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The Conference “Forestry: Bridge to the Future” (13–15 May 2010) is dedicated to the 85th anniversary of higher forestry education in Bulgaria. It is organized by the University of Forestry Sofia, Faculty of Forestry and National Project Center for Coppice Forest Management in Southeastern Europe (CoppForSEE)

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MONITORING OF PINE FORESTS UNDER AIR POLLUTION IN THE NORTH-EAST OF THE EUROPEAN PART OF RUSSIA

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Abstract

The present study aimed to estimate the dynamics of vitality of ground vegetation components under air pollution by pulp and paper industry emissions. The majority of pollutants were oxides of sulfur, nitrogen, sulfur-organic compounds, and alkaline dust. The vitality determination of tree stands was carried out according to ICP-Forests program technique, which is based on ranging trees in classes depending on crowns condition. The studies were conducted in pine forests of *Pinetum fruticulosum* type situated along the pollution gradient. From 1998 to 2009 the share of healthy trees has increased by 1.9 times. Safety and condition of pine undergrowth has deteriorated twice, versus the double increase of the safety of spruce undergrowth has increased by 2 times. Pulp and paper industry air pollution was found to increase leaf discoloration grade and shoot density of dwarf shrubs (*Vaccinium myrtillus* L. and *Vaccinium vitis-idea* L.). For the period of researches, dwarf shrubs essentially improved their condition. Mineralization degree of above-ground parts of dwarf shrubs increased compared to the background area. Species composition of ground vegetation cover significantly changed only in pine forest situated at the shortest distance from the paper plant. Pine stands in the impact area of the pulp and paper mill underwent changes during 1998–2009 which indicated improvement of their condition. Mineralization of snow and discoloration of pine needles had the highest negative relation at far distance from the polluter, versus species composition and projective cover of mosses and epiphytic lichens had the highest direct relation. It is possible to use these parameters as indicators of forest ecological condition.

Key words: pulp and paper plant emissions, tree crown condition, undergrowth, dwarf shrubs condition, chemistry.

Introduction

In a natural environment the vital condition of forest ecosystems depends on the biological features properties of tree species, site habitat conditions and natural abiotic (wild fires, windfalls, and stormbreaks) and biotic (pests and rots diseases) factors. Besides, now forest ecosystems are under a constantly increasing anthropogenic impact, connected with increasing volumes of different types of cuttings, and increasing air contamination by industrial releases. Therefore, perennial investigations of vegetation dynamics could provide additional information about changes in numerous parameters of forest ecosystems. Assessment of the vital condition of forest ecosystems being under influence of aerotechnogenic emissions of various manufactures should be carried out on the permanent sample plots disposed by emission gradient and in comparison to background areas (Yarmishko 1997).

The purpose of this study was to estimate the vital state dynamics of ground vegetation components exposed to industrial air pollution.

Material and Methods

The largest permanent source of industrial air emissions in the middle taiga zone of Komi Republic is the OJSC "Mondi Syktyvkar" pulp and paper plant (SLPK). The majority of its pollutants are oxides of carbon, nitrogen, sulfur; hydrogen sulfide, sulfur-organic compounds, and mineral dust containing calcium and potassium carbonates and sulfides. Last years, total amount of discharge was about 20 thousand t.year^{-1} that was 1.4 times less than that in 2004 and 1.6 times less than that in 1998 (Ecological report 2008). Investigation area had a mosaic cover and consisted of secondary 60–120-year-old forests after felling and fire. The studies were conducted on eight permanent experimental plots grown by bilberry pine forests, 4 of them being background plots situated 48–52 kilometers northwards from the pollution source and 4 test plots situated 1.3–11.2 kilometers north-eastwards from SLPK in the dominant wind direction (Torlopova and Robakidze 2003). Bilberry pine forests grow on illuvial-humus iron podzolic soils. They are middle-aged, maturing and mature stands formed after felling or fire. These stands are even-aged or relatively even-aged with a mean and relatively high productivity. They consist of pine (*Pinus sylvestris*), aspen (*Populus tremula*), birch (*Betula pubescens, B. pendula*), and seldom Siberian spruce (*Picea obovata*).

Table 1. Increment of pine stand parameters from 1998 to 2009, in %.

Distance from SLPK, km	Height	Diameter	Number of trees	Wood volume
1.3	106	124	79	125
6.5	114	138	59	109
11.0	111	115	86	119
11.2	97	107	66	94
48.5	118	123	83	144
50.0	89	115	87	116
51.0	107	113	94	114
52.0	100	114	84	109

Forest measurements were conducted using by common methods (GOST 1970, Zakharov 1967). Tree crown condition was assessed according to ICP-Forests (Manual… 2006). Qualitative chemical analyses of plant samples were carried out according to certified techniques.

Results and Discussion

For the study period, pine trees in stands on both background and test plots decreased in number (Table 1). However, this decrease in the impact zone was by 50% points higher, and resulted in decreasing the volume increment by a factor of 1.8.

A comparative analysis of vital status of tree stands was conducted (Figure 1). Most informative among other parameters is crown defoliation in comparison to a healthy tree. For example, the share of trees without needle loss in SLPK impact zone compared to 1998 increased by 33-fold. The share of trees with slight defoliation grew by a factor of 3.8. There were no trees with clearly expressed needle loss. These changes pointed out that vital status of the tree stands by crown defoliation improved in 2009 compared to 1998. Discoloration is an important parameter of tree status. This parameter was lowering from 1998 to 2009 but less than defoliation did. The number of trees without yellow needles decreased insignificantly. The share of trees with a low discoloration degree increased 1.4-fold. Trees with a moderate discoloration degree significantly decreased in number (8-fold). Trees without dry branches increased 4-fold in 2009 compared to 1998. The share of 1-class trees (11–25% dry branches) decreased 6-fold; 1% remained for moderately-damaged trees by presence of dry branches. Among all parameters, only tree crown-top condition worsened from 1998 to 2009. The share of trees with a healthy crown-tops

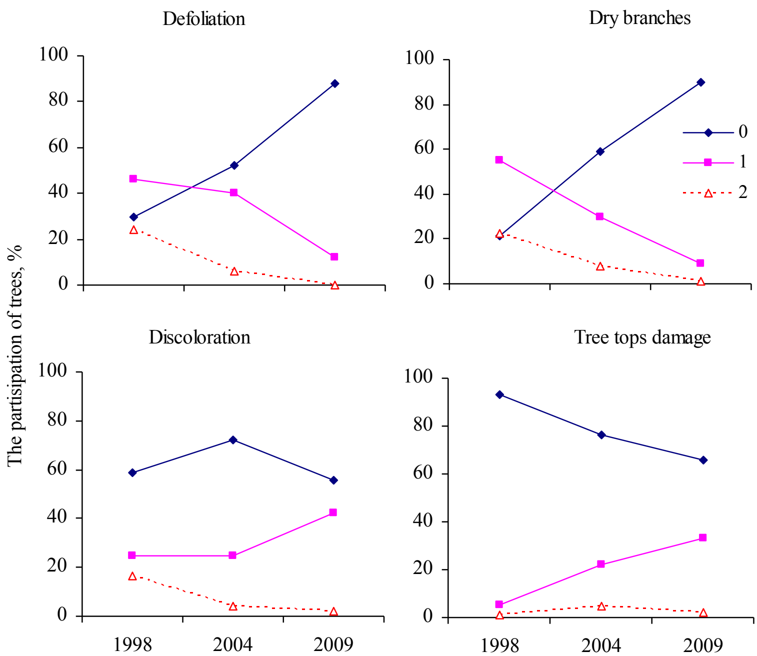

Fig. 1. Parameters of pine crown condition assessment in air pollution impact area.
Tree classes: 0 – undamaged (0–10% of damage), 1 – slightly damaged (11–25% of damage), 2 – moderately damaged (26–60% of damage), tree top damage classes: 0 – healthy, 1 – damaged, 2 – dry.

decreased 1.4-fold; 35% of the trees had damaged crown-tops which was 6 times higher compared to 1998. Tree crown-tops damage may be caused by high concentrations of pollutants in the air due to single emergency discharges.

Each tree was given a damage class accounting for defoliation, discoloration, presence of dry branches in crown and condition of tree top. The closest (1.3 km) to SLPK plot had the highest amount of slightly-damaged trees (25%) among all studied sites. Thus the share of healthy trees fell to 70% there being the lowest value in 2009. Tree stands structure by damage classes on the other plots did not correlate with distance to pollution source and did not show any significant differences between plots.

Analyzing the distribution of trees to damage classes for the observation period led to the conclusion that condition of pine forests in the SLPK impact zone improved for the last 10 years (Figure 2). The amount of healthy trees incresed 1.9-fold. The share of the slightly damaged trees lowered by 3.2-fold, moderately-damaged by 3.0-fold, and severely-damaged by 1.8-fold. In contrast to situation in 1998, the year in 2009 a reduction between the quantities of differently-damaged trees on background and impact plots was observed, whereas the distribution of trees to damage classes remained almost the same in background and test plots.

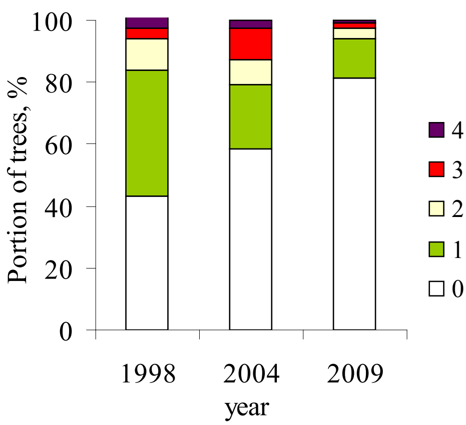

Fig. 2. Trees distribution in the air pollution impact area to damage classes: 0 – undamaged, 1 – slightly damaged, 2 – moderately damaged, 3 – severely damaged, 4 – died.

To estimate the vital state of the tree stands, the index of stand disturbance was calculated using the formula for determining the weighted-mean class of damage to the trees comprising the stand. According to Alekseev (1997) the index under 0.5 characterizes a stand as a healthy one. In the background areas, the index decreased by 1.3-fold compared to that in 1998 and by 1.2-fold compared to that in 2004 (Figure 3). The damage index in the impact zone decreased 3.1 and 2.8-fold, respectively. This happened because of the reduction of the share of trees of all damage classes tree portion (in 2004 slightly- and moderately-damaged and in 2009 moderately- and severely-damaged trees) and because died standing trees had fallen down on the ground. A comparative analysis of the damage index of woods on polluted plots in 1998, 2004, and 2009 showed an essential improvement of pine forests vital status. All studied tree stands within the SLPK impact zone were characterized as healthy in 2009.

For growth intensity comparison of tree stands the method of an evaluation of their average diameter at the age of 60 years (minimum age among the investigated pine forests) was approved. Reducing the diameters of uneven-aged pine forests to 60-aged stand has allowed us to make a correct comparative analysis of their growth. Secondary pine forests from polluted area had the least value average diameter of 60-year-old trees relative to other secondary and primary pine forests. For assessment of annual loss of wood volumes in secondary pine forests because of releases influence of pulp-and-paper mill a comparison between the mean annual single tree wood increment and that in pine stands formed after clear-cutting was carried out. It was shown that the mean annual single tree wood increment of one tree from polluted area in true moss pine forests was less than 1.6 dm^3 compared to other secondary pine forests. Taking into account the average number of trees in pine forests from polluted area (1200 trees per ha) it was calculated that the annual loss of wood reaches up to 1.9 $m^3.ha^{-1}$ per year.

Ground vegetation layer was studied by the method of registration plots 50 x 0.5 m x 0.5 m. These plots had been established regularly through forest sample plot. Ground cover of each species, and shoots number of *Vaccinium* on each plot were registered.

Undergrowth was dominated by healthy spruce trees. Pine and birch in undergrowth dies at different development stages (Table 2). Compared to 1998, the amount of healthy spruce undergrowth increased 2-fold (Figure 4).

In pine forests, individuals of *Vaccinium myrtillus* L. per area unit increased in number logarithmically closer to pollution source ($r^2 = 0.76$). Intensive appearance of new shoots is possible only on condition that other vascular plants less resistant to toxicants decrease in number which was proven in pine forests under heavy metal pollution. In 1999 polluted pine forests showed an increase in densities of *V. myrtillus* – 2.1-fold and, in density of *V. vitis-idaea* L. – 1.3-fold compared to background. In 2007 the differences between background and test areas decreased to 1.3 for *V. myrtillus* and to 1.1 for *V. vitis-idaea*, respectively. The investigation of the assimilation ap-

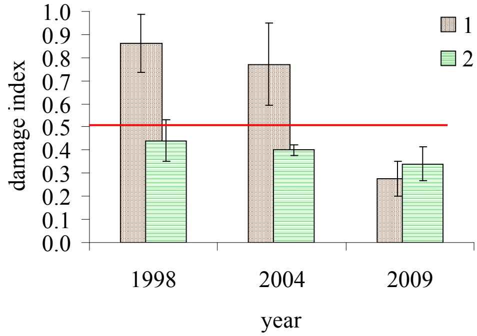

Fig. 3. Damage dynamics of pine stands under air pollution (1) and background (2). Index: healthy stand <0.5< slightly damaged stand < 1.0 < moderately damaged stand.

Table 2. Distribution of undergrowth species in pine forests in air pollution (above the line) and background (below the line) areas, individuals per ha.

Species	1999	2004	2009
Spruce	231 / 900	739 / 1061	347 / 950
Pine	280 / 210	119 / 0	44 / 33
Birch	– * / –	668 / 307	256 / 683

* in 1999 birch not detected.

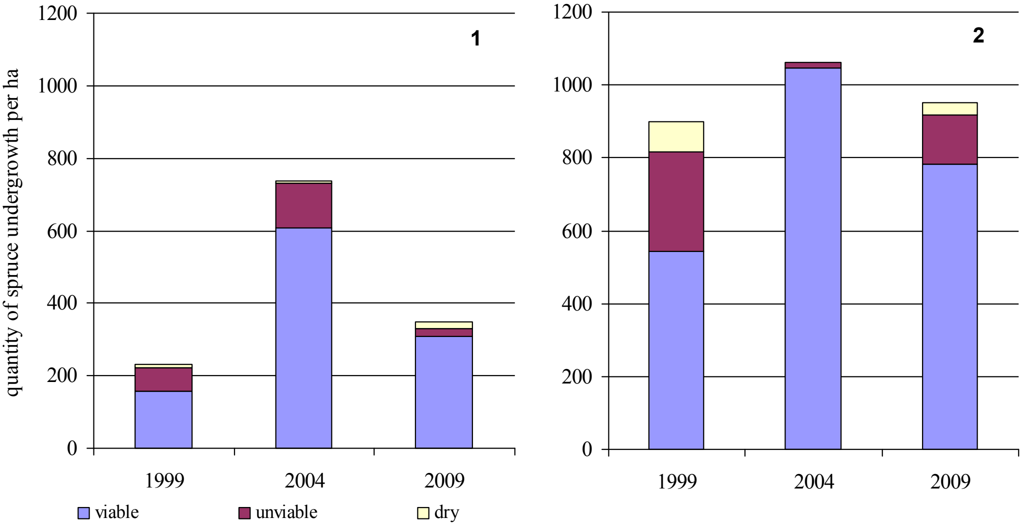

Fig. 4. Distribution of spruce undergrowth by viability in air pollution (1) and background (2) areas.

paratus of the dominating plants in the herb-undershrub layer showed a high damage grade of leaves within SLPK impact zone. Leaves were found to be yellow and brown in color, with dotty burns, and even decomposition of tissues was observed. In 1999 the share of damaged *V. myrtillus* individuals in pine forest in the impact areas was about 2.5 times higher compared to the background area while the share of damaged *V. vitis-idaea* 4.4 times. In 2007 the values for both species in the background areas remained unchanged while those in the test areas were only 1.5 time higher for *V. myrtillus* or remained the same as background for *V. vitis-idaea*.

Pulp and paper industry air pollution was found to increase leaf discoloration grade and shoot density of dwarf shrubs (*V. myrtillus* and *V. vitis-idea*). For the period 1999–2007, dwarf shrubs essentially improved their condition. Population density of dwarf shrubs did not reliably change. Mineralization degree of above-ground parts of dwarf shrubs increased compared to the background area. Species composition of the ground vegetation cover significantly changed only in pine forest situated at the shortest distance to the paper plant.

One of the principal factors which defines normal growth and development of plant is optimal supply with nutrients. Soil and atmosphere are the main source of nutrients. Air pollution is known to increase mineral elements quantity in assimilatory organs. Ash content (excluding N) of pine needles in both pollution impact zone and background area was 1.2–1.5% of dry weight. Nutrient status of pine needles was potassium-nitrogenous. In polluted area the contents of K, Ca, P, Mg and Mn in needles were lower, while the content of Al and Na was higher, compared to the background (Figure 5). Nutrient status of undershrubs leaves was distinguished by prevalence of Ca over K. Ash content of undershrubs leaves was higher, than that of pine needles. In polluted area an increase of undershrubs leave ash content was detected. The contents of C, N, and S total contents in assimilatory organs of pine as well as of dwarf shrubs were very invariable. Nitrogen and carbon shares prevailed in needles, and sulfur prevailed in *V. vitis-idaea* aboveground organs. Inverse correlation between the contents of the main nutrient elements (Ca, P, and Mg) and Fe and discoloration of *V. myrtillus* leaves was found.

Conclusion

Monitoring of bilberry pine forests under SLPK aerotechnogenic pollution started in 1998 and has shown a dynamically changing vital status of tree stands. In contrast to 1998, only 12% of pine trees had signs of needles loss in the impact zone in 2009. However, the slight discoloration of needles remained unchanged with increasing share of crown-top damaged trees. A stable increasing proportion of healthy trees in composition was found with a simultaneous reduction in the proportion of slightly- and severely-damaged trees. Aerotechnogenic pollution was harmful to ground cover plants, it reduced specific diversity of grasses, mosses and increased leaf damage of undershrubs. Grassy plants were replaced by undershrubs which lead to an increasing density of *Vaccinium myrtillus* and *V. vitis-idaea*. For the period 1999–2007, undershrubs improved their status. For the study period, both test and background plots were found for reducing ash value in needles. As in 1998, and in 2009, none of the pollutants such as carbon compounds, sulfur and nitrogen oxides do not accumulate any in pine needles (excluding 1.3-km-distance impact plot) or leaves of Vaccinium myrtillus and V. vitis-idaea, and in forest litter. Due to discharged mineral dust and solid particles, leaves of *V. myrtillus* and forest litter accumulated calcium, magnesium, potassium, manganese, and sodium, but not in pine needles. For the period 1998–2009 by applying integral damage index analysis, the vital status of bilberry pine forests in polluted plots improved by a factor of 3. As summarized by Emberson et al. (2003) sub-critical concentrations of pollutants (sulfur and nitrogen oxides, such as well as suspended particulate matter) can produce a fertilizer effect on plants, especially

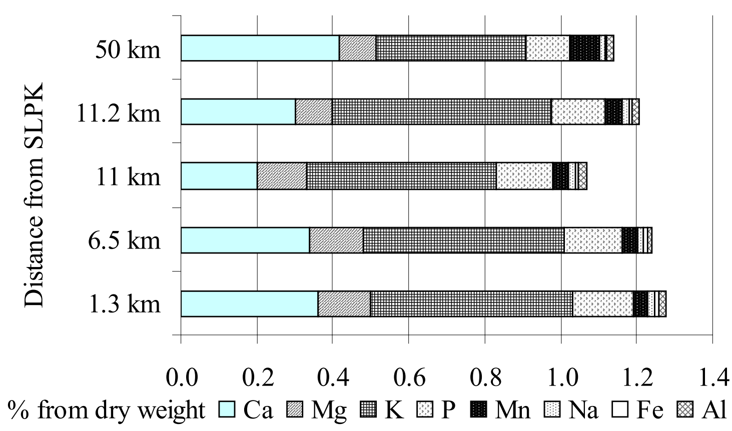

Fig. 5. Mineral composition of 2-year-old pine needles in 2009.

on those growing in poor nutrient condition, for example in the European North. Mineralization of snow and discoloration of pine needles have the highest inverse relationship with distance from the polluter, versus species composition and projective cover of mosses and epiphytic lichens have the highest direct correlation. It is possible to use these parameters as indicators of an ecological conditions. The results of the present study could be used in forest management modeling and forecasting.

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NATURAL DISTURBANCE HISTORY OF THE PRISTINE *PICEA ABIES* FOREST PARANGALITSA

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Abstract

Although understanding natural disturbance regime is very important for the management of subalpine coniferous forests, studies for Southern European Mountain ecosystems are still scarce. Among the reasons are difficulties in finding unmanaged forests that could serve as reference. We studied the disturbance history of an old Norway spruce-dominated forest in the "Parangalitsa" biosphere reserve in Bulgaria. It was declared a reserve in the 1930[s], but was protected from human intervention before that. We used aerial photo interpretation to detect disturbance areas and dendroecological methods to date the occurrence moment of the disturbance events. Over the past 150 years windthrows have been the main type of disturbance. About 20 percent of the forested area was subjected to complete blowdown. The largest proportion of the affected territory was due to medium sized windthrows with dimensions of 1 to 10 ha. Although *Ips typhographus* populations were large enough to cause mortality of some live trees, the populations did not grow to epidemic proportions. Fire disturbance was of limited importance and only two areas (4 percent of the study area) showed evidence of fire in the 20[th] century. The forest ecosystem in Parangalitsa has high resilience, which is probably due to the high landscape heterogeneity of forest structures and continuous interaction between natural disturbances.

Key words: Bulgaria, natural disturbances, *Picea abies,* windthrow.

Introduction

Disturbances are among the key processes that guide forest dynamics. They affect forest structure, composition, patchiness and resource availability (Oliver and Larson 1990, Laska 2001). To enable a real understanding of forest dynamics within a given ecosystem, their role on biodiversity and thus justify adequate forest ecosystem management, thorough knowledge of local natural disturbance regime is needed (Veblen et al. 1994, Schütz

2004, Franklin et al. 2002, Kuuluvainen 2002, Kulakowski and Bebi 2004). This is especially important in cases, when restoring of forest structure and composition to a more natural state is aimed (Schütz 2004).

Norway spruce (*Picea abiest* Karst.) is one of the main species in the subalpine forests in the Southern European mountain ranges (e.g. the Alps, the Carpathians, the Pyrenees, and the Balkan Mountains) and Northern Europe. These forests are very important for timber production, but probably even more for their protective functions against avalanches, rockfall (Brang et al. 2006), soil erosion, water-holding capacity (Raev 1986) and from the landscape functions. Several big storms in the last decades that caused wide-spread damages in high mountain forests (Lassig and Schonenberger 2000, Schelhaas et al. 2003, Zielonka et al. 2009) and expectations that climate change might increase the severity of storms (IPCC 2007), raised the attention paid to the influences of natural disturbances on Norway spruce forests and their resilience (Krauchi et al. 2000, Kulakowski and Bebi 2004). Yet, information about natural dynamics and disturbance regime of Central and South European high mountain Norway spruce-dominated forests is still limited (Korpel 1995, Krauchi et al. 2000, Splechtna et al. 2005). Among the reasons for this are difficulties in finding forest that were not managed for long enough period.

Parangalitsa forest in Rila Mts., Bulgaria, is among the few well-preserved in natural state Norway spruce – dominated forests in Southern Europe. Here we present a preliminary study of the natural disturbances in this forest ecosystem. Based on interpretation of orthophotos from 1966 and 1997 we outlined clearly detectable windthrows in the last 50 yrs. and forest patches that originated after stand-replacing disturbances within the last 100–150 years.

Material and Methods

Study site

Our study site is situated in the upper parts of Bistritsa valley in the Rila Mountains in Southwestern Bulgaria (Fig. 1). The study forest (250 ha) is situated in "Parangalitsa" reserve, which is one of the first strict forest reserves on the Balkan Peninsula. In was declared in 1933 and in 1977 it was included in the "Man and biosphere" list of UNESCO. Before that the region was considered as a protected forest and human activities were limited to hunting of local governors and pasturing on the higher grass-lands above the forest (Ruskov 1934). Timber harvesting on large scale was not allowed and the lack of roads additionally hindered any possible attempts. The lower parts of the forests are famous as some of those with highest productivity in Bulgaria (Standing volume of up to 1435 $m^3.ha^{-1}$ according to the local Forest Management Plan of 1966 (Nedyalkov 1966) and are representative of the optimum growth conditions for *Picea abies* (Raev et al. 1987).

The climate in the reserve is mountainous with influence from both Atlantic and Mediterranean cyclones. The average annual temperature at 1400 m a.s.l. is 4.9°C. It ranges from a mean

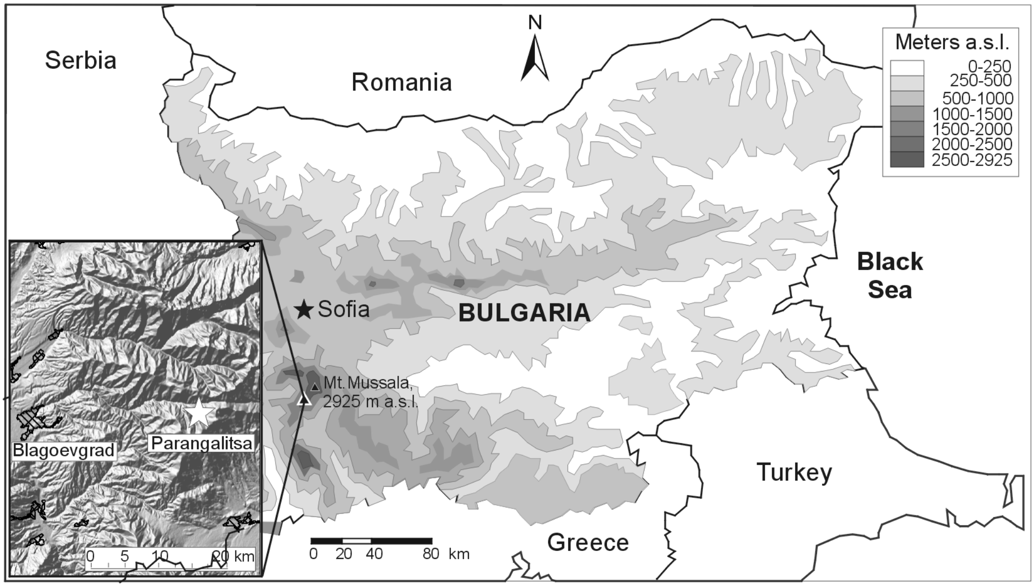

Fig. 1. Study site position.

monthly temperature of -3.7°C in January to +14.5°C in July. The annual precipitation amounts to 933 mm, with a maximum in late spring and early summer. Deep snow covers, frequently exceeding 1.5 m, are typical for the region (Raev 1986).

Data collection and analysis

The 250 ha study area was stratified by identifying internally-homogenous forest patches on aerial photos from 1966. Based on crown structure we subdivided several groups of forest patches: heterogeneous (different crown sizes, existence of empty spaces and gaps between the crowns); homogenous (densely situated crowns with similar sizes); recent disturbances (clearly visible fallen logs or cleared territories); treeline (separated trees and small groups at the treeline zone) and meadows within the forest. Because the image from 1966 reflects clearly the stand development in the previous 10–15 yrs especially in terms of disturbances, any clearly detectable blowdowns were considered as such which happened shortly before the mid 1960s (i.e. 1950s till 1960s). Homogenous patches were suspected to have originated after stand-replacing disturbances in case the age of the trees was within one age class (i.e. 20–30 years) (Oliver and Larson 1990). Additional verification of this hypothesis was done by analysis of tree ring cores from surviving older trees at the borders of the patches. Additional aerial photos from 1997 were used to detect changes between these two periods and verify the first stratification. Since the images were not originally orthorectified, we performed an absolute orientation of the aerial photos in order to generate the orthophotos. Ground control points were derived from GPS field measurements, existing satellite data as well as Google Earth software. Leica Photogrammetry Suite (LPS 9.1) was used for this purpose. To enable delineation of forest patches with reasonable size that could be defined on terrain as well as on the orthophotos we chose a minimum mapping unit (MMU) of 0.5 ha.

