stand height and F - stand form factor. Volume elements G, H and F should be determined to the required accuracy, depending on the aim of stand volume assessment. Although it is most often considered that the complete stand measurement is the most accurate volume calculation method, it is not always the case. There are several reasons, and we shall mention here only that, in this method also, mean height and stand form factor are calculated based on a sample of trees (Mirković & Banković, 1993). In addition to individual elements of stand volume, their products HF or GF can also be calculated. The former is the so-called stand form height and it is used more often than the latter. The direct application of the basic formula of stand volume calculation requires the defined data of volume elements.

The task and aim of the study are to determine the dependence of stand form factor and form height in beech high stands on other most significant stand and site elements, i.e. to define the regression models. In addition to theoretical defining of the dependences, some models should have the sufficient reliability and the prospective application in practice. This problem was partially investigated in Serbia (Panić, 1971).

MATERIAL AND METHOD

The material in this research consists of numerous data on high all-aged beech stands and their site, collected within the project "Method of evaluation of quality and assortment structure of beech high stands in Serbia". The selection of study stands, the methods of data collection and processing are described in detail in Koprivica, et *al.* (2005). Also, several papers describe the study stand characteristics (Koprivica & Matović, 2006, Koprivica et *al.*, 2006 and 2007). The research covers eleven representative high stands of beech, selected in six forest regions: Severno Kučajsko, Podrinjsko-Kolubarsko, Jablaničko, Golijsko, Donje Ibarsko and Rasinsko. A systematic sample of circular sample plots, area 500 m², square design, spacing 100 m, was established in all stands, altogether 241 sample plots. The data were processed separately for each sample plot, as the average or aggregate values per hectare (Table 1).

Table 1. Statistics of taxation elements of beech high forests in the sample (n = 241)

Element	Statistical indicators							
	X_{mean}	$X_{min.}$	$X_{max.}$	S	CV%	m%	a_3	a_4
F	0.49242	0.34314	0.56225	0.0278	5.,65	0.73	-1.31	7.23
H_LF	14.046	4.711	19.702	2.689	19.15	2.47	-0.12	2.76
V	382.88	49.96	983.92	163.52	42.71	5.50	0.93	4.25
G	26.95	6.91	54.00	9.10	33.78	4.35	0.30	2.66
N	298.34	60.0	1200.0	145.55	48.78	6.28	1.72	10.04
H_l	28.42	13.73	40.33	4.77	16.78	2.16	0.07	2.62
H	23.79	11.7	39.4	5.02	21.08	2.72	0.43	2.94
D_g	35.76	17.95	61.26	8.31	23.23	2.99	0.47	3.02
D	32.66	17.50	60.20	8.35	25.58	3.30	0.64	3.16
SK	0.84	0.14	1.00	0.163	19.41	2.50	-1.54	5.40
TN	3.34	1.0	8.0	1.47	44.16	5.69	0.34	1.95
NV	846.5	406.0	1370.0	255.53	30.19	3.89	0.13	2.17
NT	21.4	6.0	42.0	8.23	38.42	4.95	0.07	2.23
EK	4.08	1.0	8.0	2.72	66.71	8.59	0.45	1.51

Legend:

F- stand form factor
H_lF - stand form height
V - stand volume per hectare
G - stand basal area per hectare
N - stand number of trees per hectare
H_L- Lorey's mean stand height
H - arithmetic mean stand height
D_g - stand quadratic mean diameter
D - arithmetic mean stand diameter
SK - stand canopy
TN - tariff series (site class)
NV- stand altitude
NT- stand slope
EK- stand aspect

Because of a small number of stands in the sample, further analyses had to start from the assumption that the sample plot characteristics (taxation and site elements) can be conditionally equalised with the characteristics of hypothetical stands. From the statistical aspect, this can be justified because the variability of all elements in the plot sample is higher than the variability of the same elements in the stand sample. This attitude is confirmed by the papers dealing with theoretical analysis of the sample structure intended for modelling in forestry (Box & Draper, 1987; Vancelay et *al.*, 1993; Rennolls, 1997). The above papers also deal with the issue of optimal data collection, assuming that the regression method will be used for modelling. It is concluded that the best results of modelling can be achieved if the variables include all the magnitudes within the scope of their variation, taking into account also the extreme magnitudes, and not only those with minor deviations from the average. Also, for modelling at the stand level, the data should be collected on sample plots.