Forest patch ages were detected by obtaining tree ring cores. In each patch we collected samples from biggest and presumably oldest trees and from such from suspected younger cohorts. We also collected cores at the borders of detectable disturbances or uniform single-cohort patches because they could contain clues for dating of the disturbance events (Veblen et al. 1994). Tree ring samples were air-dried, mounted on wooden holders, and sanded with successively finer grades of sandpaper. Annual rings were recorded with semi-automatic video equipment at the dendrochronology laboratory of the Institute of Forest Growth (IWW) in Freiburg with precision of up to 0.01 mm. Measurement and cross-dating was performed with the Woodscan software (IWW) following standard procedures.

We dated windthrows by the presence of coincident sudden increases of radial growth (releases) and/or decreases in radial growth (suppressions) in surviving trees; and the age of a younger cohort within the disturbed area. Coincident tree establishment dates (i.e. age difference of no more than 20 years) in conjunction with rapid initial growth suggest establishment under open site conditions following a variety of stand-replacing disturbance (Kipfmueller and Baker 1998).

Results and Discussion

Disturbances in the second half of the 20th century

We could identify that more than 10% (26 ha) of the forest area was affected by windthrows shortly before the images in 1966 were taken. Of them the biggest contribution had the November 1962 windthrow, which caused complete blowdown of 18 ha in several forest patches (Fig. 2). According to the forest authorities' reports (Nedyalkov 1966) in the following few years (1962–1965) bordering areas to the 1962 windthrows were affected by sporadic tree-fall and bark-beetle mortality, which increased the total disturbance area to 22 ha by 1966. The storm also caused numerous single or small group tree falls in other regions of the forest. One zone with area of 4 ha ("N17") was affected by a windthrow in 1965. Another observed and documented windthrow that was clearly detectable on our orthophotos happened in 1983. It caused complete blowdown on a territory of about 3 ha within stand "N17". The affected area was bordering to a zone that suffered windthrow in 1965.

During the last decades there were also periods with increased release reactions in tree ring cores (e.g. in the late 1970s and late 1990s), which cannot be attributed as a consequence of these large-scale events. They reflect high intensity of small-scale disturbances with

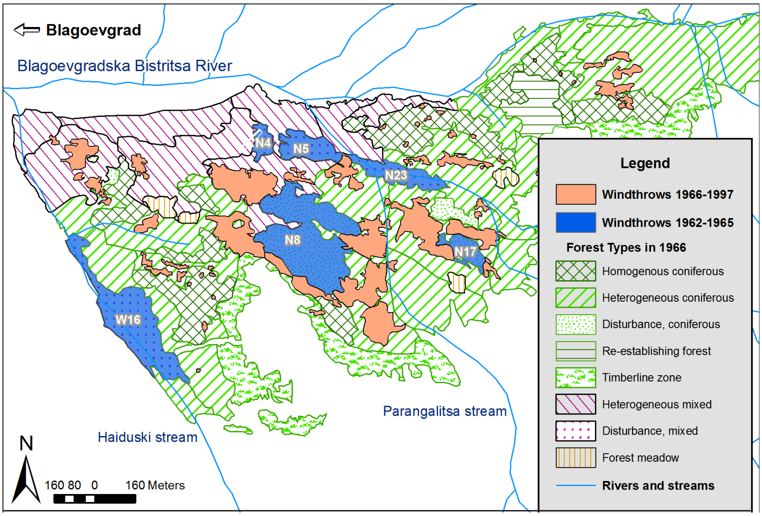

Fig. 2. Disturbances after the 1950^s.

dimensions of 0.1 to 1 ha. In the period between 1966 and 1997 (e.g. between the two sets of orthophotos) the majority of the mature trees were uprooted within small-scale disturbance patches on a total area of 9.3 ha (Fig. 2). About 30% of these new disturbance zones were bordering areas to windthrows form the period 1962–1965. In most of the cases these events were small-scale blowdowns, which later expanded. In several other patches there was continuous dieback caused by bark-beetles and single tree fall. Usually this mortality process was within small groups on the borders of recent blowdowns.

Disturbances before the 1950^s

We found 4 patches composed dominantly of one cohort whose origin could be related to a dated disturbance event (Fig. 3). The patch “W18” (territory approximately 5.2 ha) is formed mainly by trees that germinated shortly after 1873. All of these have fast initial growth suggesting good light conditions and lack of canopy layer to suppress the growth of young trees (Vablen 1994). The few older trees showed signs of radial growth releases in two periods – 1865 (with only several surviving trees) and 1873. Some older trees in neighbouring areas also displayed releases shortly after 1865, but this reaction was not found in all of the older trees. We suggest that there was an event with lower severity in the 1860^s which probably caused a medium scale windthrow with area of less than 5 ha within the territory of the current patch W18 and thus caused the release responses found in older trees. Later the territory expanded by additional falling of trees in 1873 thus forming the present borders of the patch. Such model of one initial disturbance event and later expansion of the territory by another event fits the most frequently observed one during the last 50 years.

The patch “N9” (territory approximately 2 ha) consists only of trees that reached coring height at the beginning of the 1870^s. Yet, in the few remaining older trees in the region we found only one with abrupt growth release in 1849. Several other trees in the bordering patch “N11” originated shortly after the 1840^s. While we cannot fix the date

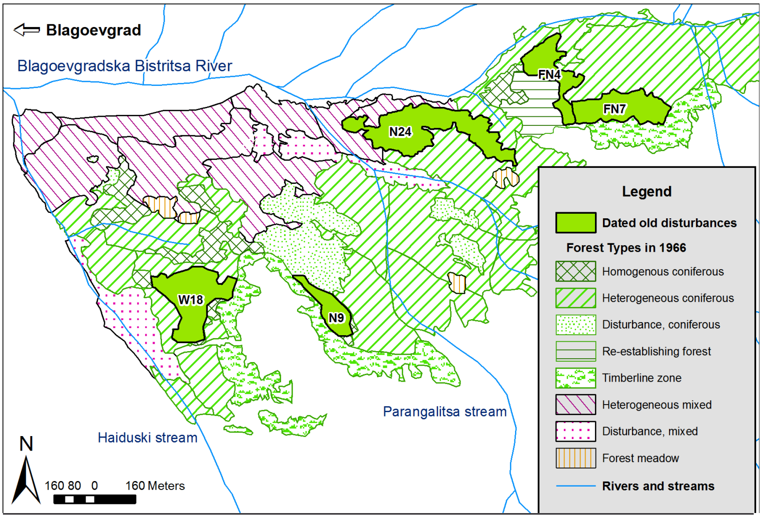

Fig. 3. Dated disturbances in "Parangalitsa" forest before 1950s.

of the event that caused the formation of this patch, the release in 1849 is a probable indicator.

Two single-cohort patches ("N24" and "FN4") were composed of younger trees that germinated at the beginning of the 20th century. Patch "FN4" (territory 3.1 ha) originated shortly after 1911. The neighbouring older trees, some of which situated at the exact borders of the patch, demonstrated abrupt growth releases after 1902. Pit-and-mound soil topography found on the territory of the patch suggests relatively recent windthrow (Ulanova 2000). Yet, there are two other findings that rise question about the character of disturbance. On one hand within the territory of the patch there are no trees that originated before 1911. There is also presence of numerous charcoal pieces straight on the surface or not fully buried by soil. Moreover, on one older tree at the border of the patch we found a fire scar, which was dated as formed in 1911. Other neighbouring older trees released at the period 1912–1914. Additionally, the first inventory reports from "Parangalitsa" forest (Georgiev 1933) mention a "fire-affected area", which according to the description matches the position of the region where our patch "FN4" is. We consider a probable explanation for the origin of patch "FN4" a combination of windthrow in 1902 followed by a low severity ground fire in 1911. Such event could explain the lack of any older trees within the patch territory since younger spruce trees would not survive even a low severity ground fire due to their thin bark and low-lying branches that easily catch fire. The other found signs also match those described as indications for fires in numerous studies of fire-driven disturbances in coniferous boreal forests (Veblen et al. 1994, Kuuluvainen 2002, Kulakowski et al. 2003).

Although we could not find an older tree with fire scar, we consider the reasons for formation of patch "N24" (territory approximately 7.3 ha) similar as those of patch "FN4". All trees germinated after 1912, with majority between 1915 and 1920. Older trees in neighbouring patches show growth releases after 1898. On the territory of the patch we found numerous pits- and mounds- soil formations. These clues suggest a windthrow disturbance at the late 1890s. Yet, here again there was presence of numerous charcoal pieces on the soil surface. This, together with the very similar age of trees and the complete lack of older trees within the patch gives us the ground to consider the reasons for its origin similar as those of patch “FN4” – e.g. a windthrow followed by low-severity fire.

In two of the other patches initially classified as “homogeneous” during the stratification process we also found that most of the trees generated within a short time period (“FN7” after 1830s, ”N7-1” after 1915). Yet, we could not date a disturbance with older neighbouring trees and even if such existed, we can only speculate about them based just on the age of the forest patch.

Conclusion

In Parangalitsa forest medium scale windthrows with dimensions of 4 to 10 ha were frequent during the last 150 years. Besides them small-scale damages with territory of up to 1 ha created a complex mosaic of forest patches with various ages and forest structures. Other disturbances such as bark-beetles and fires seem to have been less important. One of possible reasons is the high landscape heterogeneity of Parangalitsa, which limits the chances for fast spreading of fires or bark-beetles over vast territories. While a more complete description of the natural disturbance regime of the Southern European mountain forests requires further research in similar preserved ecosystems, our results contribute to better understanding of the natural range of variability of *Picea*-dominated ecosystems in this region and demonstrate that wind-driven disturbances with various sizes are normally expected.

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FORESTRY IDEAS, 2011, vol. 17, No 1 (41)

GENETIC STRUCTURE OF EUROPEAN BEECH OF MOTHER AND PROGENY STANDS IN POLAND ON THE BASIS OF DNA CHLOROPLAST MARKERS

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Abstract

European Beech (*Fagus sylvatica* L.) is one of the main forest tree species in Poland and covers 5.2% of the forest area. The most typical forest tree associations are formed at the lower forest belt in Carpathians and Sudety Mountains on the South of Poland and at the moraine landscape of Pomeranian Lake District of the northern Poland. In Poland, beech attains its north-eastern limit of natural range. The investigated six beech populations were classified according to phytosociogical characteristics to the following plant associations: *Galio-odorati-Fagetum* (Gryfino and Kwidzyn), *Dentario glandulosae-Fagetum* (Bieszczadzki National Park), *Luzulo-luzuloides-Fagetum* (Suchedniów, Tomaszów), *Dentario enneaphyllidis-Fagetum* (Zdroje). The genetic structure of these populations was analyzed. Thirty individuals per one generation (mother and progeny stands) in every provenance were investigated. The genetic variation and differentiation of mother stands and their open-pollinated progeny were characterized on the basis of DNA chloroplast markers: ccmp4, ccmp7 and ccmp10. Parameters of genetic diversity (H_S and H_T) and differentiation (G_{ST}) were counted and compared between mother and progeny generation.

Key words: *Fagus sylvatica*, genetic variation and differentiation, heterozygosity level.

Introduction

European beech (*Fagus sylvatica* L.) is one of the most important forest tree species in Poland and covers 5.2% of the forest area in Poland (Lasy Państwowe 2009). Present genetic structure of beech population was formed within last few thousand years influenced by many different factors, not only environmental (glacial epoch) nor genetic (selection) but also anthropogenic (Szafer 1935, Huntley and Birks 1983, Ralska-Jasiewiczowa 1983, Hazler et al. 1997). In Poland, beech attains its north-eastern limit of natural range, which is limited by: continental climate, soil conditions, winter temperatures and air humidity (Sławiński 1947, Jedliński 1953, Boratyńska and Boratyński 1990). The growth of beech stands outside the natural beech limit indicates that this species possess potentially wider range (Brzeziecki 1995, Tarasiuk 1999). Recent investigations of beech variation in Poland performed with isoenzyme study (Sułkowska 2002, Gömöry et al. 2003) showed high genetic diversity, similar to other neighboring European populations, slight decrease of average number of alleles per locus and lower level of differentiation towards the North of the natural range limit, which generally confirm the migration paths after glaciations. The present paper describes the genetic structure within one generation, i.e. mother and progeny beech stands in Poland assessed with chloroplast cpDNA markers.

Methods

The investigated nine beech populations were classified according to phytosociological characteristics to the following plant associations: *Galio-odorati-Fagetum* (Gryfino and Kwidzyn), *Dentario glandulosae-Fagetum* (Bieszczadzki National Park), *Luzulo-luzuloides-Fagetum* (Suchedniów, Tomaszów), *Dentario enneaphyllidis-Fagetum* (Zdroje) and were located within the natural range of beech distribution in Poland (Fig. 1). The genetic structure of these populations was analyzed. Thirty individuals per one generation (mother, progeny stands) in every provenance were investigated. The extraction of total DNA from the leaves was performed using Qiagen DNeasy™ Plant Minikit according to the manufacturer instruction (Qiagen). The quality and purity of DNA were analyzed on 1% agarose gel electrophoresis and via absorption in 230, 260 and 280 nm in NanoDrop® spectrophotometer (Wilmington, USA). The genetic variation and differentiation of mother stands and their open-pollinated progeny were characterized on the basis of three DNA microsatellite chloroplast markers: ccmp4, ccmp7 and ccmp10 according to Grivet et al. (2001) and Weising and Gardner (1999). DNA samples were analyzed in 8% acrylamide gel using automatic sequencer ALFexpress II (Amersham Pharmacia Biotech). Parameters of genetic diversity (H_S and H_T) and differentiation (G_{ST}) were counted and compared between mother and progeny generation according to Nei (1972, 1978) in PopGene 1.32 software (Yeh and Boyle 1997). The obtained results were elaborated with ALFwin Fragment Analyser™ 1.0 software.

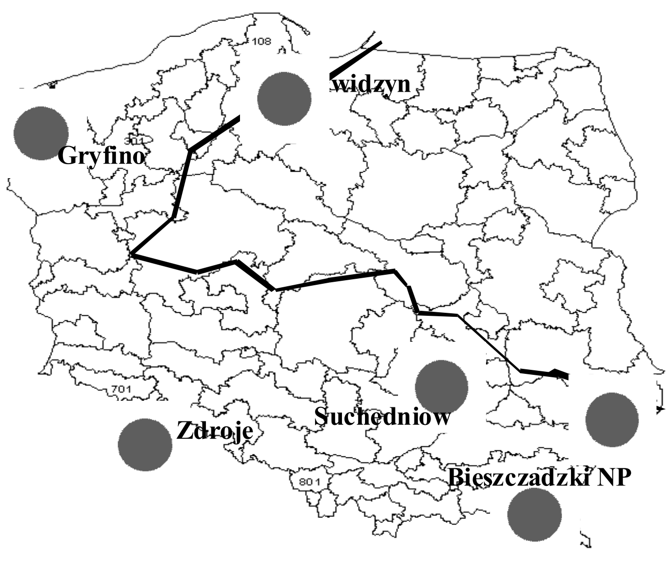


Fig. 1. Localization of investigated Polish European beech populations.

Results and Discussion

Quality and quantity of the analyzed DNA

The very high quality and purity of DNA were assessed on the basis of the ratio of absorbance at 260 and 280 nm. A ratio about ~1.8 was typical for most of the samples (Fig. 2), and proved a high purity of the extracted DNA. A ratio ~2.0 is generally accepted as „pure" for RNA. If the ratio is lower in either case, it indicates the presence of contaminants. The good quality of isolated DNA from samples was confirmed also by measurement of absorbance at 230 nm wavelength. The quantity of the genomic DNA samples balanced between 35 up to 160 ng.μl^{-1} and was fully appreciated to perform next steps of the DNA analysis procedure.

Genetic structure based on cytoplasmic DNA markers

As far as ccmp4 and ccmp10 microsatellite markers of Polish beech stands were concerned, different fragment-variants of haplotypes, i.e. from 116 to 152 base-pair size were distinguished. The ccmp7 locus was the most polymorphic, i.e. exhibited 8 allele variants: 144, 145, 147, 148, 149, 150, 151 and 152 bp. Nevertheless, some populations e.g. Bieszczadzki NP showed less polymorphism in the locus ccmp7, with only one 147 bp variant present, similarly to the previous study performed on Polish beech (Nowakowska and Oszako 2008). Another two loci, ccmp4 and ccmp10, shared the same range of 5 allele-size variants of 116, 117, 118, 119 and 120 bp. Generally, mother beech trees had more variable allele, than the

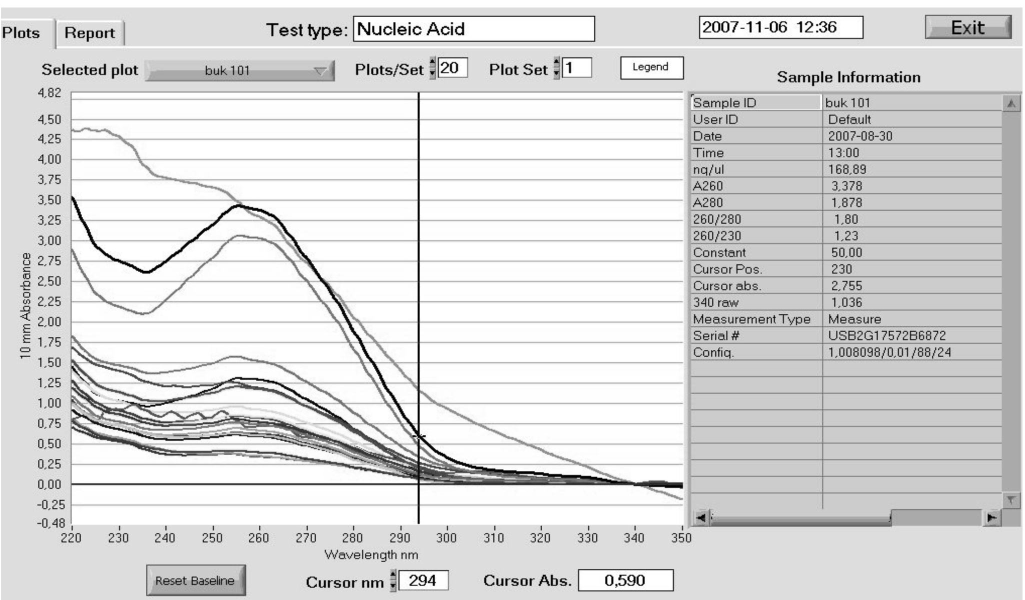
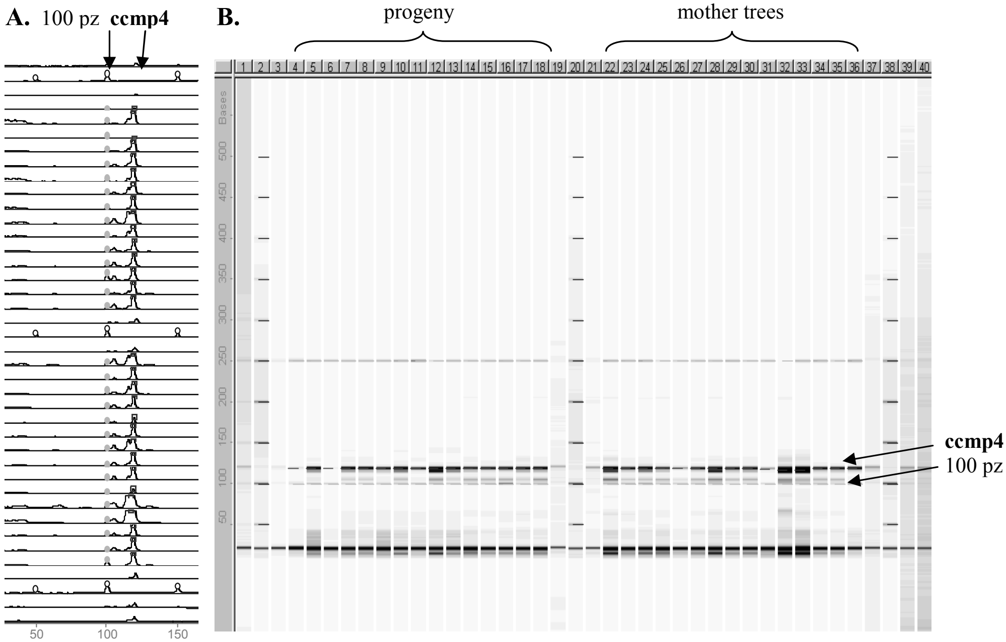


Fig. 2. Quantity and quality of the extracted DNA from 10 mother and 10 progeny beech trees from Bieszczadzki NP stand, analyzed in NanoDrop® ND-1000 spectrophotometer.



A: picks observed after automatic sequencer analysis. B: Electrophoresis on the acrylamide gel. 100 bp – size reference, ccmp4 – chloroplast locus with variable allele size around 119 base pairs. Lines 4–18: progeny trees, lines 22–36: mother trees.

Fig. 3. Amplification profile of the locus ccmp4 in Bieszczadzki NP stand.

progeny trees of the same provenance. For instance, mother trees presented alleles from 117 to 119 bp, comparatively to only one allele of 119 bp found in progeny from Bieszczadzki NP population (Fig. 3). Mean gene diversity among and within all studied mother stands (Table 1) were slightly higher (H_T = 0.4916, H_S = 0.3606 respectively) than mean genetic diversity in the progeny stands (H_T = 0.4600, H_S = 0.3375). The gene diversity level of all mother and progeny stands

Table 1. Genetic variation parameters of microsatellite loci analyzed in *Fagus sylvatica* L.

Locus	Mother Stands			Progeny Stands		
	H_T	H_S	G_{ST}	H_T	H_S	G_{ST}
ccmp4	0.5053	0.3058	0.3947	0.3957	0.3042	0.2313
ccmp7	0.3228	0.2667	0.1738	0.4650	0.2767	0.4050
ccmp10	0.6468	0.5092	0.2128	0.5193	0.4317	0.1688
Mean	0.4916	0.3606	0.2666	0.4600	0.3375	0.2663
St. Deviation	0.0264	0.0169	–	0.0038	0.0068	–

were almost at the same level: G_{ST} = 0.2666 and G_{ST} = 0.2663 respectively (Table 1). The overall haplotypic differentiation among studied Polish *Fagus sylvatica* populations was quite low (G_{ST} = 0.016), which means that most genetic diversity resides within the stands. More cpDNA variation in the chloroplast ccmp4, ccmp7 and ccmp10 loci (G_{ST} = 0.810) was reported for other 400 European beech populations (Magri et al. 2006).

High forest genetic diversity has a crucial role in maintaining biological diversity at species and ecosystem level, in the way of better forest trees survival, adaptation and evolution under changing climatic conditions. Facing several biotic and abiotic threats, variable gene pool may ensure the vitality of forests and better resistance against drought, pests, diseases and inappropriate use of forest reproductive material. Our study demonstrated almost unchanged gene pool between mother and progeny *Fagus* Polish stands, which means stable gene transmission between one generation analyzed.

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LONG-TERM EFFECT OF THINNING ON PRODUCTION AND FOREST-FLOOR CHARACTERISTICS IN SCOTS PINE STANDS IN THE POLABI LOWLAND (CZECH REPUBLIC)

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Abstract

We observed the long-term effect of management (two thinning regimes: 2a – by positive selection from above and 3b – by negative selection from below compared to 1c – unthinned control) on production characteristics and forest-floor status in the stands of Scots pine in Central Bohemia (Czech Republic). We found similar effect of treatment on production during the 45-year long period of investigation, i.e. lower periodic basal area increment of both thinned stands compared to control. About 6–9 thousand kg of dry-mass was stored per hectare in humus horizon L in the stands studied in 2008, with insignificant differences among the variants. In horizon F, we found about 14–18 thousand kg of dry-mass per hectare and differences among variants were again insignificant. Significantly higher amount of dry-mass was observed in horizon H in control unthinned plot compared to both variants with thinning (103, 45 and 60 thousand kg per ha in variants 1c, 2a and 3b, respectively). The results obtained indicate that studied thinning regimes resulted in significantly decreased amount of accumulated humus in forest-floor and consequently carbon and nitrogen under treated pine stands.

Key words: Scots pine, thinning, production, forest-floor.

Introduction

In the sustainable management of forests silvicultural measures should be directed not only at wood production, but also at sustainability of production capacity of site. In the Czech Republic, Scots pine (*Pinus sylvestris* L.) is the second most common species (about 17% in the tree species composition). Commercial forests with Scots pine stands are usually cultivated on the sandy sites in lowlands. In Central Bohemia, Scots pine stands are managed by clear-cutting system with relatively intensive soil preparation before planting (logging slash is removed completely and clear-cut area is ploughed after each rotation) and important part of forest ecosystem – humus horizons – are recreated in each rotation. Consequently, during the rotation period, foresters can influence development of stands by thinning. The aim of this study was to found possible long-term effect of thinning on production and forest floor in Scots pine stands in the Central Bohemia. Specifically, effect of thinning on stand basal area and amount of dry-mass, carbon and nitrogen in humus horizons under pine stands were investigated.

Material and Methods

Research was done on experimental series Kersko II established in 1962 in 34-year-old pine stands in site *Carpineto-Quercetum mesotrophicum* (according to Viewegh et al. 2003). Elevation of the stands is 205 m above sea level and the coordinates (in the World Geodetic System 1984) of the series are 50°08′40″ and 14°55′38″. Stands are located on arenic cambisol. According to data from the Czech Hydrometeorological Institute, during the period 1961–2000 mean annual precipitation was 501–550 mm and mean annual temperature was 8.6–9.0°C.

Intensive soil preparation by stump extraction and ploughing was done before planting. The experiment was established in 1962 at a stand age of 34 years. In the same year the first thinning was done. There were three treatments (each of an area 0.25 ha): a thinning treatments – positive selection from above (plot 2a), negative selection from below (3b) and unthinned control (plot 1c).

After the first thinning the stands were measured regularly at 5-year periods for diameter at breast height and height.

In autumn 2008 when the stands were 79-year-old, forest-floor humus horizons (L = fresh litter, F = fermented litter and H = humified litter) were investigated quantitatively and qualitatively on identical comparative plots. We used steel frames (25x25 cm) to define sampling area at six replications in each plot (1c, 2a, 3b). All samples were dried, first in open air, then in a laboratory oven at 80°C, and subsequently weighed (dry mass).

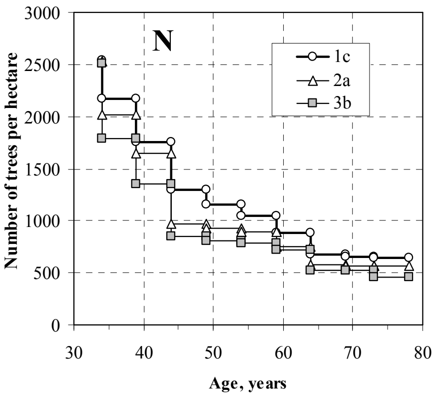
We measured the concentration of oxidizable carbon (C_{ox}) from composite samples (three per treatment) using the spectrophotometric determination of organic carbon in soil – oxidation by chromosulfuric mixture and determination colorimetrically (Walinga et al. 1992). Nitrogen content was assessed from composite samples (three per treatment) after mineralization by mineral acids and analysed using Kjeldahl procedure.

All statistical analyses were performed using multi-sample nonparametric tests (Kruskal-Wallis one-way ANOVA). Unless otherwise indicated, test levels of $p < 0.05$ were used throughout. For this analysis the Unistat (2000) statistical software package was used.

Results

Growth

During the 44-year investigation (1962–2007), the basal area of the control 1c and treated plots 2a and 3b exhibited different trends (Fig. 1). Experimental series started at the age of 34 years having initial basal area 38.7, 37.8 and 35.9 $m^2.ha^{-1}$ on the plots 1c, 2a and 3b, respectively (differences were insignificant). After the third experimen-



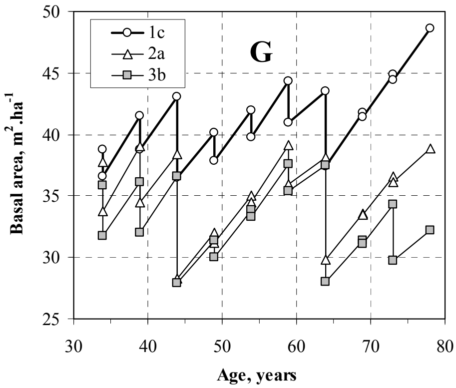


Fig. 1. Number of trees (N) and mean basal area (G) development in the pine thinning experimental series Kersko II in the Central Bohemia.
Legend: plot 1c – control treatment; plot 2a – positive selection from above; plot 3b – negative selection from below.

tal treatment (1972), the basal area of the control and thinned plots differed significantly. The basal area was 36.5, 28.3 and 27.9 $m^2.ha^{-1}$, respectively, for control 1c and thinned plots 2a and 3b. These differences continued to the end of observation period. At the age of 78 years, the control stand 1c had reached 48.7 $m^2.ha^{-1}$, whilst the basal area of the thinned stands was 38.9 and 32.3 $m^2.ha^{-1}$ on plots 2a and 3b, respectively.

Status of humus layers

In 2008 (age of 79 years), 6–9 thousand $kg.ha^{-1}$ of dry-mass was stored per hectare in humus horizon L (Tab. 1). This horizon consisted of about 2.1–4.0 thousand kg of mosses, 1.3–1.6 thousand kg of dry bark and twigs, 1.2–2.3 thousand kg of cones and 1.2–1.5 thousand kg of needles per hectare. Differences were significant only for moss amount and for total dry mass between 1c and 2a in the frame of horizon L.

In the F horizon we found 13.9–17.6 $t.ha^{-1}$ dry mass with insignificant differences between the treatments. The greatest amount of dry mass was found in the H horizon: 102.9, 45.4 and 60.1 $t.ha^{-1}$ on plots 1c, 2a and 3b, respectively. Comparison of the control 1c to the both thinned treatment 2a and 3b showed significantly greater dry mass (about 126% more compared to plot 2a and about 71% more compared to plot 3b) under unthinned stand.

Altogether, horizons L+F+H contained 123.0 (1c), 69.7 (2a) and 86.7 (3b) $t.ha^{-1}$ of dry mass. Differences between treatments were also significant. We found greater amount of dry mass under unthinned control stands (about 76% more compared to plot 2a and about 42% more compared to plot 3b), largely due to contributions of the H horizon.

Consequently, the significantly lower amount of dry mass in the H horizon resulted in lesser amount of accumulated oxidizable carbon (C_{ox}) under treated pine stands 2a and 3b (Fig. 2). While on control plot we found about 10.9 $t.ha^{-1}$

Table 1. Amount of dry mass in humus horizons in the pine thinning experimental series Kersko II in the Central Bohemia at the age of 79 years.

Horizon	Treatment						Significance p*		
	1c	S.E.	2a	S.E.	3b	S.E.	1c/2a	1c/3b	2a/3b
L (moss)	2.1	(0.42)	3.7	(0.30)	4.0	(0.48)	0.04	0.01	n.s.
L (bark + wood)	1.3	(0.28)	1.6	(0.20)	1.4	(0.45)	n.s.	n.s.	n.s.
L (cones)	1.2	(0.42)	1.3	(0.40)	2.3	(0.93)	n.s.	n.s.	n.s.
L (needles)	1.5	(0.38)	1.2	(0.13)	1.3	(0.18)	n.s.	n.s.	n.s.
L (total)	6.1	(0.36)	7.8	(0.43)	9.0	(1.3)	0.07	n.s.	n.s.
F	13.9	(2.00)	16.5	(0.46)	17.6	(1.01)	n.s.	n.s.	n.s.
H	102.9	(9.59)	45.4	(2.33)	60.1	(6.36)	0.0002	0.002	n.s.
L+F+H	123.0	(9.09)	69.7	(2.12)	86.7	(7.72)	0.0004	0.008	n.s.

*n.s. = not significant ($p>0.05$), S.E. – Standard error.
Legend: L = litter, F = fermentation, H = humus (means with standard errors) for control treatment (plot 1c) and thinned treatments (plot 2a – positive selection from above, plot 3b – negative selection from below).

of C_{ox} in the H horizon, only 5.9 and 4.2 t.ha^{-1} of C_{ox} were stored in this horizon on the treated plots 2a and 3b, respectively.

The total amount of C_{ox} in the H horizon was by 84 and 159% higher on the control 1c compared to the thinned treatment 2a and 3b.

The amount of nitrogen in the H horizon displayed similar trends as carbon, i.e. larger quantity of nitrogen was stored under control plot. The differences between treatments of about 94 and 106% (control 1c 1334 and thinned 2a and 3b 687 and 649 kg.ha^{-1} of N) were significant ($p = 0.0001$).

Discussion

In our experiment we found negative effect of thinning on the basal area of pine stands in the 35-year period of observation. This observation is partly supported by published results (Pirogowicz 1983, Huss 1983) showing that intensive thinning in 50-year-old and older pine stands did not result in higher diameter increment. An important factor in evaluating humus layer formation is the significantly different basal area of comparative plots (control and thinned) during the more than 30 years (age of 44–70 years).

No effect of thinning on dry-mass content in the L (with the only exception of moss content) and F horizon was found. Altogether, the combined L + F horizons contained 20–27 $t.ha^{-1}$ of dry mass. These data correspond with the value of 32.5 $t.ha^{-1}$ published by Komlenović (1997) or 25 $t.ha^{-1}$ reported by Podrázský (1995), although the pine stands investigated in these studies were only 30 years old.

Our study found an effect of thinning on the amount of dry mass in the H horizon. Opening the canopy (and consequently a decrease in basal area) resulted in reduction of dry mass in the H horizon at the end of the observation period. This partly corresponds with Sariyildiz (2008), who reported significant effects of tree canopy on litter decomposition rates in pine stands. His study showed that the tree canopy can significantly alter litter decomposition rates of Scots pine.

The L + F + H horizons together contained 123 $t.ha^{-1}$ of dry mass on the control plot. This amount is higher as compared to the published values of 60 $t.ha^{-1}$ in a 33-year-old stand (Podrázský 1995) and 46 t and 71 $t.ha^{-1}$ in 49- and 63-year-old stands, respectively (Muys 1995). For all horizons (L + F + H) the differences between the observed treatments were significant and consequently, the investigated thinning regime resulted in loss of dry mass (by 42–76%) in humus horizons by the end of the observation period.

Clearly, the most significant differences between treatments were in the H horizon. In this layer we found about 11 $t.ha^{-1}$ of C_{ox} on unthinned control treatment. Different amounts of carbon have been reported for other Scots pine stands: 500–2,500 $kg.ha^{-1}$ (Kurbanov et al. 2007) and 20–38 $t.ha^{-1}$ (Heinsdorf 1997), but these results represent all horizons (forest floor). However, comparison is rather complicated because different methods of carbon analysis (C_{ox} or C_{tot}) were used.

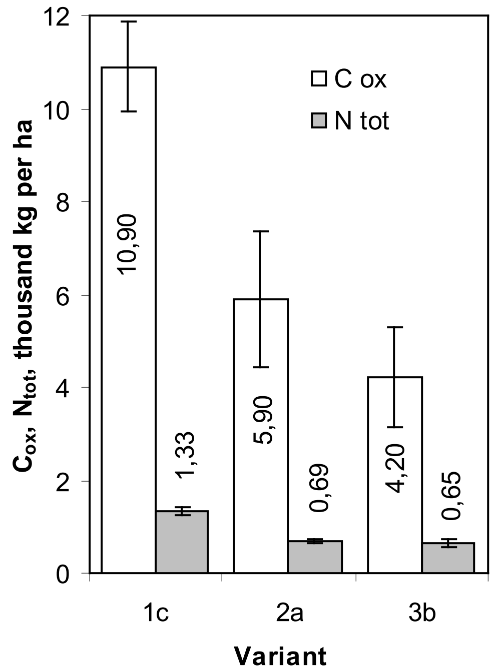


Fig. 2. Mean amount of oxidizable carbon C_{ox} and total nitrogen $N_{tot.}$ in the pine thinning experimental series Kersko II in the Central Bohemia at the age of 79 years.
Error bars denote standard errors in horizon H for control treatment (plot 1c) and thinned treatments (plot 2a – positive selection from above, plot 3b – negative selection from below).

The H horizon contained about 1,330 $kg.ha^{-1}$ of nitrogen on unthinned control. Comparable amounts (900–1,700 $kg.ha^{-1}$) were found in another study (Heinsdorf 1997) but that was for the complete forest floor. Differences between treatments for nitrogen were not as pronounced as they were for carbon content. However, the amount of nitrogen under thinned stands was lower and the difference was significant.

Conclusions

During the 46-year long period of investigation the basal area of control plot and treated plot exhibited similar trends. After the third experimental treatment (1972) the basal area of the control 1c and thinned plots 2a and 3b significantly differed.

- In terms of humus layers investigation it was basically important that more than 30 years led to significantly lower basal area on thinned variants compared to control.
- At the age of 79 years, horizon L contains about 6–9 thousand kg of dry-mass under pine stands.
- In horizon F, we found from 13.9 to 17.6 thousand kg of dry-mass per hectare.
- The highest amount of dry-mass was found in horizon H – from 45 to 103 thousands kg per hectare. Differences between variants were significant only for horizon H and consequently for the complete forest floor (L + F + H).
- Studied thinning regimes (positive selection from above and negative selection from below) resulted in decreased amount of accumulated humus and consequently carbon and nitrogen stock in H horizon under treated pine stands.

Acknowledgements

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STUDYING THE COMPETITION IN NATURAL STANDS OF *ACACIA SEYAL* DEL. VARIETY *SEYAL*

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Abstract

In this study, above ground competition was assessed for individually selected *Acacia seyal* Del. var. *seyal* trees growing in natural stands of different densities. The main objective was to develop a model to estimate the competition index (CI) depending on measuring diameter at breast height (DBH). Data were collected from Umfakarin reserve forest, south Kordofan, Sudan, between September 2007 and February 2008. A total of 286 subject trees of *Acacia seyal* were selected in stands of three different densities, related to different number of stems per hectare. A method for identifying the competitors (adjacent trees) of the subject trees was developed. Tree dimensions such as DBH, height, height of crown base and crown radii for each subject tree and its competitors were measured. Polar coordinates were used to describe the positions of competitors to each subject tree. Eight competition indices were quantified using CroCom program for each subject tree and correlated with its dimensions. The results of the study indicated a remarkable decrease of tree dimensions, especially DBH, when the competition index increased. Regression models between selected indices and tree dimensions showed significant relationships. The results of the study could serve in defining and improving silvicultural treatments, such as thinning programs, of *Acacia seyal* in natural stands.

Key words: *Acacia seyal*, competition index, CroCom, South Kordofan, Umfakarin forest.

Introduction

Competition is a process which occurs among individuals using the same limited resource (Kimmins 2004, Begon et al. 2006). In forest stands, trees often grow close together and compete for resources, such as light, space, water and soil nutrients. Trees that receive few resources will usually perform and produce less than the trees receiving enough resources. As a result of competition among trees, available growing space for each individual decreases, and very severe competition leads to mortality. Ford and Sorrensen (1992) summarized the concept of competition among trees in forest stands into five axioms (as cited in Vanclay 1994):

1) plants modify their environment as they grow, reducing the resources available for other plants;

2) the primary mechanism of competition is spatial interaction;

3) plant death due to competition is a delayed reaction to the growth reduction following resource depletion;

4) plants adjust to environmental change, responding to competition and altering the nature of the competition and

5) there are species differences in the competition process.

A competition index (CI) is any index that describes the degree of competition caused by adjacent trees to the growth and production capability of a subject tree. Various approaches have been developed in order to estimate the degree of competition. Most of these approaches (Hegyi 1974, Biging and Dobbertin 1992, Pretzsch 1995, Nagel 1999, Münder and Schröder 2001) for quantifying the competition indices can be classified in the following categories (Vanclay 1994): a) competitive influence zone;

b) area potentially available;

c) size-distance including horizontal and vertical variants, and

d) sky-view and light-interception approaches.

However, Alder (1995) classified the methods for computing the competition indices into three categories based only on spatial distribution of the trees, i.e. similar to the first three categories mentioned by Vanclay (1994).

Competition is either distance-dependent or distance-independent (Vanclay 1994, Alder 1995, Wimberly and Bare 1996). The distance-dependent or spatial competition is based on tree coordinates or locations (Hegyi 1974, Biging and Dobbertin 1992, Pretzsch 1995, Münder and Schröder 2001) while distance-independent or non-spatial competition is based on crown dimensions and other variables such as light interception (Wykoff et al. 1982, Nagel 1999).

Acacia seyal is widely distributed in eastern and central clay plains of the Sudan, often dominating the vegetation community and forming pure stands (MacAllan 1993) of different densities or mixed stands. In Sudan, *A. seyal* is managed only for firewood and charcoal and is subject to clearing for agricultural machinery. The species is also an important source of commercial gums (gum *talha*), building poles, forage, and source of nectar for honey bees (Elamin 1990, von Maydell 1990, Mustafa 1997). Additionally, *Acacia seyal* has valuable ecological functions such as reducing soil erosion and serving as a defence line for desert encroachment in many parts of the Sudan.

The objective of the study was to investigate competition among trees of *Acacia seyal* growing in natural stands of different densities and to derive different competition indices (CIs) depending on diameter at breast height (DBH), which is the simplest parameter to be measured in the forest stands.

Materials and Methods

Study area

The present study was conducted at Umfakarin Natural Forest Reserve (2,689 ha), south Kordofan State, Sudan. The forest lies between 12° 29′ and 12° 35′ N, and between

31°17′33″–31°20′ E. Annual rainfall ranging from 350 to 900 mm commences in May and lasts until September or October with a peak in August. Seasonal flooding is the most conspicuous feature of this forest. Every year most parts of the forest, especially the dense vegetation patches, are inundated for almost more than two months. Temperature ranges from 30 to 35°C. The prevailing soil types are clay plains and sandy clay locally known *Gardud* (non-cracking). In general, the forest reserve can be described as slightly undulating land surface with the exception of few seasonal streams penetrating some parts of the forest. No physical features seem to be clearly bounded the forest reserve.

The structure of Umfakarin forest can be characterized either by type and/or density of vegetation cover prevailing. *Acacia seyal* with different densities is dominating the forest. These densities can be categorized based on number of trees per unit area (hectare) into three strata i.e. dense, medium and slight stratum. Other tree species are also found such as *A. mellifera, Balanites aegyptiaca, A. polycantha, A. senegal, A. nilotica, A. sieberana, Cordia africana, Boscia senegalensis* and *Dichrystachst sinaria*.

Identifying the competitors and tree measurements

Different methods have been used to identify the competitors (adjacent trees) of subject trees. For example, Arney (1973, ex Wimberly and Bare 1996) used a radius equivalent to open-grown crown radius. Hegyi (1974) included all competitors within a radius of ten feet (3.05 m). In this study, the radius used to identify the competitors was defined as the radius of a circle equal to the height of the subject tree multiplied by a factor (1.25), i.e. $R=h*1.25$ where R is the growing space radius (m) and h is tree height (m). All trees falling within this radius were considered as competitors to the subject tree.

A total of 286 subject trees were selected in three stands of different densities (i.e. dense = 104, medium = 91 and slight = 91). The selection was based on the diameter at breast height (DBH, cm) of the subject tree. The following variables were measured for each subject tree and its competitors: tree coordinates, DBH, height, height to the crown base, crown radii, canopy class, i.e. overstory or understory and crown permeability factor (CPF, estimated as 0.4 for *A. seyal*).

Statistical computations and data analysis

Some statistical indicators for DBH, height and crown diameter were obtained. Competition indices (CIs) were quantified using the computer program CroCom (Münder et. al. 2008). The models used for quantifying competition indices by CroCom are shown in Box 1. The necessary input variables for the program (for each subject tree and its competitors) are tree code, species and number, tree location or coordinates, DBH, height, crown radii, height of crown base, canopy class and CPF. Locations of the competitors were identified by polar coordinates (θ, d), which were converted to Cartesian coordinates (x, y).

Distance-dependent competition indices

1. CI_HEGYI (Hegyi 1974, Eq. 1): Also known as "Jack-Pine-index" was found in 1974 by Hegyi and is considered as a famous and simple index (Münder 2005; Schröder 2003) for quantifying competition in forest stands. The index is based only on the DBH values of the subject tree and its competitors and the horizontal distance between each competitor and the subject tree.

2. CI_HEGYI_2 (Hegyi 1974, Eq. 2): Similar to the above mentioned index, but using basal area instead of DBH.

3. CI_KV (Biging and Dobbertin 1992, Eq. 6): The index uses crown volume based on height of intersection of search cone and tree axis.

4. CI_KF (Biging and Dobbertin 1992, Eq. 7): Based on horizontal crown area and the height of intersection of search cone and tree axis.

5. CI_PRETZSCH (Pretzsch 1995, Eq. 4): The index is part of forest growth simulator SILVA developed at the Chair of Forest Yield Science at Technische Universität München, Germany. It is used to quantify the competition based on the identification of competitors by means of the search cone method.

6. CI_VKF (Münder and Schröder 2001, Eq. 3): The index is based on the vertical crown area and the horizontal distance between the competitors and the subject tree.

Distance-independent competition indices

1. CI_C66 (Nagel 1999, Eq. 5): Based on the horizontal crown area at the height of greatest crown width. The C66 of a subject tree is calculated by summing up the horizontal crown areas of all trees cut at the height of its greatest crown width, which is fixed at 66.6% of crown length from the top (Nagel 1999 ex Schröder et al. 2007). The C66 index identifies competitors either as all trees belonging to the same stand (distance-independent mode) or by checking a fixed critical radius (distance-dependent mode) (Schröder et al. 2007). The later method was used in this study for identifying the competitors.

2. CI_BAL (Wykoff et al. 1982, Eq. 8): The index is also termed as "G_{cum}" or shading index (Schütz 2001, Schütz and Röhnisch 2003) and can be calculated by summing up the basal areas of the trees larger (BAL) than the subject tree.

Box 1 shows the models used for quantifications of various CIs in CroCom programme.

Partial correlation was used to check if any association exists between the competition index and the tree size e.g. DBH, when the effect of all other tree dimensions is removed. The association between tree dimensions (DBH, height and crown diameter) and various competition indices was tested by the following non-linear regression model (Eq. 9).

$$CI = a_0 + a_1 * \ln(x) \tag{9}$$

Where: CI = competition index; x = tree dimension; $\ln$ = natural logarithm; a_0 and a_1 = coefficients.

Results

Results of partial correlation analysis indicate that DBH is positively correlated with height and crown diameter for all three strata (different stand densities).

Box 1. Models used for CIs quantification in CROCOM Programme.

Distance-dependent competition indices

$$CI\_HEGYI = \sum_{j=1}^{nj} (d_j . d_i^{-1}).(dist_{ij} + 1)^{-1} \quad (1)$$

$$CI\_HEGYI\_2 = \sum_{j=1}^{nj} (g_j . g_i^{-1}).(dist_{ij} + 1)^{-1} \quad (2)$$

$$CI\_KV = \sum_{j=1}^{nj} KV_j (SH).KV_i^{-1} \quad (3)$$

$$CI\_KF = \sum_{j=1}^{nj} KF_j (SH).KF_i^{-1} \quad (4)$$

$$CI\_PRETZSCH = \sum_{j=1}^{nj} Beta_{ij} .KQF_i .KQF_j^{-1} \quad (5)$$

$$CI\_VKF = \sum_{j=1}^{nj} VKF_j .VKF_i^{-1}.(dist_{ij} + 1)^{-1} \quad (6)$$

Distance-independent competition indices

$$CI\_C66 = \sum_{j=1}^{nj} KF_{j(HGK_i)} \quad (7)$$

$$CI\_BAL = \sum_{j=1}^{n_{\max}} (g_j) \quad (8)$$

Source: (Schütz 2001, Schröder 2003, Schütz und Röhnisch 2003, Münder 2005, Rivas et al. 2005)

Where: *i* = focal tree; *j* = competitor; *d* = diameter at breast height (DBH); BAL = basal area of larger tree than the subject; gj = basal area, m²; Gcum = cumulative basal areas of trees larger than the subject; nmax = number of trees having basal area larger than the subject tree; *nj* = number of competitors; $dist_{ij}$ = distance $tree_i$ - $tree_j$; *Beta* = gradient of straight line connecting base of search cone and top of competitor tree; *KF* = horizontal crown area; *KQF* = *KF* at height of search-cone base; *HGK* = height of greatest crown width (fixed at 66% of crown length from the top); SH = height of intersection of search cone and tree axis; *KV* = crown volume; *VKF* = vertical crown area; *h* = height.

The partial correlation coefficient (r) ranges from 0.228 to 0.405 for height and 0.503 to 0.731 for crown diameter.

Descriptive statistics of the variables are shown in Table 1. Stands of dense, medium and slight density have mean diameter at breast height (DBH) equals to 13.9, 14.7 and 12.8 cm with mean height 7.3, 7.8 and 6.7 m respectively.

Competition index in relation to tree dimensions

The results of the data analyzed by CroCom are intra- and inter-specific competition indices. According to CroCom definition intra-specific competition is expressing the competition between trees sharing the same crown layer while inter-specific competition is between trees growing in different layers. However, in ecology, inter-specific competition refers to the competition that occurs whenever two different species attempt to utilize the same limiting resource, while intra-specific is the competition between individuals of the same species (Kimmins 2004, Begon et al. 2006). To avoid confusion between the two concepts (in CroCom and ecology), intra- and inter-layer competitions were termed referring to the competition between trees sharing the same layer and competition between trees growing in different layers, respectively. The final competition index induced by the competitors is the sum of inter- and intra-layer competition.

Table 1. Statistics parameters for some variables of *Acacia seyal* dimensions, Umfakarin natural forest reserve south Kordofan, Sudan 2007/2008.

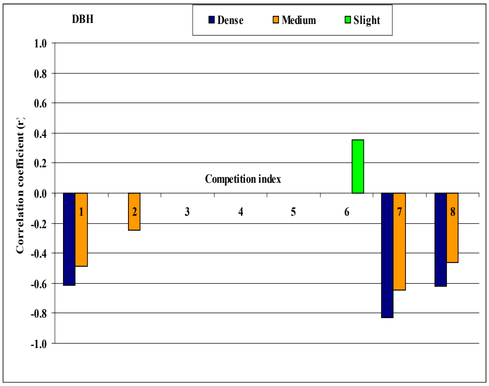
Stratum*	Variable	Parameter			
		Minimum	Maximum	Mean	Std. Deviation
Dense	DBH, cm	5.0	36.9	13.9	2.6
	Height, m	4.5	11.5	7.3	1.0
	CD, m	2.0	1.05	5.5	1.5
Medium	DBH, cm	5.8	33.4	14.7	2.3
	Height, m	4.0	9.5	7.8	1.3
	CD, m	1.7	8.4	4.6	1.3
Slight	DBH, cm	6.7	15.1	12.8	2.3
	Height, m	3.0	10.0	6.7	1.2
	CD, m	1.8	8.4	4.6	1.2

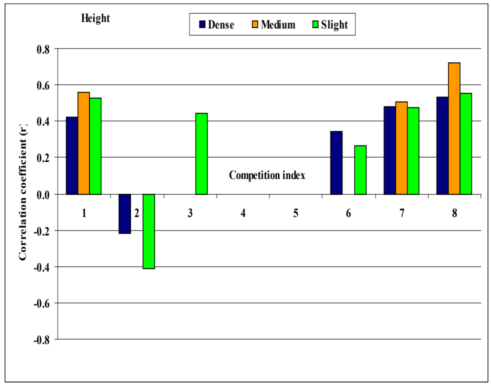
*Number of trees per hectare (dense = 396; medium = 271; slight = 209); DBH = diameter at breast height; CD = crown diameter.

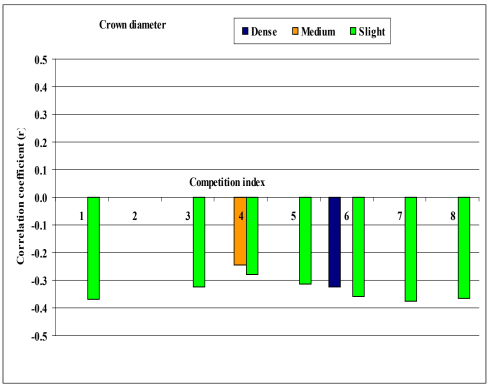
Fig. 1 shows the significance of partial correlation between various competition indices and tree dimensions. Results of partial correlation indicate that only few indices show a clear and significant correlation (CI_HEGYI, CI_PRETZSCH, CI_C66, CI_HEGY_2, and CI_BAL) with DBH in the three different strata (Fig. 1). The maximum partial correlation coefficient (–0.829) was obtained by CI_HEGYI_2 in dense stratum. With exception to CI_BD_KV and CI_VKF, tree height encountered a clear relationship with all indices in all strata with a maximum correlation coefficient (r) of 0.559 in medium stratum. Crown diameter revealed negative partial correlation with all indices except CI_PRETZSCH, which showed no correlation.

Selection of competition index

The criterion for selecting the appropriate competition index that better relates







Definition of indices:

1 = Hegyi index (CI_Hegyi)
2 = Pretzsch index (CI_Pretzsch)
3 = Biging and Dobbertin index (CI_KF)
4 = Biging and Dobbertin index (CI_KV)
5 = Münder and Schröder index (CI_VKF)
6 = Nagel index (CI_C66)
7 = Hegyi index (CI_Hegyi_2)
8 = BAL index (CI_BAL).

Fig. 1. Summary of the results about the significance of partial correlation between various competition indices (CIs) and tree dimensions of *Acacia seyal* at Umfakarin natural forest, south Kordofan, Sudan (2007/2008).

to tree dimension in a specific stratum was based on coefficient of determination (R^2). The higher the value the better index expresses the relationship. Values of R^2 vary according to tree dimension, competition index and stratum (Table 2). The maximum R^2 (0.651) was obtained as a result of relationship between DBH and HEGYI_2 index. Effect of competition on the tree dimensions is depicted in Fig. 2. The figure shows that while height is increasing, DBH and crown diameter are decreasing with increasing competition intensity.

Discussion

Tree diameter is the most sensitive variable to competitive stress. Additionally, variations in tree dimensions are caused by stand density. Tree density has been recognized as a major factor determining the degree of competition among trees in forest stands. Growth or production performance of individual trees decreases as the density per unit area increases. In dense situations, many trees may not be able to develop their crowns because of

Table 2. Results of the model used for testing the relationship between CIs and tree variables in different stand strata of *A. seyal* at Umfakarin forest, south Kordofan, Sudan.