The method of simple and multiple regression was applied for the defining of numerous statistical relations between stand form factor (F) and stand form height (H_LF) as dependent variables, and the selected stand taxation elements (G, N, H_L, H, D_g, D, SK) and site characteristics (TN, NV, NT, EK) as independent variables. "The best" regression equation (model) was selected by the method of stepwise multiple regression, and *ridge* regression method (Hadživuković et *al.*, 1982; Hadživuković, 1991) was used for the mitigation of the consequences of collinearity and multicollinearity between the independent variables.

RESULTS AND DISCUSSION

Stand form factor in beech high stands

Several models were obtained by the application of the regression method: simple regression models, multiple regression model as the best theoretical solution, multiple regression model as the best practical solution, and the model of multiple ridge regression.

Simple regression models

After the analysis of the matrix of simple and partial coefficients of linear correlation, it was concluded that stand form factor depended most on Lorey's mean height, tariff series (site class), and the stand quadratic mean diameter.

Regression equations are:

$$F = 0.878681 - 0.00584824 H_L - 6.0712 / H_L \quad (1)$$

S_e = 0.02369 R^2 = 28.04%

$$F = 0.511208 - 0.00141045 TN^2 \quad (2)$$

S_e = 0.02320 R^2 = 30.69%

$$F = 1.37336 - 0.00220329 D_g + 0.000185489 D_g^2 - 11.6156 / D_g \quad (3)$$

S_e = 0.02305 R^2 = 32.17%

With the increase of Lorey's mean height and with the increase of the stand quadratic mean diameter, the stand form factor also increases, and it decreases with the increase of tariff series (site class degradation). The dependences are professionally logical, but in all cases only about 30% of the total variation of stand form factor is explained, and standard error of regression is high. Also, there is a significant linear correlation among all three independent variables, which is especially significant in the estimation of the "net" effect of individual independent variables on the dependent variable in multiple regression models.

Multiple regression model as the best theoretical solution

This model was derived by the stepwise multiple regression method. The initial variables were the stand form factor (dependent variable) and numerous stand taxation elements and site characteristics (independent variables). The original independent variables were also taken in the transformed form, as quadratic and reciprocal values. The best solution is the following multiple regression equation:

$$\begin{aligned} F &= 1.28023 - 0.018352 H_L + 0.000105789 H_L^2 + 0.000013376 D^2 \\ &+ 1.7746 / D - 0.0333872 TN - 0.001233 TN^2 - 0.000000028571 N^2 \\ &- 0.00381379 H - 6.95341 / D_g^2 \end{aligned} \quad (4)$$

S_e = 0.00814 R^2 = 91.44% E = 0.00603

The regression equation (4) includes the independent variables whose individual and collective effect on the dependent variable is significant at probability level 99.9%: Lorey's mean height, arithmetic mean diameter, tariff series, number of trees per hectare, arithmetic mean height and the stand quadratic mean diameter. Although this model is the best theoretical (statistical) solution it cannot be simply applied in practice, because it requires the previous calculation of six independent variables. For this reason, the best solution for practical application was searched.

Multiple regression model as the best practical solution

The best multiple regression equation that can be applied in practice is:

$$F = 1.1948 - 0.0136869 H_L - 2.31108 / H_L - 0.0320255 TN - 4.1527 / D_g \quad (5)$$

S_e = 0.01301 R^2 = 78.49% E = 0.01019

Regression equation (5) includes as independent variables: Lorey's mean height, tariff series and the stand quadratic mean diameter. These independent variables can be rather simply calculated in the concrete stand, and this is to be dealt with.