Index	Dense stratum (number of target trees = 104)								
	Diameter at breast height, cm			Height, m			Crown diameter, m		
	a_0	a_1	R^2	a_0	a_1	R^2	a_0	a_1	R^2
Hegyi	7.529	-2.290	0.314			0.029	2.889	-0.641	0.081
Pretzsch			0.020	76.413	-36.878	0.074			0.002
BD_KF	96.245		0.029	149.507	-66.822	0.046			0.007
BD_KV			0.021	301.333	-140.953	0.053			0.003
VKF	7.587	-2.332	0.054	10.150	-4.187	0.064	3.230		0.026
C66			0.015	-267.331	215.252	0.122	216.584		0.031
Hegyi_2	15.749	-5.410	0.651			0.002	4.139	-1.101	0.089
BAL	12,062.205	-3,571.910	0.227		454.653	0.073	5,001.285	-1112.286	0.073
	Medium stratum (number of target trees = 91)								
Hegyi	4.632	-1.349	0.154		1.082	0.085	2.697	-0.905	0.149
Pretzsch	26.332	-9.682	0.230	13.158	-5.362	0.061	11.508	-5.861	0.181
BD_KF			0.003			0.000			0.011
BD_KV			0.008			0.001			0.016
VKF	7.116	-2.253	0.114			0.002	4.303	-1.792	0.156
C66			0.001			0.001			0.000
Hegyi_2	11.186	-3.811	0.387			0.008	4.580	-1.788	0.183
BAL	4,284.817		0.005	-2,511.140	768.168	0.356	3,721.466		0.011
	Slight stratum (number of target trees = 91)								
Hegyi	1.596		0.021		0.742	0.095	1.635	-0.562	0.109
Pretzsch	3.692		0.028	7.453	-3.660	0.241	2.764	-1.303	0.061
BD_KF			0.006		7.058	0.073	12.111	-4.748	0.066
BD_KV	17.987		0.027	13.380		0.016	16.482	-7.028	0.097
VKF	2.202		0.028	2.151	-0.746	0.042	2.084	-0.858	0.112
C66	-115.222	71.538	0.106	-83.023	76.549	0.112	55.491		0.000
Hegyi_2	3.052	-0.875	0.072			0.033	2.509	-1.033	0.184
BAL			0.009	-1,763.153	471.395	0.242	2,103.253		0.022

The non-indicated values for a parameter mean that
the parameter is not statistically significant ($\alpha = 0.05$).
a_0 and a_1 = coefficients; R^2 = coefficient of determination.

competition. Whenever the competition decreases trees start to develop their crowns and their growth and production capability increase. Sometimes in com-

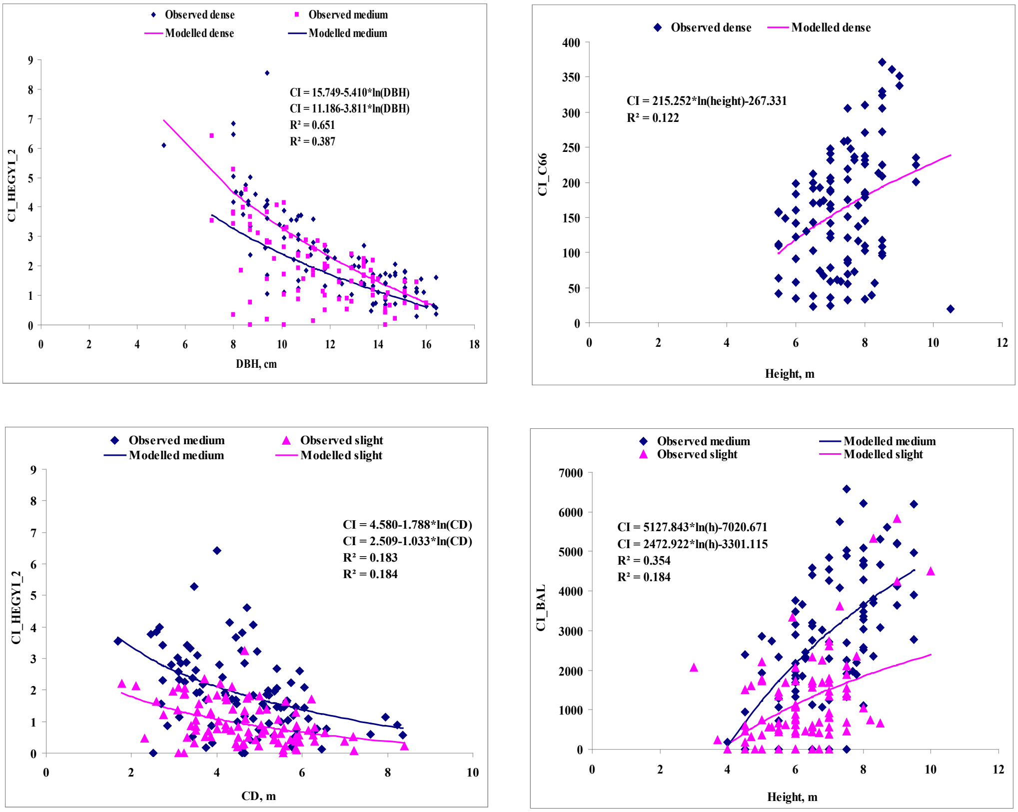


Fig. 2. Effect of competition on dimensions of *Acacia seyal* natural stands at Umfakarin forest, south Kordofan Sudan (See table 2 for models parameters).

petitive situations, trees may tend to increase in height (positive competition) in order to obtain sunlight, especially light demanding trees, like *A. seyal,* rather than enlarging cross sectional dimensions such as DBH and crown diameter (Fig. 1 and 2). This situation is clearly expressed by the positive correlation between tree height and some indices (CI_HEGYI, CI_BD_KF, CI_C66, CI_HEGYI_2 and CI_BAL). This positive association could be attributed to the method of selecting the competitors which is based on the height of subject tree. Whenever the height increases, additional competitors are included and this will increase the competition index. Competition induced by large adjacent trees may negatively affect the diameter and crown dimensions (negative competition) of individual subject trees.

As previously mentioned, non-linear regression models were used for describing the relationship between tree variables and competition indices. Based on the results of these models, CI_HEGYI_2 is considered as a suitable index to be applied for quantifying the degree of competition in natural stands of *A. seyal* of dense and medium stratum.

The results of this study could serve in defining or improving silvicultural treatments such as thinning programs for *A. seyal* trees in natural stands. Moreover, *A. seyal* is a gum Arabic producing tree in Sudan, and therefore, studying of competition among trees of *A. seyal* may play a major role in identifying the effect of competition on gum production, and hence, in developing gum production models. Establishment of permanent trial plots is necessary in order to study the effect of competition on growth of *A. seyal* and gum production as well.

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EFFECT OF SULPHURIC ACID SCARIFICATION, COLD MOIST STRATIFICATION AND GIBBERELLIC ACID ON GERMINATION OF *PALIURUS SPINA-CHRISTI* MILL. SEEDS

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Abstract

Paliurus spina-christi seeds were subjected to several treatments in order to overcome dormancy and to maximize germination. Seeds were subjected to sulphuric acid scarification for 0, 30, 60 and 90 minutes and then were stratified at 3–5°C for 0, 4, 8 and 12 weeks (1st experiment) or treated with 500, 1000 and 2000 ppm gibberellic acid (2nd experiment). In the first experiment, increasing scarification of non-stratified seeds from 30 to 60 or 90 minutes germination percentage increased significantly. Moreover, cold stratification treatments of non-scarified seeds resulted in very low germination percentages. The combination of acid scarification and cold stratification treatments improved significantly germination percentages. In the second experiment, treatment with gibberellic acid, regardless of concentration, of scarified seeds improved significantly germination percentages. The concentration of gibberellic acid was found to effect germination only in 30 minutes scarified seeds. In treated seeds with 500 or 1000 ppm gibberellic acid, a significant increase of germination percentage together with the increase of time of scarification from 30 to 60 or 90 minutes was observed. The results revealed that scarification was more effective than cold stratification in improving seed germination when treatments were applied alone. The highest germination percentages were observed when acid scarification was followed by cold stratification or treatment with gibberellic acid.

Key words: cold stratification, gibberellic acid, *Paliurus spina-christi*, seed germination, sulphuric acid scarification.

Introduction

Paliurus spina-christi Mill (Christ's thorn) is a very thorny shrub up to 4 m tall native to South Eastern Europe and West Asia. In Greece it is common in the continental part of the country and on some, more northern islands (Boratynski et al. 1992). This species is one of the most important components of overgrazed and eroded areas in Greece. The ability to withstand in dry and degraded sites makes it suitable for restoration of disturbed areas.

Regeneration from seeds is the most often used and cheapest method of propagation in many forest species. But, this propagation technique exhibits difficulty due to seed dormancy. The causes of seed dormancy can be attributed to exogenous (seed coat and other structures prevent germination) and endogenous (embryo characteristics that prevent germination) factors (Nikolaeva 1977). Scarification or stratification treatments are used to overcome seed dormancy. Scarification refers to any procedure that modifies the seed coat to permit the entry of water and the exchange of gasses so that the germination process begins. Various methods of scarification are used to break dormancy (chemical, mechanical, hot water) (Bonner et al. 1994). Moist stratification (cold or warm) is widely used for breaking embryo dormancy and enhancing the germination of seeds in numerous species (Macdonald 1993). Various solutions of hormones are used to accelerate breaking seed dormancy. Gibberellic acid (GA) is one of the hormones that can be used to partially or fully replace the necessary period of cold moist stratification (Baskin and Baskin 1998).

There are conflicting results regarding to the contribution of acid scarification and moist cold stratification in overcoming *P. spina-christi* seed dormancy. Takos et al. (2001) and Piotto et al. (2003) attributed *P. spina-christi* seed dormancy to hard seed coat and recommended sulphuric acid scarification or cold moist stratification for breaking seed dormancy. In contrast, Olmez et al. (2007) reported that treatment of seeds with sulphuric acid gave high germination percentages whereas cold stratification treatment resulted in very low germination percentages.

The assumption that both treatments overcome seed dormancy and enhance germination incurs the risk of poor germination due to inadequate treatment. Furthermore, there is a lack of published literature describing the effects of gibberellic acid on seed germination.

The purpose of this research was to: i) examine the effectiveness of acid scarification, cold moist stratification and gibberellic acid (GA_3) treatments on *P. spina-christi* seed germination; ii) describe the effects of acid scarification and cold stratification treatment combinations on germination; iii) describe the effects of acid scarification and gibberellic acid (GA_3) treatment combinations on germination; iv) propose treatments that maximize germination of *P. spina-christi* seeds.

Materials and Methods

Mature fruits (drupes) of *P. spina-christi* were collected in November 2008 from a number of plants (more than 10) in NE Greece (41º07´41´´N, 25º14´11´´E, 40 m elevation). The fruits were spread upon the floor and were then crushed by a heavy cylinder in order to break the hard exocarp and extract the seeds. Sieving and flotation were used to remove trash (parts of fruits) and also empty and broken seeds. Then, the clean seeds were spread on filter paper and left to dry. After drying, the seeds were stored in glass containers in the refrigerator (3–5ºC) until the beginning of the experiments.

Seed treatment

Two experiments were conducted in order to determine the effects of acid scarification, cold moist stratification and gibberellic acid (GA_3) treatments on *P. spina-christi* seed germination. Seeds were immersed in concentrated (95–97%) sulphuric acid for 0, 30, 60 and 90 min. After treatment the seeds were removed from the acid and were washed thoroughly under running water. Acid scarified and non-scarified seeds were subjected to the following treatments. A certain amount of the above seeds were mixed with wet sterilized river sand and were placed in plastic containers and underwent cold stratification at 3–5ºC for 4, 8 and 12 weeks (first experiment). Each container corresponded to a different duration of immersion in sulphuric acid (there were 4 plastic containers). In total, 12 treatments (combinations between acid scarification and cold stratification) were applied. Another amount of seeds were treated with gibberellic acid (GA_3) (second experiment). 120 seeds of each treatment with sulphuric acid (0, 30, 60 and 90 min) were soaked in 500, 1000 or 2000 ppm GA_3 for 24 hours. In total, 12 treatments (combinations between acid scarification and GA_3) were applied. Moreover, acid scarified (30, 60 and 90 min) and non-scarified seeds (0 min) were subjected immediately to a germination test without cold stratification (0 weeks) or GA_3 treatment (0 ppm).

Germination test

The germination experiment was conducted in the laboratory of Silviculture, in the Faculty of Forestry and Natural Environment of Aristotle University of Thessaloniki. In the first experiment, at the end of each stratification period a random sample of 120 seeds was taken out from each plastic container and randomly placed in 4 plastic Petri dishes (30 seeds per Petri dish). In the second experiment after the treatment with GA_3 the seeds (120 seeds per acid scarification duration and GA_3 concentration) were randomly placed in 4 plastic Petri dishes (30 seeds per Petri dish). In both experiments, for each treatment there were 4 replications of 30 seeds. Sterilized river sand moistened with distilled water was used as substrate in the plastic Petri dishes. Prior to the arrangement of seeds in Petri dishes they were dusted with fungicide (Captan) to avoid fungi development. The Petri dishes were randomly arranged on the shelves of the growth chamber.

The temperature in the growth chamber was set at 20ºC for a 16 h dark period and 25ºC for an 8 h light period. Seed germination was defined as the appearance of a radicle, at least 2 mm long, according to the rules of the International Seed Testing Association (1999). Germinated seeds were counted each week for 6 weeks. Finally, the germination percentage was calculated for each replication. The germination percentage of each treatment was calculated from the average of the 4 replication percentages.

Statistical analysis

In both experiments the experimental design was a completely randomised factorial design. In the first experiment the factors were the duration of acid scarification and the length of cold stratification period (4 x 4 factorial design). In the second experiment the factors were the duration of acid scarification and the concentration of gibberellic acid (4 x 4 factorial design). The data was analysed using the ANOVA method in the frame of the GLM (General Linear Model). The germination percentage data was transformed to arc-sine square root values, before analysis (Snedecor and Cochran 1980). The transformed data was checked for normality and homogeneity of variances and was then analysed by ANOVA, while the comparisons of the means were wade using the Bonferroni test (Klockars and Sax 1986).

Finally, the Duncan test was used to detect significant differences among the best 10 treatment combinations (in total 28) with a probability of 95% ($a = 0.05$) (Klockars and Sax 1986).

All statistical analyses were carried out using SPSS 12.0 (SPSS, Inc., USA).

Results

Experiment 1

The analyses of variance indicated that there were significant differences on germination percentages at $a = 0.05$ affected by: (a) acid scarification [$F(3,48) = 600.808$, $P = 0.000$], (b) cold stratification [$F(3,48) = 93.304$, $P = 0.000$] and (c) the interaction between the factors acid scarification and cold stratification [$F(9,48) = 29.032$, $P = 0.000$]. The main effects of two factors are not analyzed since there is significant interaction between them. For seeds that were not stratified, 60 or 90 min scarified seeds gave higher germination percentages than non-scarified, and those that were scarified for 30 min ($p < 0.05$) (Table 1). In each of the 3 periods (4, 8 and 12 weeks) of stratifications, seeds scarified for 30, 60 and 90 min exhibited higher germination percentages than non-scarified ($p < 0.05$), while no significant differences were observed among them ($p > 0.05$). Non-scarified seeds that were subjected to stratification for 0, 4, 8 and 12 weeks exhibited very low germination percentages without statistically significant differences among them ($p > 0.05$). In each of the 3 treatments (30, 60 and 90 min) with sulphuric acid, seeds stratified for 4, 8 and 12 weeks exhibited higher germination percentages than non-stratified ($p < 0.05$), while no significant differences were observed among them ($p > 0.05$) (Table 1).

Experiment 2

The analyses of variance indicated that there were significant differences on germination percentages at $a = 0.05$ affected by: (a) acid scarification [$F(3,48) = 858.072$, $P = 0.000$], (b) GA_3 [$F(3,48) = 199.627$, $P = 0.000$] and (c) the interaction between the factors acid scarification and GA_3 [$F(9.48) = 23.727$, $P = 0.000$]. The main effects of two factors are not analyzed since there is significant interaction between them. 30, 60 and 90 min scarified seeds, prior to GA_3 application (500, 1000, 2000 ppm), resulted in higher germination percentages compared to non-scarified seeds that were treated with GA_3 ($p < 0.05$) (Table 2). In treatments with 500 and 1000 ppm GA_3, seeds scarified for 60 and 90 min gave higher germination percentages than seeds scarified for 30 min

Table 1. Mean germination percentages (GP) of *P. spina-christi* seeds of the four periods of cold stratification (CS) in each of the four durations of acid scarification (AS) (interaction between the factors 'AS' and 'CS' – simple main effects).

CS, weeks	**0 min AS**	**30 min AS**	**60 min AS**	**90 min AS**
	GP, % ± S.D.	**GP, % ± S.D.**	**GP, % ± S.D.**	**GP, % ± S.D.**
0	4.17 a **b** ± 1.67	10.00 b **b** ± 2.72	57.50 b **a** ± 4.19	60.00 b **a** ± 4.71
4	3.33 a **b** ± 2.72	84.17 a **a** ± 3.19	85.00 a **a** ± 4.30	83.33 a **a** ± 4.71
8	3.33 a **b** ± 2.72	85.00 a **a** ± 4.30	82.50 a **a** ± 4.19	78.33 a **a** ± 6.39
12	6.67 a **b** ± 2.72	80.00 a **a** ± 5.45	79.17 a **a** ± 5.69	75.84 a **a** ± 6.31

Means, in a column are statistically different at $p<0.05$, when they share no common letter (letters in normal print). The comparisons were made using the Bonferroni test.

Means, in a row are statistically different at $p<0.05$, when they share no common letter (letters in bold print). The comparisons were made using the Bonferroni test.

($p<0.05$). In treatment with 2000 ppm GA_3, seeds scarified for 90 min gave higher germination percentages than seeds scarified for 30 min ($p<0.05$). For seeds that were not scarified, treatment with 2000 ppm GA_3 gave higher

Table 2. Mean germination percentages (GP) of *P. spina-christi* seeds of the four concentrations of gibberellic acid (GA_3) in each of the four durations of acid scarification (AC) (interaction between the factors 'AC' and 'GA_3'–simple main effects).

GA_3, ppm	**0 min AS**	**30 min AS**	**60 min AS**	**90 min AS**
	GP, % ± S.D.	**GP, % ± S.D.**	**GP, % ± S.D.**	**GP, % ± S.D.**
0	4.17 b **c** ± 1.67	10.00 c **b** ± 2.72	57.50 b **a** ± 4.19	60.00 b **a** ± 4.71
500	6.67 ab **c** ± 2.72	63.33 b **b** ± 4.71	90.00 a **a** ± 4.71	88.33 a **a** ± 4.30
1000	9.17 ab **c** ± 3.19	80.00 a **b** ± 3.85	90.83 a **a** ± 3.19	90.00 a **a** ± 2.72
2000	10.83 a **c** ± 3.19	81.67 a **b** ± 4.30	88.33 a **ab** ± 4.30	90.84 a **a** ± 4.19

Means, in a column, are statistically different at $p<0.05$, when they share no common letter (letters in normal print). The comparisons were made using the Bonferroni test.

Means, in a row, are statistically different at $p<0.05$, when they share no common letter (letters in bold print). The comparisons were made using the Bonferroni test.

germination percentage than treatment without GA_3 ($p<0.05$). In each of the 3 durations (30, 60 and 90 min) of immersion in sulphuric acid the seeds that were treated with GA_3 gave higher germination percentages than the seeds that weren't treated with GA_3 ($p<0.05$). In 30 min scarified seeds, treatment with 1000 and 2000 ppm GA_3 gave higher germination percentages than treatment with 500 ppm GA_3 ($p<0.05$). In 60 or 90 min scarified seeds, no significant differences were observed in germination percentages among treatments with 500, 1000 or 2000 ppm GA_3 ($p>0.05$) (Table 2).

In total of the 28 treatment combinations that were conducted, those that gave the best 10 germination percentages are presented in Table 3. The combination of acid scarification for 60 or 90 min and gibberellic acid (regardless of concentration) treatments gave high germination percentages that didn't differ significantly with the combinations of 60 min acid scarification plus 4 weeks cold stratification and of 30 min acid scarification plus 8 weeks cold stratification (Table 3).

Discussion

In this study, the increase of scarification duration from 30 to 60 or 90 min, without any other treatment, caused an analogous significant increase in germination percentages of *P. spina-christi* seeds. Germination percentage of non-scarified seeds was very low. This indicates that *P. spina-christi* seed have a hard, impermeable seed coat (physical dormancy) and that a 30 min acid scarification is not enough to erode the seed coat. This is in agreement with recommendations of Takos et al. (2001) that *P. spina-christi* seeds require scarification in order to germinate. They found that the increase of duration of acid scarification (30, 60, 120 and 240 min) caused an increase in germination percentages (36, 63, 69, and 92% respectively). Also, in their review regarding seed propagation of mediterranean trees and shrubs Piotto et al. (2003) recommended sulphuric acid scarification for 40 to 120 minutes to erode the seed coat. Olmez et al. (2007) reported that treatment of seeds with sulphuric acid for 40 minutes gave the best ger-

Table 3. Treatments that gave the best ten mean germination percentages (GP) of *P. spina-christi* seeds. These treatments were combinations between a) acid scarification (AS) and cold stratification (CS) and b) acid scarification (AS) and gibberellic acid (GA_3).

Treatments	GP, % ± S.D.	Treatments	GP, % ± S.D.
90 min AC + 2000 ppm GA_3	90.84 a ± 4.19	60 min AC + 2000 ppm GA_3	88.33 abc ± 4.30
60 min AC + 1000 ppm GA_3	90.83 ab ± 3.19	60 min AC + 4 weeks CS	85.00 abc ± 4.30
60 min AC + 500 ppm GA_3	90.00 abc ± 4.71	30 min AC + 8 weeks CS	85.00 abc ± 4.30
90 min AC + 1000 ppm GA_3	90.00 abc ± 2.72	30 min AC + 4 weeks CS	84.17 bc ± 3.19
90 min AC + 500 ppm GA_3	88.33 abc ± 4.30	90 min AC + 4 weeks CS	83.33 c ± 4.71

Means are statistically different at $p < 0.05$, when they share no common letter. The comparisons were made using the Duncan test.

mination percentages under both greenhouse and open field conditions (65.05 and 54.99% respectively). In contrast, they found that the increase of the duration of acid scarification (from 80 to 120 min) caused a significant decrease in germination percentages under both greenhouse and open field conditions.

In this study, germination percentages of seeds that were subjected only to cold stratification for 4, 8, and 12 weeks were very low. Similar results were reported by Olmez et. al. (2007) where cold stratification of seeds for 20, 40 and 60 days gave the lowest germination percentages under both greenhouse and open field conditions (11.42–13.47% and 3.37–8.99% respectively). In contrast, in their review Piotto et al. (2003) recommended cold moist stratification for 4 to 5 months. Takos et. al. (2001) reported that cold stratification treatment (for 2, 3 and 4 months) had the same positive effect in seed germination as acid scarification. They found that seeds stratified for 2, 3 or 4 months gave germination percentages equal to 63, 56 and 79% respectively. Possibly, during the breaking of the hard exocarp into the hammer mill that Takos et al. (2001) used for the seed extraction, the seed coat of some seeds had cracked. Consequently, these seeds germinated after cold scarification treatment.

According to the results of this study the combination of acid scarification and cold stratification treatments was necessary to maximize germination of *P. spina-christi* seeds. Cold stratification for 4 weeks of scarified seeds was enough to maximize germination. Possibly, the seeds of *P. spina-christi*, apart from the hard seed coat also have a nondeep physiological dormancy. According to Baskin and Baskin (1998) nondeep physiological dormancy in some species is broken by relative short periods of cold stratification. Takos et al. (2001) reported that seeds that were acid scarified for 30 min and then followed by 2, 3 or 4 months cold stratification gave high germination percentages.

GA_3 treatments significantly increased the germination of scarified seeds. Germination percentages of seeds that were scarified for 60 or 90 min and were then treated with GA_3 were very high. This implies that GA_3 treatment substituted cold stratification requirement in *P. spina-christi* seeds. Also, Baskin and Baskin (1998) reported that GA stimulates germination of seeds with nondeep physiological dormancy.

Conclusions

The results of this study revealed that acid scarification treatment was stronger than cold stratification in breaking the dormancy of *P. spina-christi* seeds. Cold stratification of acid scarified seeds for 4 weeks maximizes the germination of *P. spina christi* seeds. However, similar results as well as overcoming seed dormancy in 24 hours could be achieved by treatment of scarified seeds with GA_3.

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SURVEY OF THE LONGHORNED BEETLE SPECIES (COLEOPTERA: CERAMBYCIDAE) ON *ACACIA SENEGAL* L. (WILD) IN KORDOFAN REGION, SUDAN

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Abstract

Acacia senegal, the gum Arabic producing tree, is of great economic importance in Sudan. Serious biotic and abiotic factors are threatening its growth and production. Among these, the long-horned beetles (Coleoptera: Cerambycidae) are the most deleterious insect pests resulting in complete death of *Acacia* trees. This study was conducted in northern Kordofan state Sudan season 2007/2008 with aims to identify spectrum, relative abundance and phenology of long-horned beetle species acting as pests of *A. senegal* tree. Moreover, the study focuses on the assessment of tree characteristics triggering the infestation. The species spectrum and their relative abundance were determined by catch results of flight interception traps, microclimatic conditions were assessed using data loggers, tree characteristics were done by direct measurements in addition, and a questionnaire was designed to assess farmers' knowledge on long-horned beetles. The results indicated that seven species of the long-horned beetles were recorded from the flight interception traps on the study sites. The recorded species were *Anthracocentrus arabicus* (Thomson 1877), *Crossotus subocellatus subocellatus* (Fairmaire 1886) (Eisa et al. 2008), *Crossotus strgifrons* (Fairmaire 1886) *Doesus telephoroides* (Pascoe, 1862), *Titoceres Jaspideus* (Serville 1835) (Eisa et al. 2008), *Tithoes* sp. and *Gasponia gaurani* (Fairmaire 1892). The highest percentage of infestation (100%) was recorded in El Demokeya site with an average tree age of 43 years, followed by on Acacia agricultural project site (23.5%) with an average tree age of 10 years and on Elhemaira site (20%).

Key words: *Acacia senegal*, Gum Arabic, Long-horned beetles.

Introduction

The gum Arabic belt in Sudan where *A. senegal* grows naturally lies between latitudes 10º and 14º N. The two most conspicuous gum Arabic-producing areas outside these limits are in the northeast (Faw, Gedaref and Kassala areas), and in the southeast along the Blue Nile/Upper Nile states border (El Amin and Olavi 2006). A. senegal is a multifunctional tree in Sudan producing gum Arabic which is the major and most important non-wood forest product in the country. Gum Arabic is an export commodity and hard currency earner. It plays an important role as a major source of foreign exchange. Furthermore, the tree is reported to be utilized for fuel wood, forage, and medicine. It contributes to environmental protection, improvement of soil fertility, delivers shade and shelter in agro-forestry system, as well as amenity. In Sudan the main zone of gum Arabic production is the western part of the country. A. senegal is attacked by numerous insects pests, among these pests the long-horned beetles are the most serious. Long-horned beetles (Cerambycidae) are one of the largest groups of Coleoptera. Most of the species can be found in tropical and subtropical regions. These beetles are called longhorns because of their often long antennae, especially in males. Cerambycidae mostly have elongate and almost cylindrical bodies. They vary greatly in length (Evans et al. 2004). Cerambycidae family comprising about 40,000 described species worldwide (Cocquempot and Lindelöw 2010).

Study Area and Methodology

This study was conducted in North Kordofan State, Sudan. North Kordofan state is located in the dry semi-arid region between latitudes 11.15°–16.45° N and longitudes 27.5°–32.15° E. The field work had been conducted between May 2007 and October 2008. Three study sites grown by *A. senegal* were selected for the study. These sites are El Demokeya reserve forest, Elhemaira forest and Acacia agricultural project (Table 1). These sites meet the criteria of different pattern of management; a traditional system (Elhemaira), researcher managed (El Demokeya) and the mechanized system (Acacia agricultural project). Temporary sample plots technique (El Tayeb et al. 2006) was conducted in all of the study sites in this study to investigate the effects of long-horned beetles on *Acacia* tree species. A number of five circular sample plots (SPs) (each with a radius of 17.8 m and 0.1 ha in size) were taken. The SPs were located at a distance of 1 km. In each SP, observation, measurement and counting were conducted.

Flight interception traps

Nine flight interception traps were located in two of the study sites at Acacia agriculture project (AAP) and El Demokeya reserve forest with the objective to detect the presence of the longhorned beetles in addition, to verify the species spectrum of the long-horned beetles. The traps were distributed E, N, S and W directions of the forest. Traps were monitored bi-monthly and trapped insects were collected from the killing jar and conserved in 75% alcohol. The specimens collected were identified by Gianfranco Sama (Vi Raffaello Sanzio, 84, I 47521, CESENA – ITALY) in 2008.

Percentage of infestation rates

The percentage of infestation was calculated based on the ratio of the number of infested trees, as identified through inspections, and the total of trees per plot, together with the tree age.