All partial coefficients of regression in equation (5) are statistically significant at the risk level $p < 0.001$, as well as the whole regression. Standard error of regression is +/- 0.01301 and the mean absolute deviation is 0.01019. Coefficient of multiple determination is 78.49%. The reliability of the model is checked in several ways: analysis of residual, method of linear correlation and analysis of percent deviations.

The analysis of standardised residuals shows that the residuals are distributed approximately by the probability law of normal distribution. Of the total number of all residuals, there are 14 or 5.81% standardised residuals of absolute value above 2.0, and only 1 or 0.41% above 3.0. Actually, it can be expected at probability 95% that the error of the estimated stand form factor will not exceed 0.02602.

Linear correlation between original and estimated values of stand form factor in beech stands in model (5) is presented in Diagram 1. In the ideal case, the value of parameters of line equation is: a = 0 and b = 1 (Stojanović, 1976). From the statistical aspect, the values of parameters of the derived linear regression differ accidentally from the expected, so it can be concluded that the tested model is reliable for the estimation of the stand form factor in beech high stands.

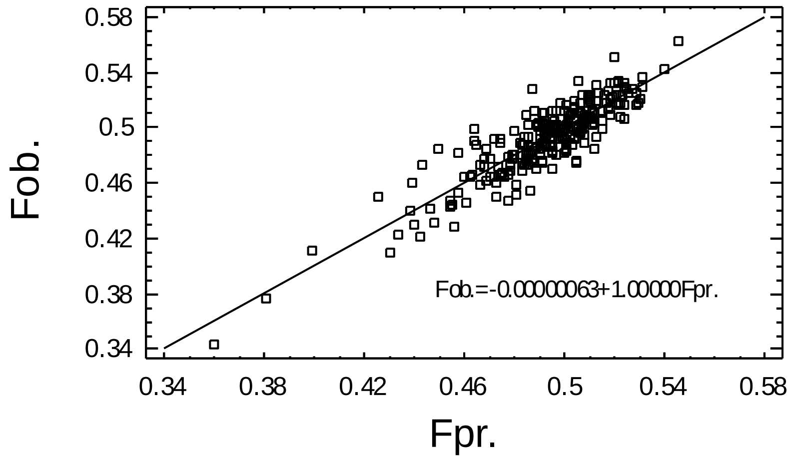

Diagram 1. Correlation between original and estimated values of stand form factor in beech stands

Percent deviation of the estimated values of beech stand form factor from the original values is obtained by the equation:

$$p = 100(F_{pr.} - F_{ob.}) / F_{ob.}$$

The accuracy of estimate is checked separately for all eleven beech stands analysed in this research and for all stands together, starting from the assumption that the stands belong to the same management class. The results are presented in Table 2.

Table 2. Accuracy of estimated stand form factor in beech high stands

Stand	n	$F_{ob.}$	$F_{pr.}$	Δ	Δ (%)
33a	23	0.50369	0.50235	-0.00134	-0.266
42a	18	0.48993	0.49170	+0.00177	+0.361
42b	10	0.48687	0.47442	-0.01241	-2.549
122a	29	0.50509	0.50003	-0.00506	-1.002
27a	20	0.50236	0.49315	-0.00921	-1.833
31a	32	0.48199	0.50919	+0.02720	+5.643
46a	28	0.49421	0.48388	-0.01033	-2.090
8a	16	0.48386	0.49453	+0.01067	+2.205
8b	10	0.49828	0.49696	-0.00132	-0.265
44a	22	0.50332	0.49416	-0.00916	-1.820
116a	33	0.48875	0.50043	+0.01168	+2.390
All	241	0.49446	0.49845	+0.00399	+0.807

Percent deviation for individual stands varies from -2.55% to +5.64%. and for all stands together it accounts for +0.81%. Only in one stand, the deviation absolute value is higher than the standard error of regression (+/-0.01301).

All methods of checking the accuracy of the regression model (5) show that the model is highly reliable. In the study material for the model construction, all stand and site elements were determined to exceptionally high accuracy. However, the accuracy of this model in practical application depends exclusively on the accuracy of the calculated mean height, mean diameter and tariff series of the concrete stand.

Multiple ridge regression model

In the equation of multiple regression (5) there is a statistically significant linear correlation between the independent variables. Between H_L and TN, correlation coefficient is -0.87, between H_L and D_g 0.64, and between TN and D_g -0.36. In the analysis of the model structure, the collinearity of the variables is especially significant (Koprivica, 1982).The *ridge* regression method is applied to mitigate the multicollinearity problem.

Regression equation (6) is calculated with the additional value of coefficient $k = 0.05$, with the same independent variables as in equation (5). The aim of the application of *ridge* regression method is to obtain the equation of multiple regression with stable partial coefficients of regression (Hadživuković, 1991).

The following equation of multiple regression is obtained:

$$F = 0.815428 - 0.00585862H_L - 0.307044/H_L - 0.0192758TN - 2.74227/D_g \quad (6)$$

S_e = 0.01614 R^2 = 55.11% E = 0.01250

The comparison of regression equations (5) and (6) shows that there was a significant change in the value of the partial coefficients of regression, but mathematical signs of the parameters with independent variables remained the same. The greatest change was in the value of the standard error of regression, i.e. the coefficient of multiple determination. This means that regression model (5) is better for the estimation of stand form factor, and model (6) for the estimation of the “net“ effect of independent variables on the dependent variable.

Form height of beech high stands

Form height of beech high stands was analysed in the same way as the stand form factor.

Simple regression models

After the analysis of the matrix of simple and partial coefficients of linear correlation, it was concluded that in beech high stands, form height depended most on Lorey’s mean height, tariff series (site class), and the stand quadratic mean diameter.

Regression equations are:

$$H_L F = -6.33797 + 0.89749H_L - 0.00618669H_L^2 \quad (7)$$

S_e = 0.6838 m R^2 = 92.60%

$$H_L F = 20.3476 - 2.18971TN + 0.076042TN^2 \quad (8)$$

S_e = 1.1509 m R^2 = 81.84%

$$H_L F = 18.3054 + 0.00100635D_g^2 - 190.153/D_g \quad (9)$$

S_e = 1.8667 m R^2 = 52.23%

With the increase of Lorey’s mean height and with the increase of the stand quadratic mean diameter, the stand form height also increases, and it decreases with the increase of tariff series (site class degradation). All the dependences are professionally logical. However, despite the statistically significant value of determination coefficients, standard error of regression is high, there is a significant linear correlation among all three independent variables.

Multiple regression model as the best theoretical solution

This model was derived by the stepwise multiple regression method. The initial variables were the beech stand form height (dependent variable) and numerous stand taxation elements and site characteristics (independent variables). The original independent variables were also taken in the transformed form, as quadratic and reciprocal values. The best solution is the following multiple regression equation:

$$H_L F = 11.1449 + 0.223593 H_L + 60.095 / H_L - 195.577 / D_g + 0.0761118 D$$
$$+ 94.338 / D - 1.20169 TN - 0.00238052 H^2 \quad (10)$$

S_e= 0.2469 m R^2 = 99.18% E = 0.1839 m

The regression equation (10) includes the independent variables whose individual and collective effect on the dependent variable is significant at probability level 99.9%: Lorey's mean height, the stand quadratic mean diameter, arithmetic mean diameter, tariff series, and arithmetic mean height. Although is this model the best theoretical (statistical) solution, it cannot be simply applied in practice, because it requires the previous calculation of five independent variables.

Multiple regression model as the best practical solution

The best multiple regression equation that can be applied in practice is:

$$H_L F = 15.5566 + 0.176377 H_L - 0.929592 TN - 115.761 / D_g \quad (11)$$

S_e = 0.3823 m R^2 = 98.00% E = 0.2973 m

Regression equation (11) includes the independent variables: Lorey's mean height, tariff series and the stand quadratic mean diameter, i.e. the same variables as in the case of stand form factor in regression equation (5). These independent variables can be rather simply calculated in the concrete stand, and this is to be dealt with.