Statistical analysis methods

The data collected during field survey on *Acacia* species were coded, computerized and analyzed using the Microsoft Excel programme and the SPSS for windows; version 15 for statistical analysis, computations and functions.

Table 1. Study sites.

Site name	Elevation, m	Plot, No	Number of surveyed trees
Acacia agricultural project	569	1	26
Acacia agricultural project		2	17
Acacia agricultural project		3	22
Acacia agricultural project		4	25
Acacia agricultural project		5	23
Elhemaira forest	543	1	21
Elhemaira forest		2	10
Elhemaira forest		3	17
Elhemaira forest		4	10
Elhemaira forest		5	15
El Demokeya reserve forest	543	1	5
El Demokeya reserve forest		2	5
El Demokeya reserve forest		3	7
El Demokeya reserve forest		4	2
El Demokeya reserve forest		5	5

Results

Seven species of longhorned beetles were recorded on the study plots of El Demokeya site (Table 2).

Distribution of the recorded species:

***1. Anthracocentrus arabicus* (Thomson 1877)**
Saharan and Sahelian countries from southern Morocco and Mauritania to Central Eastern Africa (Sudan, Somalia, Eritrea); also known from the Arava Valley in southern Jordan and Israel, the Sinai Peninsula and the whole Arabian peninsula from the United Emirates to Yemen (Sama and Rapuzzi 2006).

2. *Crossotus strigifrons* (Fairmaire 1886)
Described from “Soudan”, widespread in Saharan and Sahelian countries from Mauritania and Southern Morocco to Upper Egypt and Sinai Peninsula, as well as in desert areas of southern Israel and Jordan; also known in Somalia, Eritrea, Ethiopia and Arabian Peninsula (Breuning 1942, Holzschuh and Téocchi 1991, Sudre et al. 2007).

3. *Doesus telephoroides* (Pascoe 1862)

India: Central provinces: Jabalpur (Gahan 1906); from Senegal au Laos; Sénégal, Bénoué (Cameroun), Bahr-Sara (Congo Français) (Lepesme 1948); Nigeria, French Equatorial Africa, (Ubangui-Chari), Western French Africa (French Sudan, currently Mali), Senegal, Cameroon (Ferreira and da Veiga Ferreira 1959); Uganda, Sudan (Darfur), Chad (G. Sama personal collection).

Table 2. Long-horned beetle catches at flight interception traps on the El Demokeya site (*Acacia senegal*).

Species of longhorned beetles	**Host plants** according to literature (Sama, in preparation, personal communication)	**Synonyms**	**Date of recording**	**Length**
Anthracocentrus arabicus (Thomson 1877)	*Tamarix articulata*, *A. raddiana* and *A. scorpioides*	Unknown	April/2008	Length: 45–81 mm
Crossotus strgifrons (Fairmaire 1886)	*Acacia raddiana*; *A. ehrenbergiana*, and *A. seyal*.	*Crossotus albicans* (Breuning 1942) *Dichostethes nebulosus* (Fairmaire 1892) *Dichostates strigifrons* (Fairmaire 1886)	April/2008	Length: 12–18 mm
Doesus telephoroides (Pascoe 1862)	Unknown	*Doesus telephoroides* (Breuning and Villiers 1972) *Doesus telephoroides* (Lepesme 1948) *Doesus telephoroides* (Lepesme 1952)	November/2008	Length: 16–20 mm
Tithoes sp.		Unknown	August/2008	Length: 30–70 mm
Gasponia gaurani (Fairmaire 1892)	*Acacia nilotica*	Unknown	June/2008	Length: 9–12 mm

4. *Tithoes* sp.

The distribution area of this species covers the whole Tropical Africa (probably except rain forest areas) from Mali to South Africa (Gilmour 1956); it is also known from the Arabian Peninsula (Fuchs 1969, Holzschuh 1993) and Upper Egypt (Sama in print).

5. *Gasponia gaurani* (Fairmaire 1892)

The genus includes three species: *G. fascicularis* (Fairmaire 1887) from Tanzania; *G. gaurani*, described from Obock (Djibouti) and recorded from several countries in Sub-Saharan

Africa (such as Somalia and Kenya) and from the Arabian Peninsula (Yemen); *G. penicillata* (Gahan 1906), sometimes regarded as a synonym (Breuning 1942, 1962) or a subspecies of *G. gaurani* (Téocchi 1998), but, very likely, a distinct species (Adlbauer 2001) widespread in south African countries from Malawi to South Africa.

Table 3. Infestation percentages for long-horned beetles at the Acacia Agricultural Project site.

Plot, No	Number of observed trees	Number of infested trees	Average of age	Infested trees, %
1	26	4	10	15.38
2	17	4	10	23.53
3	22	1	10	4.55
4	25	0	10	0.00
5	23	1	10	4.35

Infestation rates of the long-horned beetles on study sites of *A. senegal*

Acacia agricultural project site

At the Acacia agricultural project study site 5 study plots with a total of 113 Acacia trees were under investigation. All trees of the five plots observed for the infestation of *A. senegal* with the longhorned beetles, had an average age of 10 (S.D. ± 0.0). Infestation rates of trees varied between no infestation (0.00%) and 23.53%, Table 3.

Elhemaira study site

At the Elhemaira study site 5 study plots with a total of 73 Acacia trees were under investigation. Trees of the five plots observed for the infestation of *A. senegal* with the longhorned beetles, had an average age range between 10 and 17. Infestation rates of trees varied between no infestation (0.00%) and 20%, Table 4.

The results of the first plot with a total number of 21 trees, and with no infested trees, showed zero percentage of infestation, (0.00%) and corresponded to an average age of 10 (S.D. ± 4.9). In the second plot with a total number of 10 trees, and with no infested trees, the percentage of infestation was 0.00% and corresponded to an average age of 17 (S.D. ± 3.86), whereas the results of the third plot

Table 4. Infestation percentages for long-horned beetles at the Elhemaira forest site.

Plot, No	Observed trees, number	Infested trees, number	Tree age, years Mean ± S.D.	Infested trees, %
1	21	0	10 (S.D ± 4.9)	0.00
2	10	0	17 (S.D ± 3.86)	0.00
3	17	1	13 (S.D ± 5.75)	5.88
4	10	2	12 (S.D ± 6.81)	20.00
5	15	0	15 (S.D ± 0.0)	0.00

with a total number of 17 trees, and with one infested tree, the percentage of infestation was 5.88% and the average age was 13 (S.D. ± 5.75). The results of the fourth plot with a total number of 10 trees, and with 2 infested trees, showed a percentage of infestation that reached 20% with an average age of 12 (S.D. ± 6.81). The fifth plot with a total number 15 trees showed no infested trees, the percentage of infestation was zero (0.00%) with an average age of 15 (S.D. ± 0.00).

El Demokeya study site

At El Demokeya study site 5 study plots with a total number of 24 Acacia trees were under investigation. All trees of the five plots observed for the infestation of *A. senegal* with the longhorned beetles, had an average age of 43 (S.D. ± 0.00). Infestation rates of trees varied between no infestation (0.00%) and 100%, Table 5.

The results of the first plot with a total number of 5 trees, and with no infested trees, showed zero percentage of infestation, (0.00%) and corresponded to an average age of 23 (S.D. ±0.00), while on the second plot with a total number of 5 trees, and with 4 infested trees, the percentage of infestation was 80% and corresponded to an average age of 43 (S.D. ± 0.00). On the third plot with a total number of 7 trees, and with 4 infested trees, the percentage of infestation was 57.14% and the average age was 43 (S.D. ± 0.00). The results of the fourth plot with a total number of 2 trees infested, showed a percentage of infestation reaching 100% with an average age of 43 (S.D. ± 0.00).

Table 5. Infestation percentages for long-horned beetles at the El Demokeya site.

Plot, No	Number of observed trees	Number of infested trees	Average Age, years	Infested trees, %
1	5	0	43	0.00
2	5	4	43	80.00
3	7	4	43	57.14
4	2	2	43	100.00
5	5	3	43	60.00

Discussions

At the study sites, seven long-horned beetle species were captured using flight interception traps. According to the literature, most of the species have a more or less extended distribution range. *Anthracocentrus arabicus* (Thomson 1877) occurs in Saharan and Sahelian countries, from southern Morocco and Mauritania to Central Eastern Africa (Sudan, Somalia, Eritrea). It also occurs in the Arava Valley in southern Jordan and Israel, the Sinai Peninsula and the whole Arabian Peninsula from the United Emirates to Yemen (Sama and Rapuzzi 2006). *Crossotus strigifrons* (Fairmaire 1886) is also widespread in Saharan and Sahelian countries, from Mauritania and Southern Morocco to Upper Egypt and the Sinai Peninsula. It is also spread across the desert areas of southern Israel and Jordan and in Somalia, Eritrea, Ethiopia and the Arabian Peninsula (Breuning 1942, Holzschuh and Téocchi 1991, Sudre et al. 2007). Individuals of *Crossotus subocellatus* Fairmaire (*Crossotus subocellatus subocellatus* (Fairmaire

1886) are found in the southern part of the Arabian Peninsula, Sudan, Somalia, Ethiopia, Eritrea, and Kenya. In Northern Africa, the species is only found in Egypt (common in South-Eastern Desert and Sinai), Libyia, Senegal, Saudia Arabia, Morroco, Chad, Mauritania, and Niger. Sama (personal communication) argues that *Doesus telephoroides* (Pascoe 1862[1]) was recorded for the first time in Sudan. It is widely distributed throughout central provinces of India and Jabalpur (Gahan 1906). In Africa, it can be found in Senegal, Cameroon (Ferreira and da Veiga Ferreira 1959), Bahr-Sara (Congo Français) (Lepesme 1948), Nigeria, French Equatorial Africa (Ubangui-Chari), Western French Africa (French Sudan, currently Mali), Uganda, Sudan (Darfur), and Chad (G. Sama personal collection). *Titoceres jaspideus* (Audinet Serville 1835) is a common species throughout Africa, extending from South Africa northward. The *Tithoes* sp., very likely *T. confinis* (Castelnau 1840), has a distribution area that covers the entire tropical African region (probably except rain forest areas) from Mali to South Africa (Gilmour 1956). It has also been found on the Arabian Peninsula (Fuchs 1969, Holzschuh 1993) and Upper Egypt (Sama in print). Jamal (1994) recorded the presence of this species, using the synonym *Acanthophorus confinis* Cast (Cerambycidae, Prioninae). This pest exerted several attacks on the branches of *Acacia* trees. *Gasponia gaurani* (Fairmaire 1892) was first recorded in Sudan. In the present study *A. senegal* trees differ in their infestation rates with the long-horned beetles in different sites (Table 1, 2 and 3). These results could be demonstrated, as site conditions or factors in relation to pest infestation were discussed by many investigators. Coulson and Witter (1984) mentioned that site condition is an important consideration because the site contains the host material (tree species in various stages of growth) that pest species utilize for food and habitat. The availability of suitable preferred and alternate hosts is a primary requisite for development of insect populations. Different sites vary in plant species composition, density, and age classes. Therefore, the opportunity for insect populations to grow in size varies on different sites. Site condition, in combination with weather, determines the rate of growth and general vigor of the host trees present, moreover Speight and Wylie (2001) mentioned that feeding of the larvae of *Celosterna scabrator*, the babul borer (Cerambycidae) causes cessation of growth and sometimes death of the plant above ground. Incidence of attack of up to 80% has been recorded for trees growing on unsuitable sites and they also mentioned that *Strongylurus decoratus* (McKeown) (Cerambycidae) has affected trees ranging in age from 4 years to more than 30 years and in height from 3 to 31 m. This description was agreed with the findings of infestation rates of *Acacia* trees with longhorned beetles in different sites with different age range, where highest infestation coincided with the highest average of tree age for Acacia company project site and El Himaira forest site respectively. El Demokeya reserve forest site indicated the highest percentage of infestation with the minimum average of tree age (Table 1, 2 and 3 for *A. senegal*);

[1] First record from Sudan

moreover the height of *A. senegal* ranged from 1 to 9 m. The positive correlation of tree age and tree infestation was mentioned for other long-horned beetle species. Investigation on other long-horned beetles also demonstrate for the obtained results about *Acacia* species infestation. The locust borer *Megacyllene robiniae* (Forst.) (Cerambycidae) a wood – boring beetle attacks the host when stems are 3 to 5 years old with sufficient diameter to support the extensive wood mining done by the larvae (Thatcher 1961). The greatest damage by the locust borer *Megacyllene robiniae* (Forst.) (Cerambycidae) is coincident with drought conditions since drought enables a larger number of larvae to survive. The attacks are most serious on trees under 6 inch in diameter since breakage due to structural weakening of narrow stems is proportionally greater but dry periods may allow the larvae to successfully and severely attack larger trunks (Thatcher 1961). Moreover, Dajoz (1992) stated that temperature under bark is a function of the exposure of the tree to sunlight, and the structure of the bark. At sunny sites, daily variation in temperature is much bigger than those recorded at sheltered trees. Under bark sheltered from sunlight fluctuations of temperature are slighter than those of the ambient air. The same applies to the temperature inside the trunk. Thus, temperature plays an important part in the localization of insects that live in tree trunks. As an example, the life cycle of the Cerambycid *Monochamus scutellatus* lasts one year in trunks exposed to sunlight, and three years in shaded trunks. In this study, the least number of infestation holes of *A. senegal* was located on the south direction, which has the lowest sun exposition during the course of a day.

Conclusions

More than one species of the long-horned beetles had been identified from *Acacia* species as the species *Acacia* reported as an important from ecological perspectives for family Cerambycidae. Further research is needed to verify other host tree species and to study about larval behavior due to lack of ecological data of these species in the savanna low rainfall zone in the study area.

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RECONSTRUCTION OF PHYLOGENY OF THE GENUS *RHODODENDRON* L. FROM RUSSIA BASED ON THE MOLECULAR GENETIC DATA

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Abstract

As a result of the analysis performed, a genealogical network representing evolutionary relations between *Rhododendron* L. species was obtained. Phylogeny of *Rhododendron* species reconstructed on the basis of ITS1-ITS2 sequence corresponds to anatomical-morphological classification of D. Chamberlain (1996).

Key words: anatomical-morphological classification, evolutionary relations, genealogical network.

Introduction

One of the tasks of plant systematics is construction of a natural classification pattern of a taxon; which should reflect evolutionary history. Data on DNA nucleotide composition are best suited for analyzing micro-changes of the species. ITS1-ITS2 (internal transcribed spacer 1, gene 5.8S ribosomal RNA, internal transcribed spacer 2) section of nuclear DNA of *Rhododendron* L. species growing in Russia was studied. Based on data on nucleotide sequences and statistical analysis, one of alternative classification schemes was justified.

Materials and Methods

Material for molecular genetic analysis was taken from living plants cultivated in Central Siberian Botanical Garden, SB RAS and those brought from the natural habitats. Isolation of total DNA was performed from the leaves dried in silica gel with the help of Diamond DNA Kit (ABT Llc., Russia) by the directions of the firm-manufacturer. ITS1-ITS2 locus was amplified by N. Friesen's modified method (2007) with the use of direct primer - 5′-AAGGTTTCCGTAGGTGAAC-3′ – and reverse primer – 5′-TATGCTTAAACTCAGCGGG-3′ (Desfeux and Lejeune 1996). For amplification reaction the following PCR mixture was used: 31,5 μL of H_2O, 2 μL of DNA, 5 μL of 10x buffer, 5 μL of 25 mM $MgCl_2$, 2 μL of each 10 mM primer, 2 μL of 20 mM dNTPs. 0,2 μL (5 u / μL) Taq polymerase of was added directly to PCR mixture (so called "hot start") after the first cycle of warming up, which made it possible to increase yield of amplification, to get rid of non-specific annealing of primers and formation of primer dimmers (Real time PCR 2009).

Amplification was carried out as follows: 1 cycle: 95°C for 3 min; 35 cycles: 95°C – 20 sec, 56°C – 30 sec, 72°C – 80 sec; closing stage: 72°C – 10 min., cooling at 4°C.

Before sequencing, the amplified product was cleaned by NucleoSpin® Extract II Kit (Macherey-Nagel) following the instructions of the manufacturer.

Sequencing was carried out using automatic sequencer ABI Prism 3130xl. For the first time the sequence of the studied section was obtined for *Rh. dauricum* L., *Rh. ledebourii* Pojark., *Rh. parvifolium* Adams, *Rh. smirnowii* Trautv., *Rh. adamsii* Rehd. (Tuva population) = *Rh. burjaticum* Malyschev (Malyshev 1961), *Rh. sichotense* Pojark, *Rh. fauriei* Franch., *Rh. caucasicum* Pall., *Rh. japonicum* (A. Gray) Suring, and *Calluna vulgaris* L. The rest of the sequences used in the analysis were taken from GenBank, NCBI. Accession Numbers of used sequences: HM854157, HM854158, HM854159, HM854160, HM854161, HM854162, HM854163, HM854164, HM854165, HM854166, HM854167, X97415, AF393409, AF393412, X96814, AF404816, X97426, AF393418, X96806, AF393413.

Results and Discussion

A low rate of variability of ITS1-ITS2 was determined for the species studied. A cluster analysis of nucleotide sequences was performed by ME method (minimum evolution) in MEGA 4.1 (Kumar et al. 2008) and a bootstrap-consensus tree was constructed. Nucleotide sequences were aligned in a combined manner – automatic by ClustalW with correction "by hand". The total aligned length of leveling was 638 nucleotide pairs. There were 10000 replications of the bootstrap test. As an out group was taken *Calluna vulgaris* L. which demonstrated the closest relationship with *Rhododendron* species on the base of the analysis of nucleotide sequences by BLAST (NCBI). As a result, a reconstruction of phylogeny of ITS1-ITS2 sequences reflecting evolutionary relationship between *Rhododendron* species was obtained (Fig. 1). It is worth noting that cladograms constructed by MP method (maximum parsimony) had similar topology and bootstrap support of clades.

The phylogenetic tree resulting of Rhododendron species corresponded to the anatomical-morphological classification accepted in West Europe (Chamberlain 1996), as opposed to alternative classification by the Russian botanists (Alexandrova 1975, Koropachinskiy and Vstovskaya 2002).

Rh. adamsii and *Rh. parvifolium* manifested close relationship, and therefore, it is doubtful to assign them to different sections: *Pogonanthum* and *Rhododendron*, respectively.

A low degree of differentiation among *Rh. mucronulatum*, *Rh. dauricum*, *Rh. ledebourii* and *Rh. sichotense* is observed in the subgenus *Rhododendron*, section *Rhododendron* and subsection

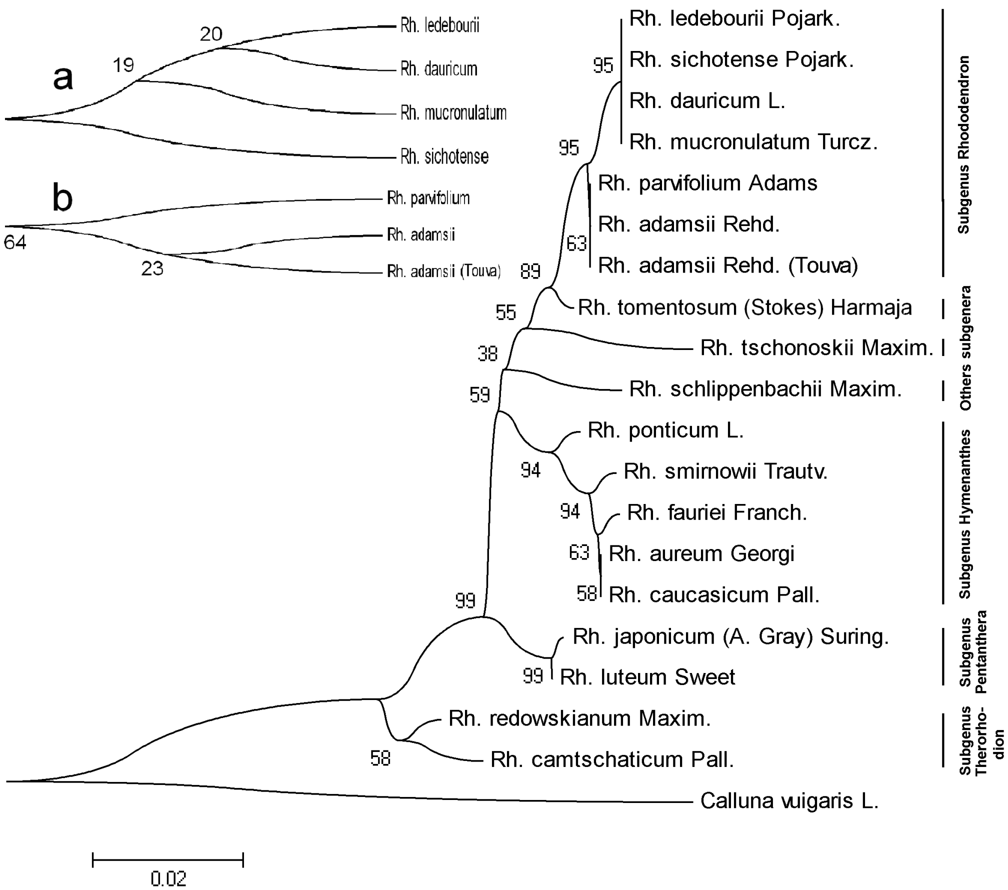

Fig. 1. ME-cladogram of *Rhododendron* L. representatives based on ITS1-5.8S-ITS2 sequences.

Rhodorastra (according to Chamberlain), which suggests recent isolation of these species (Fig. 1a). Moreover, low levels of support are evident in this clade, which does not allow establishing true phylogenetic relationships.

It is impossible to place *Rh. schlippenbachii* Maxim. and *Rh. tschonoskii* Maxim. in the phylogenetic diagram with confidence as the clade formed by these species has a low level of bootstrap support. Concerning these two species, we should follow the point of view of Alexandrova (1975) who separated them into independent taxa of subgeneric rank.

Noteworthy is a clear-cut distinction of the species of the subgenus *Therorodion*: *Rh. redowskianum* and *Rh. camtschaticum*, which were considered by some researchers to be the same species: *Rh. redowskianum* as a high-mountain ecological form of *Rh. camtschaticum* (Alexandrova 1975).

Independence of the genus *Ledum* L. is doubtful. *Ledum palustre* groups in the center of the tree of the genus *Rhododendron*, consequently, it belongs to this genus, which was already noted in Chamberlain's classification where this species was called *Rh. tomentosum* (Stokes) Harmaja.

Conclusion

Thus phylogeny of *Rhododendron* L. species reconstructed on the basis of ITS1-ITS2 – sequence corresponds to anatomical-morphological classification of Chamberlain (1996) with small additions. The reconstruction of phylogeny based on ITS1-ITS2 sequences is only possible for wall isolated *Rhododendron* L. species. To establish isolation of species in the Section *Rhododendron*, it is necessary to conduct population genetic research based on the methods of screening the whole genome (ISSR, RAPD or AFLP).

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TREE RING AND ANATOMICAL STUDIES IN *PINUS HELDREICHII* FORESTS IN PIRIN MOUNTAINS, BULGARIA

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Abstract

Pinus heldreichii forests in pristine condition can still be found in Pirin National Park in Bulgaria. Although they are of high conservational value still numerous questions for their structure, the physiological and genetic peculiarities of the species exist. Here we present results for several tree ring chronologies constructed along altitudinal gradient and different exposures. We also studied the variation of anatomical leaf parameters like number of resin ducts and stomata. We found that the oldest trees in the valley reach 800 years and are situated on isolated by rock bands sites close to the local treeline. Forests on the slopes at the bottom of the valley are 200–300 years old with single older trees. Many of them are probably shaped by fires in the past. We found similar variability in tree ring chronologies from different exposures. Only one chronology constructed from a mixed coniferous forest was found to differ at certain periods from the other chronologies. The most probable reason for this is exogenous disturbance like a fire. We did not find enough evidence that the anatomical traits of the needles are altitude dependent, although number of resin ducts and the number of stomata on the outer surface were found to increase with altitude.

Key words: *Pinus heldreichii*, tree rings, leaf anatomy.

Introduction

Pinus heldreichii Christ (*Pinus leucodermis* Antoine) is a tertiary relic species occurring in isolated subalpine and treeline locations in some mountains within the Balkan Peninsula and Southern Italy (Barbero et al. 1998). In Bulgaria, it grows only in the Pirin and Slavyanka Mountains on soils formed on marble and limestone bedrocks. All of its locations are under strict protection being included in nature reserves and Pirin National Park, which is in UNESCO's World natural heritage list. Especially in Pirin Mts. hardly accessible steep slopes helped the conservation of numerous forests in pristine state, which provides the chance to conduct studies in ecosystems that evolved under small or without any human influence. Although *Pinus heldreichii* is of high conservational value, still many topics related to its habitats are not well studied. Among them is morphological variation. Research was mostly locally based and focused on single parameters (Gudeski et al. 1975, Yurukov et al. 2005, Panayotov and Yurukov 2007, Todaro et al. 2007, Guerrieri et al. 2008, Panayotov et al. 2010a). Therefore further studies are needed to fill in some of these gaps. Moreover, besides pure conservational research needs the species additionally provides high potential for proxy climate studies due to its longevity (Panayotov et al. 2010a).

Here, we aim at studying the variation of tree ring and leaf morphology along altitudinal gradient within one valley. Additionally we compare the influence of exposure on these parameters.

Material and Methods

Study area

The study area was situated in the Banderitza valley in the Pirin Mountains, Bulgaria, 41°45′ N, 23°26′ E (Fig. 1). We selected 3 study sites at different elevations for collecting tree ring cores, 2 on the eastern slope of Vihren peak and one on the north-western slope of Todorka peak (Table 1). For leaf morphology we additionally collected samples from two

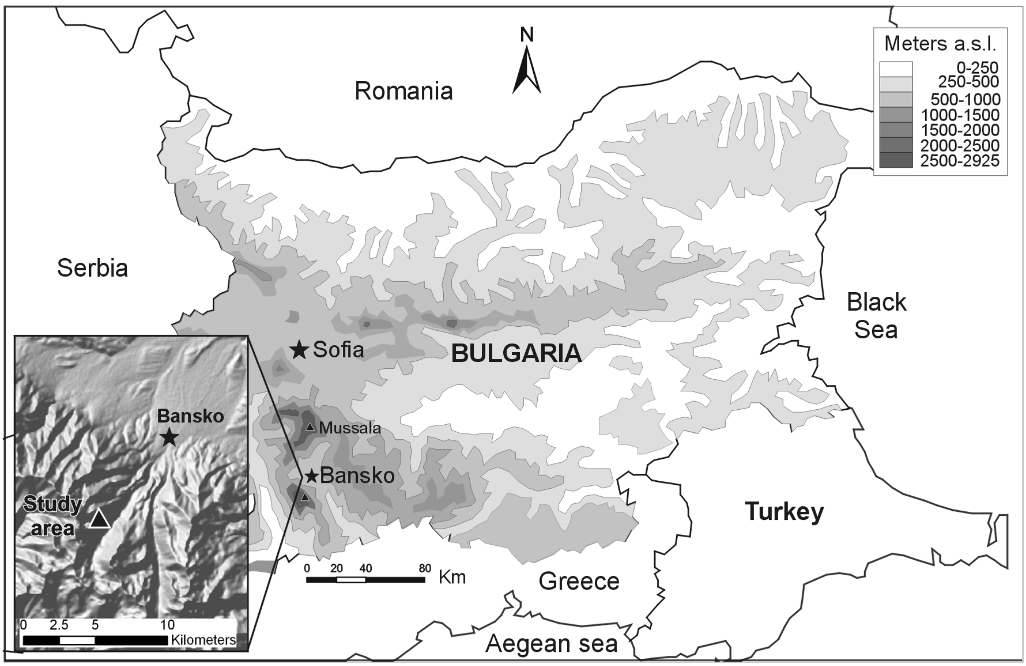

Fig. 1. Study area.

Table 1. Position of study plots.