All partial coefficients of regression in equation (11) are statistically significant at the risk level $p < 0.001$, as well as the whole regression. Standard error of regression is +/- 0,3823 m, and the mean absolute deviation is 0.2973 m. Coefficient of multiple determination is 98.00%. The reliability of the model is checked in the same way as the reliability of the model (5). The analysis of standardised residuals shows that the residuals are distributed approximately by the probability law of normal distribution. Of the total number of all residuals, there are 14 or 5.81% standardised residuals of absolute value above 2.0, and 3 or 1.24% above 3.0. This practically means that it can be expected at probability 95% that the error of the estimated stand form height will not exceed 0.7646 m

Linear correlation between original and estimated values of stand form height in beech stands in model (11) is presented in Diagram 2. From the statistical aspect, the parameters of the derived linear regression differ accidentally from the expected (a = 0 and b = 1), so it can be concluded that the tested model is reliable for the estimation of the stand form height in beech high stands.

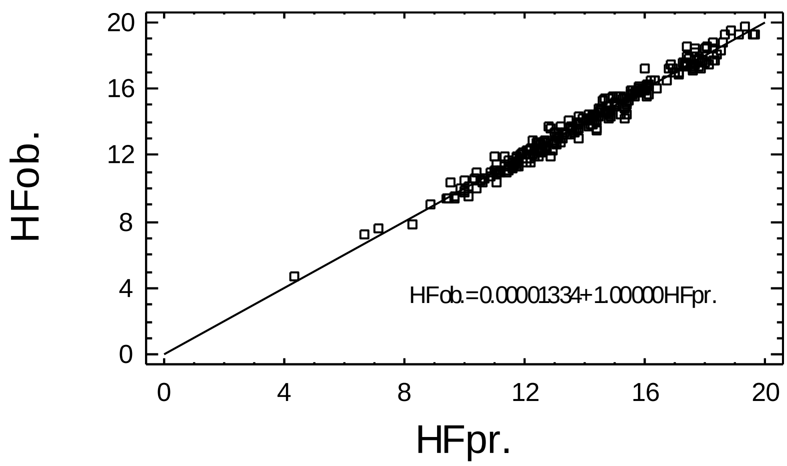

Diagram 2. Correlation between original and estimated values of form height in beech high stands

Percent deviation of the estimated values of beech stand form height from the original values is obtained by the equation:

$$p = 100(H_L F_{pr.} - H_L F_{ob.}) / H_L F_{ob.}$$

The accuracy of estimate is checked separately for all eleven beech stands analysed in this research and for all stands together, starting from the assumption that the stands belong to the same management class. The results are presented in Table 3.

Table 3. Accuracy of estimated form height in beech high stands

Stand	n	$H_LF_{ob.}$	$H_LF_{pr.}$	Δ	Δ (%)
33a	23	15,6343	15,5430	-0,0913	-0,584
42a	18	11,9910	12,0621	+0,0711	+0,593
42b	10	10,5681	10,3215	-0,2466	-2,333
122a	29	17,3498	17,1571	-0,1927	-1,111
27a	20	15,1855	14,8896	-0,2959	-1,949
31a	32	13,5004	14,2573	+0,7569	+5,606
46a	28	13,6156	13,3090	-0,3066	-2,252
8a	16	12,5125	12,8030	+0,2905	+2,322
8b	10	12,2543	12,3107	+0,0564	+0,460
44a	22	16,1734	15,8646	-0,3088	-1,909
116a	33	13,0568	13,3744	+0,3176	+2,432
All	241	14,1604	14,2295	+0,691	+0,488

Percent deviation for individual stands varies from -2.33% to +5.61%, and for all stands together it accounts for +0.49%. Only in one stand, the deviation of the absolute value is higher than the standard error of regression (+/-0.3823).

All methods of checking the accuracy of the regression model (11) show that the model is highly reliable. In the study material for the model construction all stand and site elements were determined to exceptionally high accuracy. However, the accuracy of this model in practical application depends exclusively on the accuracy of the calculated mean height, mean diameter and tariff series of the concrete stand.