Site name	Altitude	Exposure	Number of tree ring cores	Number of needles studied
SHADE-LOW	1800	NW	–	50
SHADE-MID	1950	NW	17	50
SUN-LOW	1750	E	15	50
SUN-MID-1	1900	E	29	50
SUN-MID-2	1950	E	–	50
HIGH-1	2000	E	–	50
HIGH-2	2100	E	55	–

more locations, one on Vihren slope and the other on Todorka slope.

The slopes were steep, with inclination of 20-50° and covered mostly with thin Rendzic Leptosols and Regosols formed on marble bedrock. Forests were pure *Pinus heldreichii* at higher elevations (i.e. sites “SUN-MID-1”, “SUN-MID-2”, “HIGH-1” and “HIGH-2”) and mixed with *Picea abies, Pinus sylvestris*, and the Balkan endemic *Pinus peuce* at lower elevations.

The climate in the region is typically mountainous, with strong influence of the Mediterranean air masses. The mean annual temperature (Vihren chalet climate station, 1970 m a.s.l.) is 3.5°C. It ranges from a mean monthly temperature of -4.7°C in January to +12.2°C in August. The annual precipitation amounts to 1378 mm, with a maximum in autumn and winter. Deep snow covers are characteristic for the region. At the same time, the summer precipitation minimum combined with shallow soil profiles on steep rocky sites might cause local drought conditions on sites with eastern and southern exposure (Panayotov et al. 2010a).

Data collection and analysis

Tree ring cores were collected with increment borer at breast height (1.3 m) from dominant trees that were not affected by avalanches or rock-falls. They were mounted on wooden boards, air-dried and sanded. Tree ring widths were measured in the dendrochronology laboratory at the University of Forestry in Sofia following standard procedures with precision of up to 0.001 cm. Obtained tree ring width series were cross-dated with the use of visual clues (Stokes and Smiley 1968) and the computer program COFECHA (Holmes 1983). Then the data were standardized with the software package ARSTAN (Cook 1985) using negative exponential function. In cases with higher low-frequency tree ring variation, which could be due to non-climatic forcing (Fritts 1976) and was typical mostly for the lower altitude sites, we also used cubic splines to perform the standardization. The final chronologies were composed by calculating bi-weighted robust means of annual ring widths. The highest altitude chronology (HIGH-2) was previously published (Panayotov et al. 2010a) and thus we compare newer obtained tree ring series to it.

For the study of leaf morphology we collected 5 two-year old needles from the lower southern parts of the crowns of 10 trees at each site (e.g. 50 needles per site). We measured the number of resin ducts (NRD), number of stomata per unit length on the abaxial leaf surface (NSO) and the number of stomata per unit length on the adaxial leaf surface (NSI). Anatomical traits were observed and recorded on micro-sections obtained from the mid-length of the needle.

Results and Discussion

Tree ring chronologies

Oldest trees were found at site "HIGH-2" (Fig. 2). The longest series had its first tree ring in 1026 AD (i.e. 803 tree rings). Though this is below the expected maximum longevity for the species as the famous tree "Baikushevata mura" is believed to be about 1300 years old, our core produced the longest dated tree ring series available in Bulgaria. Other trees at that site were also close to the age of 800 years, which allowed the tree ring chronology to reach a replication of at least 5 cores at 1250 AD (Panayotov et al. 2010a). This tree ring chronology was found to be very well correlated with others available from *Pinus heldreichii*. The highest coefficients ($R = 0.66$, $p<0.001$, common period 1600–1981; GLK = 70%; $t = 16$) were found in a tree ring chronology produced by Schweingruber (1981) for Olympus Mts. in Greece (Panayotov et al. 2010a).

The second-oldest site was "SHADE-MID". There the longest series had 566 tree rings. Yet, because

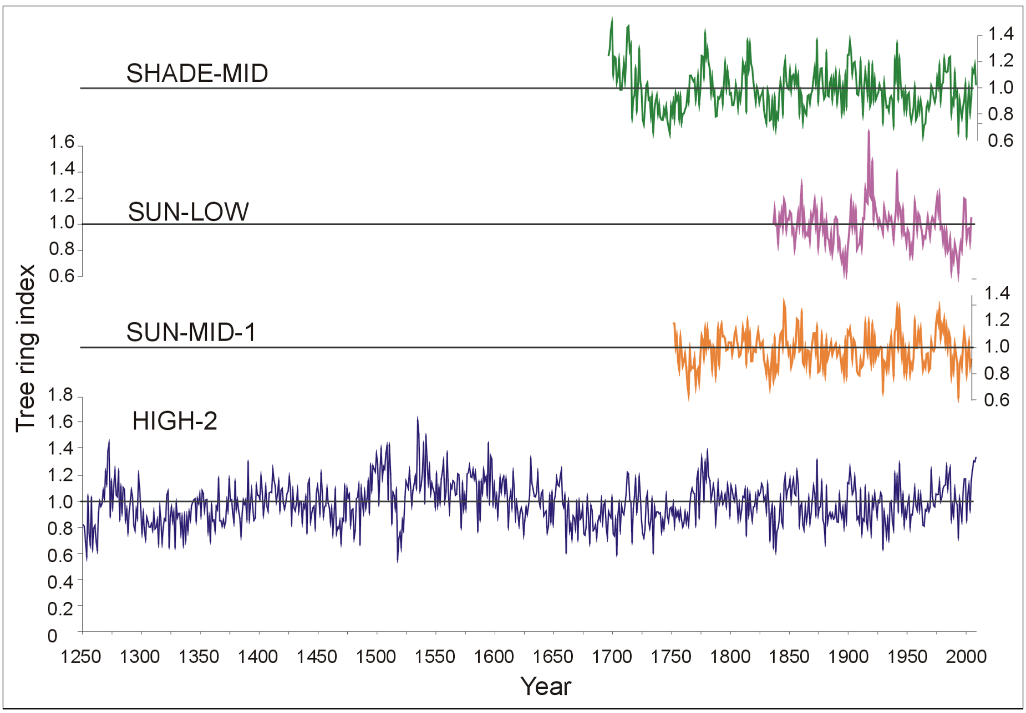

Fig. 2. Tree ring chronologies.

other trees at that site were younger the tree ring chronology was truncated at 1696. The trees at the other studied sites were found to be much younger. Few of them were older than 250 years. Youngest were the trees at site "SUN-LOW", which was a mixed *Pinus heldreichii-Picea abies-Pinus peuce* forest found at the bottom of the valley close to the Banderitza river. We suggest two possible reasons for the found differences in the age of the trees at the studied sites. One is heavy human management in the past at the lower sites. This could explain the lack of older trees at the more accessible lower parts of the slopes (sites "SUN-LOW" and "SUN-MID-1". Yet, 100 to 200 years ago mechanization and wider road was unavailable which would hinder attempts to transport numerous larger-sized trees. This would suggest that if the forests were influenced by cuttings, such could be rather more irregular selective ones. Other possible influence could be fires, either natural or set by local shepherds to clean up pastures. Such could easily escape the valley bottom and cause burning of forests on the slopes above. In that case only isolated by high rocks sites could remain intact. The position of site "HIGH-2" is exactly the same. It is completely isolated by a 30 to 100 meter high rock band from the valley below. One further fact that would support the hypothesis of a huge fire 250 to 300 years ago in Banderitza valley is the complete lack of older trees, besides the "Baikushevata mura", below the rock band that isolates site "HIGH-2". Such older trees would be expected on more inaccessible locations, which are present on the slopes, if the forest age was due only to cuttings.

High and low frequency variation was rather similar between the composed chronologies. This was expressed by the correlation coefficients, which were found to be higher than 0.6. An exception was chronology "SUN-LOW", which had lower correlations ($R < 0.31$) with chronologies "HIGH-2" and "SHADE-MID-1". This was probably due to differences in two periods in chronology "SUN-LOW". It had "a peak" in tree ring indices starting from 1914 and finishing in 1923 and a decrease starting at the beginning of the 1980^{-s}. They were not matched by other chronologies. The 1914 sharp increase of index values was due to the presence of numerous "releases" in the tree ring width series of the trees at site "SUN-LOW". Such releases could be due to exogenous disturbances, which normally cause a sharp change in tree ring widths that is not matched by other regional tree ring chronologies (Fritts 1976). In our case several types of disturbances could be expected – fires, cuttings or avalanches. Because the location of site "SUN-LOW" is close to an avalanche couloir, a tourist path and former trade route, any of these reasons is possible. Numerous fire-scars in the region and age of much of the younger trees of about 90 years could be a clue for a fire about 100 years ago, but further studies are needed to confirm or reject such hypothesis.

We expected to find difference in the tree ring chronologies composed from trees situated at sun-exposed and shady slopes. Yet, chronology "SHADE-MID-1" is rather similar in variation to the chronologies from the sun-exposed eastern slope. A comparison of the correlation coefficients for the climate-growth relationship of these chronologies also demonstrated similar reactions to the general climate variability (Panayotov et al. 2010b).

Leaf anatomical features

Previous results for morphological parameters of *Pinus heldreichii* leaves published by Yurukov et al. (2005) and Panayotov et al. (2010b) demonstrate that basic leaf features like length, width and thickness vary much between sites, but do not necessarily demonstrate altitude or exposure dependent variability. Therefore we chose to present and comment results for anatomical parameters like number of resin ducts and stomata. Our data show that the number of resin ducts (NRD) in leaves from sites in Banderitza valley was higher on the sun-exposed eastern slope than on the shady western slope (Table 2). The mean and maximum values increased with increase of the altitude till 1950 m a.s.l. and then decreased slightly at sites close to the local treeline. Existence of differences between altitudes is also demonstrated by the results from the ANOVA test (Table 2). Yet, no clear trend could be observed, which raises the question whether the found differences were site or altitude dependent. The number of resin ducts was also much smaller (up to twice) than indicated by previous results from Bulgaria presented by Yurukov et al. (2005). This is probably due to differences in measurement equipment, which was with lower quality in the previous study. Moreover, at

Table 2. Anatomical leaf parameters.

Site name	Altitude, m	Exposure	NRD			NSO			NSI		
			Mean	min	max	Mean	min	max	Mean	min	max
SHADE-LOW	1800	NW	2.6	0	6	14.0	10	20	14.2	10	17
SHADE-MID	1950	NW	2.4	0	6	14.9	11	20	14.6	11	18
SUN-LOW	1750	E	2.8	1	6	13.9	10	16	13.5	9	16
SUN-MID-1	1900	E	2.9	2	7	14.5	10	20	13.9	10	16
SUN-MID-2	1950	E	3.9	1	10	14.9	12	19	15.1	12	18
HIGH-1	2000	E	3.0	0	8	14.4	8	18	14.5	10	18
	ANOVA		$p<0.0001$			$p<0.0001$			$p=0.477$ n.s.		
	Regression against altitude		$R^2=0.08$; $p=0.49$			$R^2=0.012$; $p=0.93$			$R^2=0.011$; $p=0.80$		

Legend: NRD – number of resin ducts; NSO – number of stomata per unit length on the abaxial surface; NSI – number of stomata per unit length on the adaxial leaf surface.

least one of the locations for collection of leaves for the previous study was close to our locations and therefore less difference is expected.

We found similar tendency for increase of the number of stomata on the abaxial leaf face per length unit (i.e. NSO) with the increase of altitude (Table 2). Highest mean values were found again for the sun-exposed slope at altitude of 1950 m a.s.l. Values were higher on the shady slope at comparable altitudes. No altitude or exposure – dependent variability was observed for the parameter number of stomata per unit length on the adaxial leaf surface (NSI). Because the results for stomata are novel for the country, we cannot comment them in respect to previous findings.

Conclusion

Old-growth *Pinus heldreichii* forests with age of the dominant trees of up to 800 years can be found in Banderitza valley. They allow construction of reliable tree-ring chronologies, which can be used for proxy climate reconstructions. The lower parts of the valley slopes are occupied by younger forest. Among the possible reasons for this are disturbances such as fires and avalanches. Our data provide a clue for a possible fire at the 1910-s. No differences were found between tree ring chronologies obtained from sun-exposed and shady slopes. This is a clue that the climate-related variability of *Pinus heldreichii* tree rings is rather stable and less dependent on factors like exposure.

We could not find evidence that leaf anatomical features are altitude or exposure dependent. Although the number of resin ducts and the number of stomata on the abaxial leaf surface were found to increase with the increase of altitude, further studies from other locations are needed to draw more sound conclusions.

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VARIABILITY IN MAGNESIUM CONCETRATION IN NEEDLES OF DIFFERENT DOUGLAS-FIR PROVENANCES

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Abstract

Magnesium concentration in the needles of Douglas-fir (*Pseudotsuga mensiesii* /Mirb./ Franco) was investigated in a provenance test under homogeneous site conditions (Ass. *Fagetum submontanum* Rud. on acid brown soil on gneiss) . The established quantities of magnesium in needles are indicative for different capabilities of certain Douglas-fir provenances to adopt this nutritive element from the soil. Significant differences among the examined provenances were found in mean height, mean diameter, tree volume and basal area. However, regression analysis revealed that there is no significant relation between magnesium concentration in needles of different Douglas-fir provenances and any of the examined forest stand parameters. Differences in mean height, mean diameter, tree volume and basal area are not the consequence of different capability of certain Douglas-fir provenances to adopt magnesium from the soil but are of genetic nature.

Key words: Douglas-fir, provenances, magnesium, foliar nutrion, stand height, stand diameter, Serbia.

Introduction

Douglas-fir (*Pseudotsuga menziesii* /Mirb./ Franco) is one of the most thriving coniferous species in its native range of North America and Canada (Brodie et al. 1987, Campbell 1979). As a fast growing species (Curtis et al. 1974), with the potential to produce high quality wood with wide usability it is regarded as an important component of the economy and wood industry (Cleary et al. 1978). It was introduced into Europe in 1827 and since then it has become the most prospective coniferous species introduced into plantations and mixed stands in Central Europe (Hermann 1987). The wide native range of the species implies the choice of a suitable provenance as a precondition for successful introduction (Campbell 1979, Hermann 1985).

One of the important elements of site suitability is the state of mineral foliar nutrition. If a plant is supposed to reach its optimum production determined by its genetic potential it needs to be adequately supplied with mineral nutrients.

Mineral nutrients have numerous physiological functions in a plant: forming of plant tissues, serving as catalysts of numerous reactions, osmoregulators, elements of buffer solutions, regulators of membrane permeability etc. For instance, Ca is a constituent of the cell wall, Mg of the chlorophyll molecule, S of proteins, P of phospholipids, nucleoproteids etc.

Magnesium ion (Mg^{2+}) is constituent of the chlorophyll molecule where it is placed in the centre of the chlorin ring. Magnesium also plays an important role in organic matter exchange by activating specific enzymes, which take part in photosynthesis and respiration (reactions which involve ATP-enzymes for carbon fixation). It participates in the metabolism of phosphorus and nitrogen and is important for the preservation of ribosome structure, as well as for the transport of products of photosynthesis and accumulation of reserve substances, particularly starch.

Magnesium participates in the process of neutralization of the surplus of organic acids by forming hardly soluble salts such as Mg-oxalate. It affects plant water regime through swelling of the protoplasmic colloids and improves the strength of the cell membrane by forming Mg-pectinate.

Material and Method

The object of the study is a Douglas-fir provenance test located in the Juhor mountain range in Central Serbia (Lavadinović et al. 2001, Lavadinović and Isajev 2003): Douglas-fir seedlings were produced in the nursery of the Institute of Forestry in Belgrade from seed imported from USA. The seed originated from 20 provenances, which differ in latitude, longitude and altitude and cover a part of the native range of the species (Table 1). The test was established in 1982 (seedling age 2+2) on a site classified as beech forest (*Fagetum moesiaca montanum* Jov. 1976) on acid brown soil (dystric cambisol) on gneiss.

The altitude of the site is 660–700 m a.s.l. on a southern slope. Each provenance was represented by 12 plants in each block with six replications, i.e. in total 72 trees per provenance. The site conditions in the entire test area were fairly uniform.

Stand parameters were measured at a stand age of 19 years. On the basis of these measurements the following values were calculated: mean diameter and mean height, basal area, tree volumes and volume increment of each Douglas-fir provenance.

Needle samples for foliar analysis were collected in the upper third part of sample trees of all provenances. Magnesium concentration in the needles was determined by the analysis of ash after dry ashing using the complexometric method.

Results

Average magnesium content in oven-dry needles of all the examined Douglas-fir provenances was 0.40% needles (Table 2, Fig. 1). The highest magnesium content (0.88%) was observed in the provenance with mark 30 (code Oregon 204-04), and the lowest one in the provenance marked 15 (code Washington 202-17). Standard deviation

Table 1. Geographical co-ordinates of the tested Douglas-fir provenances.

Provenance	Our mark, No	Latitude, °N	Longitude, °E	Altitude, m asl
Oregon 205-15	1	43.7	123.0	750
Oregon 205-14	2	43.8	122.5	1200
Oregon 202-27	3	45.0	122.4	450
Oregon 205-38	4	45.0	121.0	600
Washington 204-07	9	49.0	119.0	1200
Oregon 205-13	10	43.8	122.5	1050
Oregon 205-18	11	44.2	122.2	600
Oregon 202-22	12	42.5	122.5	1200
Washington 202-17	15	47.6	121.7	600
Oregon 201-10	16	44.5	119.0	1350
Washington 201-06	17	49.0	120.0	750
Oregon 202-19	18	45.3	123.8	300
Oregon 205-11	20	45.0	123.0	150
New Mexico 202-04	22	32.9	105.7	2682
New Mexico 202-10	23	36.0	106.0	2667
Oregon 202-31	24	44.3	118.8	1500
Oregon 205-29	26	42.6	122.8	900
Oregon 205-08	27	42.7	122.5	1050
Oregon 204-04	30	45.0	121.5	900
Washington 205-17	31	47.7	123.0	300

was 0.1617 and the coefficient of variation 40.87%. Magnesium content of provenance Oregon 204-04 significantly deviated from the average of all the examined provenances at the threshold of significance of 95%. Inter-provenance variabil–ity of magnesium content in Douglas-fir needles was not the consequence of different nutritive conditions of nutrition, because the provenances were grown under the same site conditions on the same type of soil, the same altitude and exposition. Stand conditions were also made uniform for all provenance groups of trees. The differences in the quantity of magnesium adopted by Douglas-fir trees are thus genetically preconditioned.

Regression analysis (table 3) revealed no significant relationship between magnesium concentration in Douglas-fir needles and the mean diameter of provenances. Maximum mean diameter was recorded for provenance Oregon 202-27. Magnesium concentration in the needles of this provenance was 0.49%. The minimum mean diameter was found in provenance Washington 204-07 and magnesium content in the needles was 0.33%.

Regression analysis revealed that there was no significant relationship between magnesium concentration in needles and mean height of the Douglas fir provenances. The largest mean height (11.0 m) was found in provenance Washington 205-17 in which the magnesium concentration was 0.25%. The smallest

Table 2. Magnesium content in needles of various Douglas-fir provenances hand forest estimation elements.

Provenance	Our mark	Mg concentration in needles	Standard deviation of [Mg]	Aritmetic mean diameter	Aritmetic mean height	Basal area	Volume	Periodic volume increment (15-18 years)
	No	%		cm	m	$m^2 \cdot ha^{-1}$	$m^3 \cdot ha^{-1}$	$m^3 \cdot ha^{-1}$
Oregon 205-15	1	0.45	0.3370	14.8	10.8	35.8	179.5	20.1
Oregon 205-14	2	0.45	0.3370	14.6	10.2	34.9	174.7	22.2
Oregon 202-27	3	0.49	0.5844	15.5	10.6	39.3	201.0	35.1
Oregon 205-38	4	0.42	0.1515	15.0	10.3	38.8	187.8	26.3
Washington 204-07	9	0.33	-0.4051	6.8	4.8	8.7	30.9	3.3
Oregon 205-13	10	0.54	0.8936	14.5	10.3	34.4	170.4	26.4
Oregon 205-18	11	0.39	-0.0340	14.6	9.9	34.9	173.0	23.1
Oregon 202-22	12	0.22	-1.0853	15.1	10.0	37.3	181.2	28.0
Washington 202-17	15	0.10	-1.8275	14.8	10.4	35.8	177.4	27.9
Oregon 201-10	16	0.51	0.7081	10.9	7.2	19.4	86.2	9.4
Washington 201-06	17	0.45	0.3370	11.3	7.1	20.9	90.8	13.3
Oregon 202-19	18	0.19	-1.2709	15.0	10.7	36.8	186.3	27.7
Oregon 205-11	20	0.31	-0.5288	15.2	10.0	37.8	179.8	22.8
New Mexico 202-04	22	0.41	0.0897	11.5	7.7	21.6	102.8	16.5
New Mexico 202-10	23	0.40	0.0278	11.3	7.8	20.9	94.4	11.9
Oregon 202-31	24	0.47	0.4607	8.3	5.6	11.3	47.4	5.7
Oregon 205-29	26	0.34	-0.3432	15.1	10.1	37.3	183.5	21.1
Oregon 205-08	27	0.31	-0.5288	14.7	10.6	35.3	175.9	23.6
Oregon 204-04	30	0.88	2.9963	14.0	9.7	32.1	157.3	26.8
Washington 205-17	31	0.25	-0.8998	14.5	11.0	34.4	177.7	28.6
Average		0.40	0.0000	13.38	9.24	30.39	147.90	20.99
St deviation		0.1617		2.4927	1.8435	9.4953	51.5632	8.4290
V		40.87		18.64	19.95	31.25	34.86	40.16
Min		0.10		6.80	4.80	8.70	30.90	3.30
Max		0.88	2.9963	15.50	11.00	39.30	201.00	35.10

mean height (4.8 m) was found in provenance Washington 204-07, which also has the smallest mean diameter.

The largest basal area was found in the provenance Oregon 202-27, which had also the largest mean diameter, and the

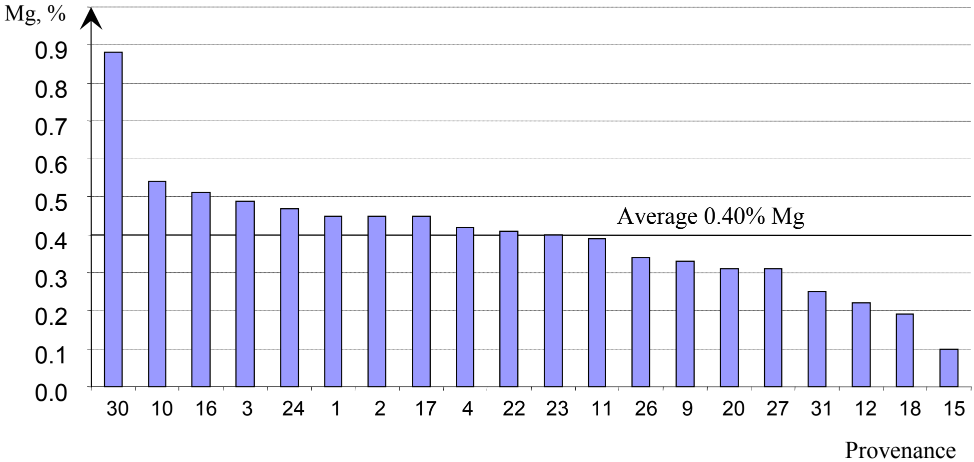

Fig. 1. Inter-provenance variability of magnesium concentration in Douglas-fir needles.

smallest in the provenance Washington 204-07 with the smallest stem diameter. Inter-provenance variability of magnesium concentration in needles had no impact on the inter-variability of stand basal area, which is derived from stand diameter.

Tree volume was also derived from diameter and height. No relation between stand tree volume and magnesium concentration in needles was found.

The statistical analysis showed that inter-provenance variability of magnesium concentration in needles has no impact on the volume increment of Douglas-fir. The highest volume increment on the examined site was recorded in prov-

Table 3. Regression analysis.

Forest stand parameter	Regression equation	Degrees of freedom n-1	Coefficient of determination, r^2
Mean diameter	d = -2.2624 Mg + 14.27	19	0.0215 ns*
Mean height	h = -1.9263 Mg + 10.002	19	0.0285 ns
Basal area	G = -10.321 Mg + 34.467	19	0.0309 ns
Volume	V = -56.264 Mg + 170.15	19	0.0311 ns
Periodic volume increment (15-18 years)	Iv = -56.264 Mg + 170.15	19	0.311 ns

* Not significant at 0.05 probability level

enance Oregon 202-27 and the lowest one - in provenance Washington 204-07.

Conclusion

Based on the examinations carried out as a part of a test of the 19-year-old Douglas-fir provenance on a beech site (*Fagetum moesiaca montanum* Jov. 1976) on acid brown soil (dystric cambisol) on gneiss, it can be concluded that inter-provenance variability of magnesium concentration in Douglas-fir needles is genetically preconditioned and is not a consequence of different conditions of nutrition with this element.

Inter-provenance variability of magnesium content in Douglas-fir needles is not the cause of inter-provenance variability of the examined forest stand parameters (mean diameter, mean heights, basal area, volume and volume increment). Like in the case of magnesium concentration in needles, differences in the examined forest estimation elements among certain provenances are genetically preconditioned.

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FUNGI PHYLUM *BASIDIOMYCOTA* ON WILLOWS IN THE AREA OF CENTRAL DANUBE BASIN

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Abstract

The results of three-year's research of fungi phylum *Basidiomycota* on *Salix* sp. in the area of Central Danube Basin (45°08′18″ and 45°42′50″ north latitude and 17°10′10″ and 17°58′50″ east longitude from Paris), are presented in this paper. During the research, the fruiting bodies were collected in the field and determined based on their reproductive organs. Twenty-eight species of macrofungi have been identified on the willows of which *Fomes fomentarius* (L.: Fr.) Fr., *Phellinus igniarius* (L.: Fr.) Quél. and *Trametes suavealens* (L.: Fr.) Fr. have the greatest pathogenic significance as wood rotting agents. Four of the above 28 species are used in human nutrition: *Pleurotus ostresus* (Jacquin: Fr.) Kumm., *Flammulina velutipes* P. Karst., *Auricularia auricula-judae* (Bull. :m Fr.) Wettst. and *Polyporus sulphureus* (Bull.) Fr.), and one is used in pharmacy (*Ganoderma lucidum* (Curt.: Fr.) P. Karst.). During this research, 17 species of macrofungi were first found on willow, which have not been so far identified from Serbia and Montenegro. Of 17 newly identified fungi, 7 species have never been identified from Serbia and Montenegro, and 10 have been identified, but on other hosts (e.g. poplars).

Key words: Basidiomycota**,** Central Danube Basin, *Salix* sp. pathogenic fungi, white rot, brown rot, medicine, edible fungi.

Introduction

Limited wood production in natural forests and rapid increment of industrial processing of lumber have resulted in wood deficit. This problem could be alleviated by production of fast growing trees in plantations (first of all poplars and willows).

In the Autonomous Province of Vojvodina, considerable areas of land are marked as unsuitable for agricultural production and establishment of plantations of economic important trees such as oak or poplar. This situation emphasizes the importance of willow plantations. Species of willows are recognized as pioneer plants on the lowest parts of the alluvial terraces along the rivers.

Opposite to poplar diseases, the diseases on willows did not attract attention until now. This problem became fully expressed by the establishment of new willow plantations and made investigations like this necessary.

The aim of this study is to recognize the main willow wood destroyers, decay causers, and to determine the possibilities of the use of fungal sporophores in human nutrition and in medicine.

Materials and Methods

Field research and collecting of material have been done on several localities in the Middle Danube region, in natural willow populations and plantations in floodplain areas along the Danube River. The investigated area is situated between 45°08′18″ and 45°42′50″ north latitude, and 17°10′10″ and 17°58′50″east longitude, elevated from 73 to 79 m above see level. This region has a moderate continental climate (Katić et al. 1979).

The sampled sporophores were preserved using standard methods, and determined according to appropriate keys in the laboratory.

Results and Discussion

A list of macrofungi (Phylum Basidio–mycota – Cl. Basidiomycetes) detected on willows is given in Table 1.

During the research, twenty-eight species of macrofungi have been de-

Table 1. A list of macrofungi detected on willows.