Multiple ridge regression model

In this case too, ridge regression model is derived with the additional value of the coefficient *k = 0.05,* with the same independent variables as in model (11). Multiple regression equation is:

$$H_L F = 14.3004 + 0.200463 H_L - 0.836164 TN - 106.873 / D_g \qquad (12)$$

$S_e = 0.3894$ m $R^2 = 95.85\%$ E = 0.3057 m

The comparison of regression equations (11) and (12) shows that there was no significant change in the partial coefficients of regression, and mathematical signs of the parameters with independent variables remained the same. Also, there is no significant change in the value of the standard error of regression and the coefficient of multiple determination. This means that both regression models are equally good for the estimation of beech stand form height, as well as for the assessment of the "net" effect of independent variable on dependent variable.

APPLICATION OF RESULTS

Regression models (5) and (11) are intended for practical application. If necessary, they can be used for the construction of three-way tables for the calculation of stand form factor and form height of beech high stands. For the regression equation to be applied, it is necessary to determine: tariff series (site class) for beech, Lorey's mean height and stand quadratic mean diameter. The problem can be solved by the implementation of *relascopy method.*

The procedure is in short as follows: First the stand structure should be evaluated (per tree diameter and per stand area) and then the tree enumeration factor should be selected, so that the average number of trees per sample plot is not lower than 15 to 20 (Van Laar & Akca, 2007). Sample plots should be located in the stand based on professional evaluation, aiming at the best representation of the stand volume. The number of sample plots should be 3 - 5. On each sample plot, the trees eligible for measurement should be marked (sighting from the sample plot centre, the diameter at breast height is larger than the width of the measurement scale). The diameter at breast height and the height of all selected trees should be measured. Based on these data, height curve can be compiled and tariff series (TN), i.e. site class, should be determined. Based on the classification of trees per diameter classes, the stand basal area per diameter classes is obtained and total per hectare, i.e. G_1 , G_2 . . . G_k and G. Mean heights of trees per diameter classes h_1 , h_2, . . . h_k should be read off from the height curve, and Lorey's mean height (H_L) should be determined. The stand quadratic mean diameter (D_g) is derived based on the number of trees per hectare. This should be done separately for each diameter class (by dividing the number of enumerated trees in diameter class, i.e. the class basal area per hectare G_i, by basal area of mean tree in diameter class g_i), and then the results should be added. After that, mean basal area of the stand is determined from the ratio G/N, i.e. mean diameter D_g. By this procedure, all stand elements necessary for the estimation of stand form factor (F) or stand form height (H_LF) are determined.

Stand volume and its approximate structure can be calculated, as the stand basal area G and its structure by diameter degrees or classes are known. Evidently, the stand volume calculated in this way lacks the satisfactory accuracy. The stand volume accuracy can be additionally improved by establishing an additional number of sample plots on which only basal area per hectare should be determined, without measuring tree diameters and heights. This sample type is two-way, i.e. in the first phase stand basal area is determined on a larger sample, and in the second phase, stand form factor or stand form height are determined on a smaller sample (Kangas & Maltamo, 2006). This is statistically justified, because in the sample of plots, basal area (CV = 33.78%) is the most variable of all volume elements, then form height (CV = 19.15%) and Lorey's mean height (CV = 16.78%), and the least variable is the beech stand form factor (CV = 5.65%). The coefficient of volume variation is 42.71%.

Example: In a beech high stand, the following elements were determined by the described procedure: tariff series 5 (site class III), G = 30 m^2/ha , H_L = 25 m and D_g = 35 cm. By the regression equation (5) F = 0.48141, and by equation (11) H_LF = 12.0106 m. Therefore, stand volume is V = 30 x 25 x 0.48141 = 361.05 m^3/ha, i.e. V = 30 x 12.0541 = 360.32 m^3/ha. The classical method of stand measurement, by sampling intensity 5% of stand area, shows that stand volume amounts to 380 m^3/ha, with double relative error +/- 11.0%. The real average stand volume ranges in the interval from 338 to 422 m^3/ha, with probability 95%. The average stand volume calculated by the described method also ranges within these values.