Fungus name	Family	Decay	Importance potential use *
Ordo Auriculariales			
Auricularia auricula-judae (Bull.) Quél. (photo 1)	Fam. Auriculariaceae Genus *Auricularia*	White	+ ●
Ordo Agaricales			
Flammulina velutipes P. Karst. (photo 2)	Fam. Tricholomataceae	White	+ ●
Mycena sp.		-	-
Panellus ringens (Fr.) Romagn.		White	+
Pluteus salicinus (Pers.:Fr.) Kummer	Fam. *Pluteaceae*	White	+ +
Pholiota adiposa (Batsch.:Fr.) Kumm.	Fam. Strophariaceae	White mottled	+ +
Pholiota squarrosa (Pers. ex Fr.) Kumm.		White	+
Ordo Cortinariales			
Crepidotus variabilis (Pers. ex Fr.) Kummer	Fam. Crepidotaceae Genus *Crepidotus*	White	+ +
Ordo Ganodermatales			
Ganoderma adspersum (Schulz) Dank	Fam. Ganodermataceae	White	+ +
Ganoderma lucidum (Curt. ex Fr.) Karst (photo 5)		White	+ + ◘

Ordo Hericiales			
Creolophus cirrhatus (Pers. Ex Fr.) Karst	Fam. Hericiaceae	White	+
Ordo Hymenochaetales			
Phellinus contiguus (Fr.) Pat.	Fam. Hymenochaetaceae	White	+
Phellinus igniarius (L. ex Fr.) Quel. (photo 6)		White layered	+ + +
Ordo Poriales			
Daedaleopsis confragosa (Bolt.: Fr.) J. Schroet	Fam. Coriolaceae	White	+ +
Fomes fomentarius (L.: Fr.) Fr.		White mottled	+ + +
Trametes gibbosa (Pers. ex Fr.) Fr.		White	+
Trametes hirsuta (Wulf. ex Fr.) Pilat		White	+ +
Trametes suavealens (L.: Fr.) Fr.		White	+ + +
Trametes versicolor (L.ex Fr.) Pilat		White	+ +
Lentinus tigrinus (Bull.: Fr.) Fr.	Fam. Lentinaceae	White	+ +
Pleurotus ostreatus (Jacquin : Fr.) Kumm. (photo 3)		White	+ ●
Polyporus sulphureus (Bull.) Fr. (photo 4)	Fam. Polyporaceae	Brown prismatic	+ + ●
Ordo Stereales			
Cytidia salicina (Fr.) Burt.	Fam. Corticiaceae	White	+
Irpex lacteus (Fr.:Fr.) Fr.	Fam. Steccherinaceae	White	+
Phanerochaetae filamentosa (Berk. & Curt.) Burdsall	Fam. Meruliaceae	White	+
Stereum subtomentosum Pouzer	Fam. Stereaceae	White	+ +
Ordo Schizophyllales			
Schizophyllum commune Fr.: Fr.	Fam. Schizophyllaceae	White	+ +
Ordo Tremellales			
Exidiopsis calcea Wells	Fam. Exidiaceae	-	-

*+++ Appearance of fungus is abundant and causes serious damages on host plant
+ + Appearance of fungus is rare and causes slight damages on host plant
+ Appearance of fungus is rare and without practical significance
- Appearance of fungus is abundant without practical significance
● Edible fungus
◘ Medical fungus

Photos 1-6. Macrofungi on willows: 1. *Auricularia auricula-judae* (Bull. ex St-Amons) Wetts.; **2.** *Flammulina velutipes* P. Karst.; **3.** *Pleurotus ostresus* (Jacquin : Fr.) Kumm.; **4.** *Polyporus sulphureus* (Bull.) Fr.; **5.** *Ganoderma lucidum* (Curt. ex Fr.) Karst.; **6.** *Phellinus igniarius* (L. ex Fr.) Quel.

tected on willows, of which twenty-four fungi cause white, while one brown prismatic decay.

The first group expressing the highest impact (+ + +) encompasses the fungi which have the greatest pathogenic influence as wood rotting agents. *Fomes fomentarius* (L.: Fr.) Fr., *Phellinus igniarius* (L. ex Fr.) Quél. and *Trametes suaveolens* (L.: Fr.) Fr. are recognized as the major pathogens, causing serious decay.

During this research, 17 species of macrofungi were first found on willow, which have not been so far identified from Serbia and Montenegro. Of 17 newly identified fungi, 7 species have never been identified from Serbia and Montenegro, and 10 have been identified, but on other hosts (e.g. poplars).

A list of macrofungi detected on willows is given in Table 2.

Fomes fomentarius (L. : Fr.) Fr.

This fungus appears as saprotrophe or parasite on broadleaved trees and causes white mottled decay. It grows on standing, living trees, and develops further on fallen trees, but only untill they are wet. It rarely develops its colonies on dried and processed wood.

Karadžić and Anđelić (2002) cite that the species can be found in Europe, Asia, Africa and Northern America. The infection is done through bark contu-

Table 2. A list of macrofungis first time detected in Serbia and Montenegro and on willows.

Name of fungus	The first time found in Serbia and Montenegro	The first time Found on willow
Flammulina velutipes P. Karst.	-	+
Panellus ringens (Fr.) Romagn.	+	+
Pluteus salicinus (Pers.:Fr.) Kummer	+	+
Pholiota adiposa (Batsch.:Fr.) Kumm.	-	+
Pholiota squarrosa (Pers.ex Fr.) Kumm.	-	+
Crepidotus variabilis (Pers. ex Fr.) Kummer	+	+
Ganoderma adspersum (Schulz) Dank	-	+
Ganoderma lucidum (Curt. ex Fr.) Karst	-	+
Creolophus cirrhatus (Pers. Ex Fr.) Karst	-	+
Phellinus contiguus (Fr.) Pat.	+	+
Daedaleopsis confragosa (Bolt.: Fr.) J. Schroet	-	+
Trametes gibbosa (Pers. ex Fr.) Fr.	-	+
Pleurotus ostreatus (Jacquin : Fr.) Kumm.	-	+
Cytidia salicina (Fr.) Burt.	+	+
Phanerochaetae filamentosa (Berk. & Curt.) Burdsall	+	+
Stereum subtomentosum Pouzer	-	+
Exidiopsis calcea Wells	+	+

sions, and the process of rotting is very quick. After the degradation of the core, the fungus progress into the sapwood, and it proceeds with the destruction.

According to Krstić (1962) the emission of the spores from one sporophore is 887 millions in one our, or 139 millions per 1 cm^2 in one day, so we can conclude that the potential for contamination is very high.

Phellinus igniarius (L. : Fr.) Quél.

Karadžić and Anđelić (2002) cite that this fungus occurs as a parasite on broadleaved trees, and it occurs specially often on alder, wild cherry, beech, sycamore, ash, hornbeam, poplars, birch, willows, elm and on *Sorbus* sp. It causes white layered decay which is localised in the hearthwood and rarely grows into the sapwood. The infection is done through contusions or dead branches, from where it spreads towards the core of the stem. The sporophores appear individually or 2–3 samples incorporated, and they can be found the whole year (photo 6).

According to Krstić (1962) this fungus destroys both the cellulose and the lignin. It is a great problem for the broadleaved tree species, and it continues its development on the trunks even after the felling of the trees.

Trametes suavealens (L.: Fr.) Fr.

This species is registered on standing willow trees and it causes white, soft decay of the core of willows and poplars.

It's widespread in Europe, Asia and Northern America on broadleaved tree species. Sporophores are annual, and they can be found during the whole year.

The sporophores are console like, broadly fixed to the substratum, from 2–12 (15) cm wide, and 1.5–4 cm (on the fixation with the wood 1.5–8 cm). The surface is flat or widely wave-like, piled, white or grey-white, with sharper edges. The hymenophore consists of pores which are round-coined or elongated, dimensions from 1–3 mm, white, ocher or brown.

The trama is white; when fresh it is soft with a cork like consistence, when dry it is hard, tough and light. The body has a strong smell of. The sporophores are single or, more often, grown together in series (Karadžić and Anđelić 2002).

The following fungi are used in human nutrition *Pleurotus ostreatus* (Jacquin : Fr.) Kumm., *Flammulina velutipes* (Curtis) Singer, *Auricularia auricula-judae* (Bull.) Quél and *Polyporus sulphureus* (Bull.) Fr.

Pleurotus ostreatus (Jacquin : Fr.) Kumm. syn. *P. columbinus* Quél.

It can be found in broadleaved forests and in mixed broadleaved-coniferous forests. It is especially often recorded on beech and on poplars, and it can also be found on older willow stems. It attacks all constituents of wood and therefore causes white decay. The sporophores often grow in groups (photo 3). The body is flesh like, white, it has nice smell and taste. The print of spores is pale grey-purple (Marković 2006b).

It can be found in Europe, Asia, Australia, Northern Africa and America.

Flammulina velutipes (Curtis) Singer

The fungus spreads in the hearthwood of several broadleaved tees, both in living trees and in dead fallen wood. It causes soft, hollowed, yellow-white decay of sapwood (Josifović 1952). It is especially often recorded in flatland areas, where it appears during winter months, from October until March, rarely outside of this period.

Karadžić and Anđelić (2002) cite that this fungus appears on death wood material, rarely on healthy or injured living stems of broadleaved trees, especially on *Salix*, *Tilia*, *Fagus* and *Alnus*, but with the remark that Breitenbach and Kranzlin (1991) also noticed this fungus on some coniferous tree species (*Abies*).

It does not have great importance as wood destructor. The Mushrooms are edible, and its importance is enhanced because it apperas during tre winter months when other edible fungi cannot be found (photo 2).

Polyporus sulphureus (Bull.) Fr.

The fungus often grows on stems of living broadleaved trees. It spreads in the corewood, very rarely in the sapwood, and causes brown prismatic decay.

It is an edible fungus, and in Germany and America it is considered a delicacy.

Ganoderma lucidum (Curt. : Fr.) P. Karst.

According to Krstić (2002) this fungus appears on roots and on the bottom part of the stem on oaks, rarer on other broadleaf tree species. It causes white decay. The carpofores are formed during the whole year. It can be found in Europe, Asia, Australia and Northern America.

It appears very rarely on willows. The sporophores are shaped like a kidney, in the beginning the surface is bright-yellow, later dark blood-red to red-brown with a purplish tinti, polished, the edges are white or yellow, 10–25 cm in diameter and 3 cm broad with a typical, rather long, excentric, brownish or almost black, polished stem (photo 5).

It is considered to be a medical fungus, especially in Asia (China) where it is also grown (Karadžić and Anđelić 2002).

Conclusion

During the three-year's research of Basidiomycota on willows, 28 species of macrofungi have been detected (Marković 2006a). Twenty-four species cause white, while one brown prismatic decay (Karadžić and Anđelić 2002). The following fungi have the greatest pathogenic importance: *Fomes fomentarius*, *Phellinus igniarius* and *Trametes suavealens*.

Pleurotus ostreatus, *Flammulina velutipes*, *Auricularia auricula-judae* and *Polyporus sulphureus* are used for human nutrition (Keizer 1996).

During this research, 17 species of macrofungi were first found on willow, which have not been so far identified from Serbia and Montenegro. Of 17 newly identified fungi, 7 species have never been identified from Serbia and Montenegro, and 10 have been identified, but on other hosts (e.g. poplars).

Based on the comparison of the mycoflora occurring on willow trees in natural stands and that occurring in plantations on clonal material, it was concluded that, on the clonal material, the dominant parasitic fungi are those which infest the leaves and bark, while on the old trees in natural stands, the dominant fungi are the agents of wood decay. To protect the juvenile willows in plantations (primarily against the parasites on the foliage) it is necessary to apply the chemical measures of protection, i.e. to apply the fungicides during the critical period for parasite infection.

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PRODUCTION OF SEEDLINGS OF WHITE WILLOW (*SALIX ALBA* L.) ON EUGLEY SOIL

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Abstract

The paper presents research results of morphological, physical and chemical properties of eugley soil in a protected part of alluvial plains of the Danube River. Research was conducted in the nursery "Kacka suma", where the seedlings of white willow (*Salix alba* L.) were produced. The following hydrological soil characteristics were studied in detail during the vegetation period: immediate moisture content by depth profile, the dynamics of movement of groundwater and its interaction with water level of Danube. In order to assess the benefits of this soil type for the production of willow seedlings, the height of seedlings of five white willow genotypes (*S. alba*: cl. '107/65/9', cl.'347', cl.' V-158', cl. '79/64/2' and cl. 'V-55') was measured at the end of the vegetation period. Genotype '107/65/9' exhibited the largest amount of seedlings with proper dimensions and genotype 'V-55' exhibited the smallest one. The results suggest that high quality *S. alba* seedlings may be successfully used on eugley soils.

Key words: eugley soil, soil moisture, seedlings, plant height, *Salix alba*.

Introduction

The genus Salix includes about 450 willow species: shrubs or trees, some reaching 40 m in height (Keoleian and Volk 2005*)*. In European flora there are 70 species of allochtonos and autochtonos willows, (Rechinger 1964, ex Krstinic 1986a). According to Krstinic (1986a) only two autochthonous species (*S. alba* L. and *S. fragilis* L.) can grow as tall trees in lowlands of Europe, white willow being considered of the greatest economic significance. It is due to the great areas it covers, rapid volume growth in natural stands and plantations, and the high revenue from timber production (Krstinic 1986b)*.* Zalesny and Bauer (2007) showed that some willow genotypes exhibited goodgrowth on polluted soils, and considered them suitable for phytoremediation of polluted areas. Because of the mentioned advantages of the willow, this study focused on the production of white willow seedlings. Volk et. al. (2004) found that the phase related to establishment of quality stands of white willow genotypes was critical for the biological and economical success, and that special attention should be paid, among other things, to the storage of reproduction material.

In order to obtain quality willow planting material, it is necessary to choose a soil suitable for the considered species. Since willows are part of alluvial hygrophilic forests (Jovic et al. 1991) occupying narrow or wide belts along the larger rivers, the production of willow planting material is organized in that very area. Barsoum (2002), studying the natural regeneration of willows and poplars mentioned that the unseasonal flood waters in the area of river terraces harmed the regeneration from seed, but helped the vegetative regeneration. Soils to be used for production of willow seedlings should have a high fertility potential, reflected in their textural composition (Roncevic et al. 2002).

According to (Roncevic et al. 2002), the eugley soils and hypogley moistening system with increased clay content are used for production of willow planting material. These soils usually contain more silt and clay than the soils in poplar nurseries, and most often belong to the class of clay loam (table 1). Since these soils are found on the lowest micro cites they are exposed in nature to increased moistening by flood or ground water.

Due to the construction of protective embankments along the Danube River, eugley soils in the central Danube basin are protected from flooding, and are mostly exposed to excessive moistening only by underground waters. Underground water level is generally high with approximate depth 1 m below surface, depending mainly on the Danube water level.

The aim of this study was to investigate properties of eugley soils in the protected part of alluvial plain in the central Danube basin, and the possibility of nursery production of white willow (*Salix alba* L.) clones.

Materials and Method

The experiment was located in the protected part of the alluvial plain in the central Danube basin in the Experimental estate of the Institute of lowland forestry and environment. The field experiment was carried out in spring 2006 using five *S. alba* genotypes free grown on eugley soil. The following clones were used in the experiment: *S. alba*: cl. ‘107/65/9’, cl. ‘347’, cl. ‘V-158’, cl. ‘79/64/2’ and cl. ‘V-55’. The experiment consisted of five replications with 30 plants per plot and random design. Spacing was 0.8 m between the rows, and 0.3 m within the row, tus resulting in 41.625 plants per hectare. At the end of 2009 vegetation period the heights of one year old sprouts from four year old root stocks were measured. Soil profile was created at the experimental site and its morphological characteristics were described. Soil samples were analyzed in the Laboratory for soil properties of the Institute of lowland forestry and environment, and physical and chemical properties of the soil samples were determined using the following methods:

- Mechanical soil composition according to B-pipette method – extracted with sodium-pyrophosphate;
- Moisture soil content up to the depth of 60 cm – thermogravimetric method;
- Differential porosity calculated from total porosity value and moisture retention using different procedures;
- Humus content in soil using Tjurin method modified by Simakov;
- Soil $CaCO_3$ content was analyzed volumetrically using a Scheibler calcimeter;
- Soil chemical reaction, pH of water determined electrometrically using a glass electrode;

Table 1. Mechanical soil composition.

Horizon	Depth, cm	Total sand > 0.02 mm, %	Total clay <0.02 mm, %	Textural Classes
Aa,p	0–30	24.28	75.72	Dusty loam
AaGso	30–60	34.84	65.16	Loam
Gso	60–90	88.08	11.92	Sandy loam

- Nitrogen according to Kjedahl;
- Easily available phosphorous and potassium according to Al-method, Egner-Riehm–Dominigo;
- Ground water levels measured in the piezometers.

Depth of ground water was measured and soil samples for immediate moisture determination were taken each month. Heights of seedlings were measured at the end of vegetation period. Data were processed using standard method of statistical analysis for mean value comparison (ANOVA, LSD-test at the probability level of 0.05).

Results

Soil characteristics

Analysis of eugley soil composition (Table 1) revealed high content of total clay in surface horizon and in transition AaGso horizon down to the depth of 60 cm. The lowest content of total clay was found in the gleyic subhorizon (Gso). On the other hand, total sand content was the lowest in the surface and transitional horizons, and was extremely high in Gso subhorizon. Due to the share of mechanical fraction the soil of these horizons were classified as silty loam, loam, and loamy sand textural classes, respectively.

Table 2. Differential porosity.

Horizon	Depth, cm	Pore share, % vol.		
		Coarse (>10 μm)	Medium (0.2-10 μm)	Fine (<0.2 μm)
Aa,p	0–30	2.86	21.58	33.15
AaGso	30–60	5.17	28.92	23.66
Gso	60–90	27.25	16.10	6.82

Differential porosity (Table 2) was closely related to the share of granulometric fraction. In the surface Aa,p horizon fine pores (<0.2 μm) prevailed; in transition AaGso horizon the medium pores (0.2–10 μm), while in Gso subhorizon the coarse pores (>10 μm) prevailed.

The analysis of the soil chemical composition (Table 3) revealed a higher share of carbonates in the transitional AaGso horizon in comparison to the humus horizon and the Gso subhorizon. It is thus classified as very limy. Soil alkalinity increased with depth, and on average, was low. Organic matter content was highest in the surface horizon, and its share declined with depth. Due to its average organic matter content this soil is classified as poor in humus. Also, nutrient concentration was the highest in the surface horizon and declined with soil depth.

Table 3. Chemical soil composition.

Horizon	Depth, cm	$CaCO_3$, %	pH of H_2O	Humus, %	Total N, %	P_2O_5, mg per 100g	K_2O, mg per 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

Since the soil moisture content in the first 10 cm

Table 4. Hydrological properties.

Date of observation		15.06.	01.07.	03.08.	07.09.	23.09.	09.10.
Danube water level, cm		**246**	**442**	**282**	**140**	**205**	**185**
Soil profile layer	**Depth, cm**	**Soil moisture, vol. %**					
Aa,p	0–30	26.26	35.60	23.59	22.73	23.11	28.24
I Gso	30–60	32.17	36.41	23.59	29.76	28.79	27.92
II Gso	60–90	36.41	43.72	32.33	24.11	44.64	40.17
Underground water level, cm		120	120	180	210	190	200

was low, roots could not absorb any water. At the depth of 30–60 cm the content of current soil moisture was available to seedlings and at the depth of 60–90 cm, current soil moisture was also easily available to the seedlings.

Correlation between Danube water level and underground water level at the examined site was significant and high throughout the growing period ($r = 0.74$). The correlation of soil moisture in the first two examined layers of profile was also significant and high with the underground water level ($r = 0.65$ for Aa,p and $r = 0.69$ for I Gso), but for the lowest horizon it was insignificant ($r = 0.40$). This could suggest constant water supply by capillary flow in the horizon.

Plant height

The main result of this study is that plants exhibited different height at the end of the vegetation period (Table 5). Genotypes '107/65/9', 'V-158', '347' and '79/64/2' had mean height ranging from 196.5 cm to 227.8 cm and geno-

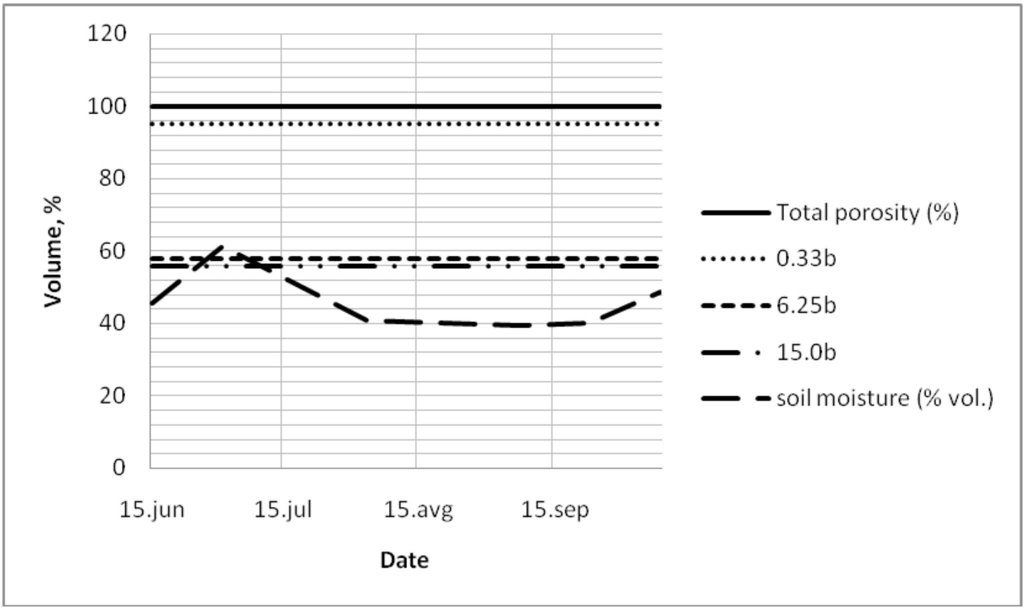

Fig. 1. Current soil moisture dynamics at 10 cm.

type 'V-55' was 156.6 cm high. Since the height limit for production

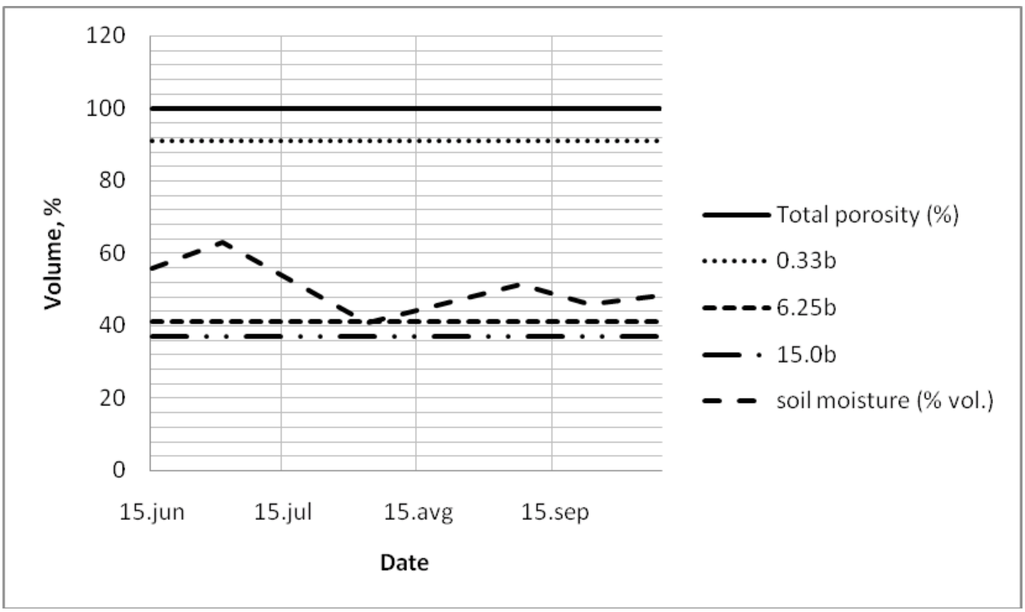

Fig. 2. Current soil moisture dynamics at 30–60 cm.

of the white willow planting material type 1/0 is 2.5 m (Markovic and Roncevic 1986), it can be concluded that genotypes '107/65/9', 'V-158' and '347' reached the production limit. According to the height structure of the seedlings (Figure 4), the share of seedlings more than 2.5 m high ranged from 2.56 to 30.76%, those of 2.0–2.5 m from 33.33 to 53.84%, and those below 2.0 m ranged from 15.38 to 64.1%; for genotype 'V-55' it was even 100%. From the height structure recorded it can be seen that the genotype '107/65/9' had the highest percentage of seedlings higher than 2.5 m (30.76%), and the highest percentage of seedlings from 2.0–2.5 m high (53.84%). Genotypes 'V-158', '347' and '79/64/2' had significantly lower share of seedlings over 2.5 m (2.56–19.8%), and equal participation of seedlings from 2.0–2.5 m high (33.33–37.73%). According to the lowest final height sizes, seedlings of genotype 'V-55' were all below 2.0 m (100%).

Obtained planting material measured at the end of vegetation period had mean heights mainly lower than 2.0 m, which was the limit for the white willow seedlings classification. Although majority of seedlings were below pre-

Table 5. Mean values of total heights (h), results of ANOVA, and the least significant difference LSD-test at the level of 5%.

Clone	h, cm
107/65/9	227.84 a
V-158	210.61ab
347	206.75 abc
79/64/2	196.50 bc
V-55	156.60 d
F-test	30.057*

* – very statistically significant.

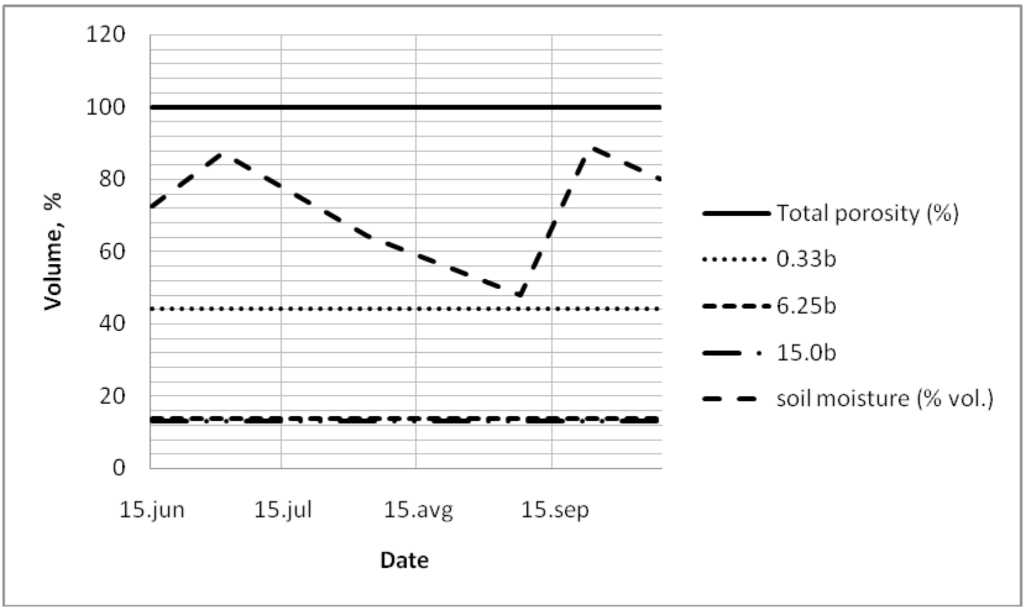

Fig. 3. Current soil moisture dynamics at 60–90 cm.