In exceptional cases, when a fast assessment of beech stand volume per hectare and total volume, on the entire area, is necessary, the described procedure should be maximally simplified. Then, mean stand height, mean diameter and tariff series should be calculated with minimal measurements. However, this requires a high professional experience of the taxator.

CONCLUSION

The dependence of stand form factor and form height on the most significant site and stand factors in beech high stands was defined by simple and multiple regression methods. Models (4) and (10) have the highest theoretical significance and models (5) and (11) have the highest practical significance.

Stand form factor and form height of beech high stands can be estimated rather simply by using the models (5) and (11), based on three factors (Lorey's mean height, tariff series and the stand quadratic mean diameter). Model (11) is superior, from the aspect of model reliability and efficacy.

The described procedure of application of the derived models is intended for fast and low-cost estimation of beech high stand volume, as well as for a rough control of the calculated stand volume by a classical method. Despite the high precision of regression models (5) and (11), the accuracy of the calculated beech stand volume in all cases depends exclusively on the accuracy of the determined volume elements.

In the evaluation of the quality and efficacy of the presented method, it should be taken into account that most often, even the classical methods, with economically justified sample size, cannot achieve the satisfactorily accurate beech stand volume assessment for the drawing of reliable forest management plans (Koprivica, 1996).

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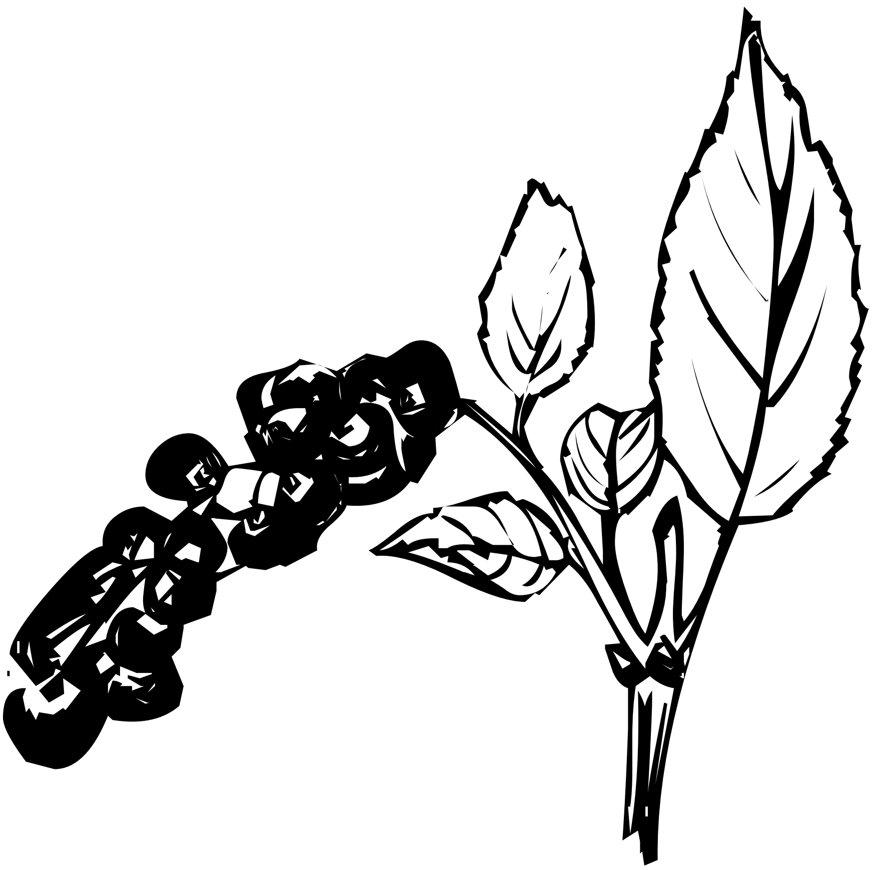

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