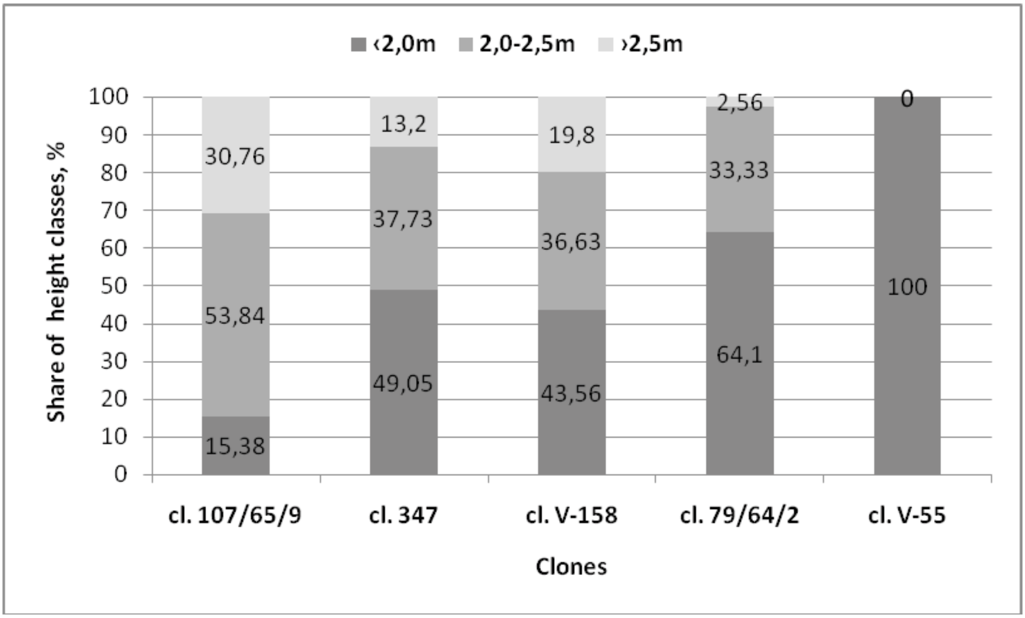

Fig. 4. Share of different height classes in the total number of seedlings.

scribed height, these which ranged from 2.0–2.5 m could be used for plantation establishment.

Discussion

Lower heights of white willow genotypes grown in studied eugley soil could be related to the characteristics of this soil type. By analyzing the content of current soil moisture (Figure 1, 2, 3) it can be concluded that at depth of 10 cm the moisture content in the soil was unavailable to plants for most of the time of the growing period. At the depth of 30–60 cm, in the root system area the moisture content increased, but in the best conditions 15–45% of capillary pores were water filled for a short period of time, while during some periods the moisture content was almost close to lento-capillary moisture. Even non-capillary pores were water filled. Since the study area was the protected part of alluvial plain the primary form of eugley soil moisturing was by underground water. Roncevic et al. (2002) mentioned that these soils should be in close contact with underground water to be suitable for production of willow seedlings. Underground water level ranged from 120–210 cm, or on average 165 cm from the surface soil, which is in fact a low level of ground water, and moisturizes only the lowest layer in the area below the rhizosphere. The low underground water level has resulted in the small amount of water available to seedlings in the soil, which resulted in smaller height of white willow seedlings. Several studies have shown that the early life stages of floodplain forest species are susceptible to the fluctuating hydrological conditions (Sacchi and Price

1992, Segelquist et al. 1993, Shafroth et al. 1994, Douglas 1995). Rapidly declining river levels and underground water levels can result in limited water availability for development of seedlings of white willow (Van Splunder et al. 1996).

Conclusion

The tested eugley soil was characterized by high content of total clay fraction, which ranged from 65.16–75.72% down to the depth of 60 cm. Textural class ranged from loam to dusty loam. Decreased level of water available to plants in the rhizosphere zone, linked with heavier mechanical composition resulted in smaller height of seedlings. Genotypes '107/65/9', 'V-158' and '347' reached the production limit, and confirmed the fact that one-year old seedlings of appropriate quality could be grown on this soil. According to height structure, genotype '107/65/9' had the greatest number of seedlings over 2.5 m (30.76%), and genotipes 'V-158', '347' and '79/64/2' had much lower share of such seedlings (2.55-19.8%). All seedlings of genotype 'V-55' were below 2.0 m. Continuous production process for obtaining two-year old sprouts is needed to provide sufficient quality of planting material of the studied clones.

Acknowledgement

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OCCURRENCE AND DISTRIBUTION OF ALIEN INVASIVE TREE SPECIES IN THE ITALIAN FORESTS

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Abstract

The spread of alien plants, intentionally or accidentally introduced by human activity into areas outside their native ranges, is one of the threats to natural ecosystems. Particularly the alien plant species defined as "invasive" may alter ecosystem processes and threaten the survival of native species in natural ecosystems or cause a critical economic impact in agriculture. The paper presents some data about the occurrence and the distribution of alien invasive tree species in the Italian forests derived from the Italian national forest inventory.

Key words: alien invasive species, biodiversity, forest inventory.

Introduction

The uncontrolled spread of alien species, defined as "plant species whose presence in an area is due to intentional or accidental introduction by man" (Richardson et al. 2000), represents an important cause of the loss of biodiversity at the global level.

With reference to their current invasion status, Richardson et al. (2000) divide the alien species into three categories: casual alien plants, naturalized non-invasive plants and invasive plants.

This paper focuses on the third category, the invasive alien species, defined as "naturalized plants that produce reproductive offspring, often in very large numbers, at considerable distances from parent plants, and thus have the potential to spread over a considerable area" (Richardson et al. 2000).

At the European level, several inventories of invasive alien species have been compiled. The international project DAISIE (Delivering Alien Invasive Species Inventories for Europe 2008), funded by the VI Framework Programme of the European Commission, has provided a global inventory of alien plant species in the pan-European region.

Due to its specific climatic conditions, Italy is one of the European countries most affected by the invasion of alien species. Its territory has been a centre of intense exchange and colonization of non-native biota as a result of human trade and migrations ever since ancient times (Blasi et al. 2007).

The project "A survey of the non-native flora of Italy", funded by the Italian Ministry for the Environment in the years 2005–2008, produced a database on non-native plant species in Italy. This inventory listed 1,023 alien species, of which 154 invasive.

The aim of this paper is to present some data on the occurrence and the distribution of alien invasive tree species in Italy derived from the analysis of the national forest inventory data. As a first step, the list of alien species published by Celesti-Grapow et al. (2009) within the above mentioned project was compared with the list of tree and shrub species recorded during the inventory surveys. The collected data were processed to estimate the number of stems, the basal area and the volume per hectare of invasive alien species and to give some information on their distribution in the Italian forest stands.

Material and Methods

The second Italian National Forest Inventory (National Inventory of Forests and forest Carbon pools – INFC) is an important source of data for forestry and natural ecosystems management. It provides reliable and updated information, consistent with international standards, about the forest resources at national and regional level, including forest biodiversity.

The INFC adopted a three-phase sampling design for stratification (Gasparini et al. 2010), with three samples of approximately 300,000, 30,000 and 7,000 samplig units. The first sample was used to assess the land cover/land use by photo-interpretation of digital orthophotos (first inventory phase). The classification system adopted is consistent with the first level of the CORINE Land Cover System (European Commission 1993) and with the FAO (2001) – Forest Resources Assessment (FRA 2000) definition of Forest and Other Wooded Land (OWL) (UN-ECE/FAO 1997). The quantitative measurements (counts of stems by species, diameter, tree height, etc.) were taken in the field during the third phase. The field plots were randomly selected from the second phase sample and stratified by administrative district (21 regions) and forest type (23 types). The forest type was classified in the field on the basis of the prevailing tree species in the sample plot (INFC 2003). Figure 1 shows the inventory plot design. The measurements were

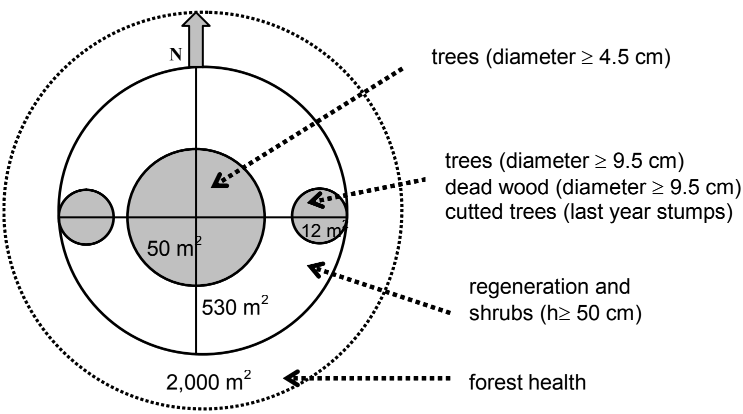

Fig. 1. National Forest Inventory ground plot configuration.

taken in different sized plots: two circular concentric plots for dendrometric measurements (50 m^2 and 630 m^2), two small satellite plots for forest regeneration and shrubs (12 m^2), a larger plot (2000 m^2) for the forest health survey and qualitative measurements (INFC 2006, Gasparini et al. 2010). Trees with a diameter at breast height (DBH) larger than 4.5 cm and 9.5 cm were measured respectively within the two concentric plots for dendrometric measurements; forest regeneration and shrubs include all stems with a DBH smaller than 4.5 cm and a height larger than 50 cm.

The total and per hectare estimates of the observed or derived variables (number of stems, basal area, growing stock, etc.) were obtained using the estimators proposed by Fattorini et al. (2006).

For this study, the field data concerning invasive alien species were processed separately to estimate the number of stems, the basal area and the stem volume (total and per hectare values) of these species at national and regional level and by forest type. Moreover, data on regeneration of invasive alien species were used to estimate the number of regeneration stems, for comparison with the same estimates on non-invasive alien species. The alien species classification adopted is the one proposed by Celesti-Grapow et al. (2009).

Results and Discussion

Comparing the list of Celesti-Grapow et al. (2009) with the list of the species recorded during the INFC field surveys, a noteworthy feature is that 34 alien species were found, of which 15 naturalized, 10 casual and 9 invasive. Table 1 gives the list of the invasive alien species surveyed by INFC.

In 440 of the 6,685 plots surveyed during the inventory, one or more stems of invasive alien species (DBH $\geq$ 4.5 cm) were counted (Fig. 2). Within "forest" category of INFC, the total number of alien species stems represented 4.43% of overall total and 2.7% was the proportion of invasive alien species. In terms of basal area and volume, the proportion of invasive alien species was 1.9% and 1.8%, respectively. Focusing on "tall trees forests", the most important inventory category (8,582,968 ha on 8,759,200 ha of the total forest area), table 2 gives the total and per hectare estimates of the number of stems, basal area and volume for invasive alien

Table 1. List of invasive alien species recorded during the Italian National Forest Inventory surveys.

Species	Common name	Habitus
Acacia dealbata Link	silver wattle	shrub
Acer negundo L.	ash leaved maple	tree
Ailanthus altissima (Mill.) Swingle	tree of heaven	tree
Amorpha fruticosa L.	false indigo	shrub
Opuntia ficus-indica (L.) Mill.	prickly-pear cacti	shrub
Prunus laurocerasus L.	laurel cherry	shrub
Prunus serotina Ehrh.	wild black cherry	tree
Quercus rubra L.	red oak	tree
Robinia pseudacacia L.	black locust	tree

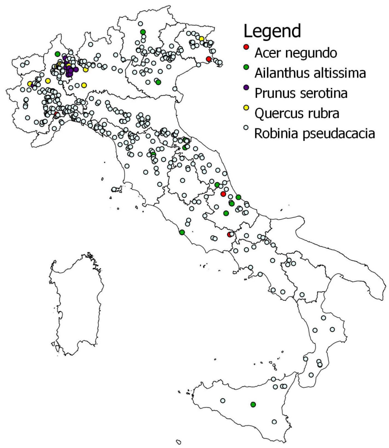

Fig. 2. Map of National Forest Inventory plots with invasive alien tree species.

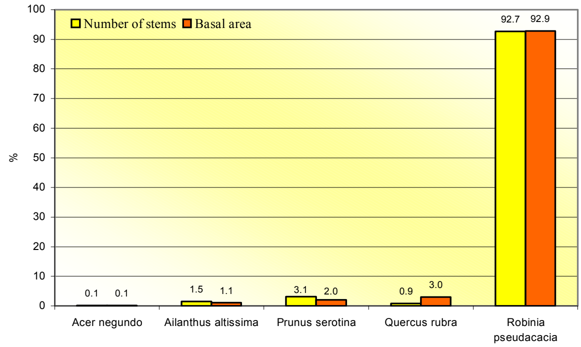

Fig. 3. Proportion of invasive alien tree species in terms of number of stems and basal area ("tall trees forest" category).

tree species, compared with the same estimates for other species. Figures 3 and 4 show the proportion of invasive alien tree species respectively by species and forest type. The forest type with the highest value of alien species was the "other deciduous broadleaved forests"; one fourth of this forest type (233,553 ha, 2.7% of tall trees forests) was represented by invasive alien tree species dominated mainly by black locust stands. Coniferous forest types and cork oak stands were almost not affected by invasive alien species.

Figures 5 and 6 show the total volume and the number of stems of inva-

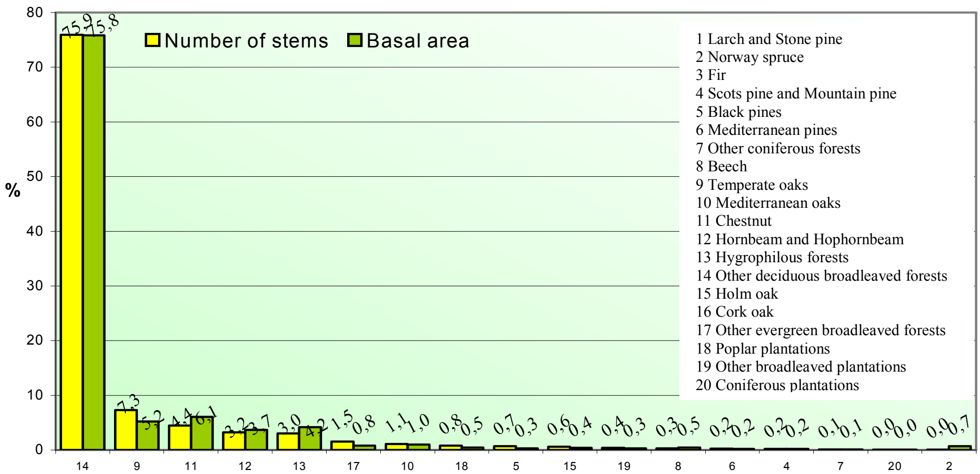

Fig. 4. Proportion of invasive alien tree species in terms of number of stems and basal area by forest type.

Table 2. Total and per hectare estimates at national level of dendrometric parameters ("tall trees forests" category).

Species	Number of stems		Basal area		Volume	
	n	n.ha^{-1}	m^2	m^2.ha^{-1}	m^3	m^3.ha^{-1}
Invasive alien species	314,520,116	36.6	3,343,897	0.4	22,618,478	2.6
Other species	11,556,715,954	1,346.5	173,380,117	20.2	1,233,481,015	143.7

sive alien species in the Italian administrative regions.

As shown in figure 3, the most widespread species, within "tall trees forest" category, is *Robinia pseudacacia* L., (more than 92.7% of the invasive alien species stems counted by INFC, 92.9% of the total invasive alien species basal area). *Prunus serotina* Ehrh., *Ailanthus altissima* (Mill.) Swingle, *Quercus rubra* L. and *Acer negundo* L. are the other main invasive alien species.

The highest values for the number of invasive alien stems were found in Piemonte, Lombardia, Toscana and Emilia Romagna. These results are consistent with those obtained by Celesti-Grapow et al. (2010).

Finally, figure 7 shows the percentage of invasive alien species regeneration and its distribution by species. Invasive alien species were found in 220 of the 4,850 plots with regeneration. The total and per hectare number of invasive alien species regeneration stems compared with the same statistics for other species are given in table 3.

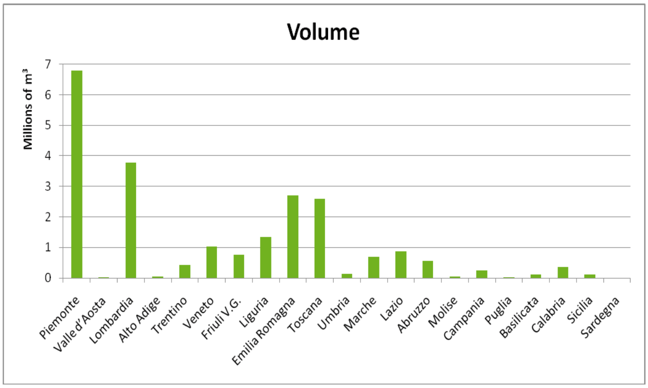

Fig. 5. Total growing stock of invasive alien species by regions.

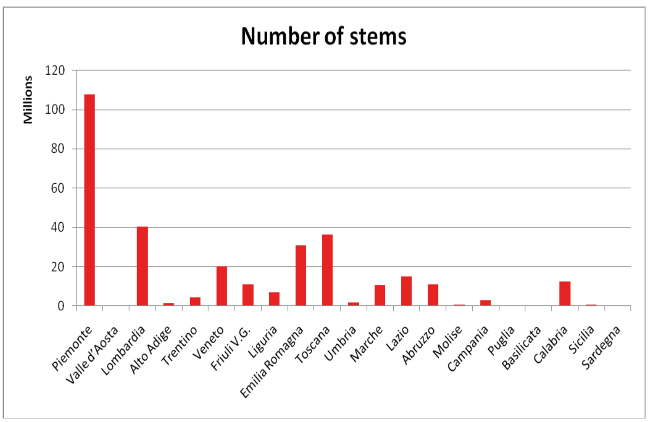

Fig. 6. Total number of stems of invasive alien species by regions.

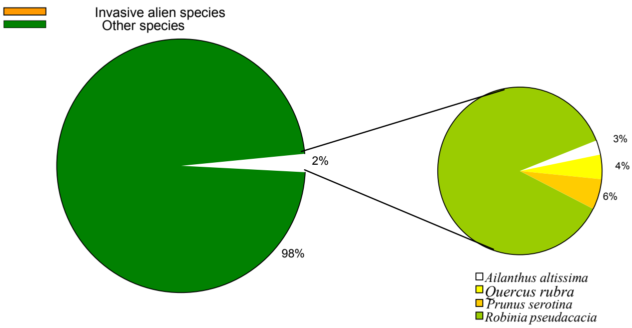

Fig. 7. Percentage of invasive alien species regeneration and its distribution by species.

The main statistics concerning invasive tree species produced by the national forest inventory give a general picture of the occurrence and distribution on these species in Italy. The percentage standard error of estimates is generally high (80% or more) as a consequence of relatively low sampling intensity, that is suitable for the purposes of the forest inventory and not specifically for a invasive alien species inventory.

The data presented in this paper seem to suggest a limited occurrence of these species, both in the overstorey and in the regeneration storey. The data obtained by Celesti-Grapow et al. (2010) confirm that the number and density of non-native species is highest in artificial land use types.

Closed-canopy forests have long been cited as highly resistant to invasion. However, while invasion of forests (in particular by shade-tolerant exotics) may be a slower process than the establishment of exotic species in disturbed or open ecosystems, the long-term effects are likely to be just as pervasive (Martin et al. 2009).

The level of knowledge on the alien flora in Italy varies considerably within regions. In order to identify management priorities and gain a better understanding of plant invasion processes, it is crucial to distinguish, among the high number of plants that make up non-native floras, those species that pose a major threat to the environment or other aspects of human life owing to their high rate of spread and current or potential impact. One essential, basic step in this direction consists of gathering information on the distribution and invasion status of each species, on the differences in the land use in which they occur (e.g. man-made or natural) and on the type of impact they cause (e.g. ecological or socioeconomic) (Celesti-Grapow et al. 2010).

Table 3. Total and per hectare number of regeneration stems (“tall trees forests” category).

Species	Number of stems	
	n	n.ha^{-1}
Ailanthus altissima (Mill.) Swingle	58,461,747	6.7
Quercus rubra L.	88,914,371	10.2
Prunus serotina Ehrh.	124,984,909	14.3
Robinia pseudacacia L.	1,808,001,266	206.4
Other species	86,283,872,360	9,850.7

The forest inventory data give a general picture of invasive alien species occurrence and distribution in Italy. Further analysis could be carried out at plot level to study the effects of site features and management practices.

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POSSIBILITY OF GYPSY MOTH CONTROL IN CERTIFICATED FORESTS BY CHEMICAL INSECTICIDES OF THE THIRD GENERATION

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Abstract

In certificated forest ecosystems in Serbia, *Bacilus thuringiensis* ssp. *kurstaki* insecticides are well-accepted and most widely used pesticides for control of one of the most important economically harmful defoliators of *Lepidoptera* order – gypsy moth (*Lymantria dispar* Linne, 1758), in progradation phase, when number of pests is relatively small. When number is greater, it is assumed that so-called "soft" ecotoxicologically favourable preparations Avaunt®, Alverde®, Coragen®, registered for application in agriculture, but not in forestry, can be used for inhibition of multiplication. The experiments of biological efficacy of those pesticides were established in the period 2009–2010, during the third larval instar of the gypsy moth. From the beginning of feeding till the end of the two groups of experiments the caterpillars were fed with natural (Pedunculate oak leaves) and synthetic food, but from the third instar the food was shortly soaked in water solutions of the analysed doses of the preparation. Results of laboratory studies of biological efficiency of above preparations showed that they have preconditions for application in forest ecosystems. The high biologic efficiency (90.52% and 100% for Avaunt®, 99.53% and 100% for Coragen®, 97.16% and 100% for Alverde®), mechanism of rapid inhibition of larvae feeding, and thereby inhibition of leaf mass damage, resistance to water rinsing, high selectivity, and small quantities of application, anticipated a bright future for them.

Key words: Avaunt®, Alverde®, Coragen®, biological efficiency.

Introduction

The rapid growth of the world population, accelerated industrialisation and urbanisation, imposed the need for thinking about the use and management of forests in a completely new way.

Forest certification is one of the fastest growing methods which can be used for the adaptation of forestry to the modern concept of "sustainable management", by meeting the requirements of the FSC (Forest Stewardship Council) standard. There is a general understanding in the world and in this country that the management of the forest resources should be sustainable, economically profitable, ecologically acceptable and socially justified, which also implies the improvement of the methods of work and development of responsibility. By joining the process of forest certification, Serbia was obligated to meet the principles of FSC policy in the use of pesticides (Forestry Stewardship Council Pesticides Policy).

In certificated forest ecosystems in Serbia, biological insecticides based on *Bacillus thuringiensis* var. *kurstaki* (*Btk*) have been generally accepted and lately most applied pesticides in the suppression of one of the most important economically harmful species of defoliators of *Lepidoptera* order – the gypsy moth (*Lymantria dispar* L.) during its progradation phase, when the density is low (Tabakovic-Toshic 2006). This is in correlation with the fact that their biological efficacy is never 100% and that a part of the target population always remains alive. During the culmination phase of the outbreak, when the density of the target insect is maximal (several tens of thousands of egg masses per hectare), if biological preparations are applied even with the excellent efficacy, the part of the population which remains alive often causes the damage to such an extent that, at the first glance, it makes the efficacy of the preparation doubtful (Tabakovic-Toshic 2005).

Btk insecticides which are used for the control of gypsy moth show the best efficacy on the younger larval instars (L_1 and L_2). The older instars require higher lethal doses, so very often the applied rates of the preparation cause sublethal effects and the loss is even greater than one caused by the larvae in the untreated areas, because one of the consequences of sublethal doses is the prolonged larval development. This fact is especially emphasised during the unfavourable meteorological conditions which lead to prolonged hatching and to washing down of a part of the applied preparation. As a consequence, the age structure of the treated population larvae is most often from L_1 to L_4, and the rate of the active ingredient is reduced so that it does not have the lethal potency (Tabakovic-Toshic 2005, 2008).

It is hypothesised that the above problems can be solved by using the chemical (biotechnical) insecticides of the third generation such as Avaunt®, Alverde® and Coragen®.

Material and Method

Avaunt® 15 SC (active ingredient – indoxacarb), Coragen® 20 SC (active ingredient – chlorantraniliprole) and Alverde® 240 SC (active ingredient – metaflumizone) are modern chemical (biotechnical) non-systemic pesticides of the third generation, active by ingestion, less by contact. Indoxacarb and metaflumizone inhibits the sodium ions entry into nerve cells, which paralyses the larvae and causes the cessation of feeding and insect death (US EPA 2009, Klein and Oloumi 2005). This mode of action requires no metabolism for toxicity to target insects. Chlorantraniliprole is a diamide insecticide. The mode of action of chlorantraniliprole is the activation of insect ryanodine receptors. This activation stimulates the release of calcium from the internal stores of smooth and striated muscle which causes impaired muscle regulation, paralysis and insect death (Ioriatti et al. 2009).

After foliar applications, most of the active ingredients remain on the leaf

Table 1. Variants (rates) of the applied preparations in the laboratory tests of their biological efficacy.

Code*	Name	Dose
1a, 1b	Avaunt® 15 SC	250 ml.ha^{-1} + 750 ml.ha^{-1} white oil + water up to 1000 l.ha^{-1}
2a, 2b	Coragen® 20 SC	200 ml.ha^{-1} + 800 ml.ha^{-1} white oil + water up to 1000 l.ha^{-1}
3a, 3b	Alverde® 240 SC	100 ml.ha^{-1} + 900 ml.ha^{-1} white oil + water up to 1000 l.ha^{-1}
4a, 4b	distilled water	1000 l.ha^{-1}

*a – natural food; b – synthetic food.

surface and a small amount penetrates into the leaf tissue. They are highly potent and active at low rates on target species, which are mainly Lepidoptera and also some Coleoptera and Diptera. The Reduced Risk Committee categorized indoxacarb, chlorantraniliprole and metaflumizone as the "reduced risk" pesticides.

The experiments of biological efficacy of pesticides presented in Table 1 were established in May 2009, February and April 2010, during the third larval instar of the gypsy moth. From the beginning of feeding till the end of the two groups of experiments the caterpillars were fed with natural (Pedunculate oak leaves) and synthetic food, but from the third instar the food was shortly soaked in water solutions of the analysed doses of the preparation (Table 1). During the experiments, temperature and light conditions were constant (temperature 21°C, light regime – 10 hours night, 14 hours a day) The potency was controlled 72, 144 and 216 hours after the establishment of the experiments, and the individuals which survived in the treated and control groups were monitored till the end of the development. All experiments were established in the complete random block pattern in four repetitions (Scheme 1), where the blocks for each variant present 3 Petri dishes with the corresponding number of larvae (15)

Scheme 1. Plan of experiments - the complete random block pattern in four repetitions.

I Blok			II Blok			III Blok			IV Blok		
Avaunt® 15 SC			Avaunt® 15 SC			distilled water			distilled water		
12*	**31**	**41**	**2**	**39**	**46**	**14**	**20**	**48**	**8**	**19**	**29**
Alverde® 240 SC			distilled water			Avaunt® 15 SC			Coragen® 20 SC		
1	**21**	**23**	**3**	**13**	**32**	**4**	**33**	**44**	**26**	**45**	**47**
Coragen® 20 SC			Coragen® 20 SC			Alverde® 240 SC			Alverde® 240 SC		
6	**22**	**35**	**11**	**15**	**24**	**10**	**28**	**42**	**5**	**7**	**25**
distilled water			Alverde® 240 SC			Coragen® 20 SC			Avaunt® 15 SC		
16	**17**	**30**	**27**	**38**	**40**	**9**	**34**	**43**	**18**	**36**	**37**

* number of Petri dish

(EPPO/OEPP 1990). The statistical processing consisted of the analysis of variance, the calculation of the mean value of the number of alive larvae, efficacy by Abbott (E), as well as the testing of the differences of mean values of survival (LSD test).

Results and Discussion

In the 2009 and 2010, the biological efficacy of chemical insecticides of the third generation (Avaunt®, Alverde®, Coragen®), were studied in the Institute of Forestry in Belgrade, in the aim of the maximal increase of biological efficacy in the suppression of gypsy moth (*Lymantria dispar*) outbreak at the density when the application of the microbiological insecticide does not produce the satisfactory results. The study results are presented in Tables 2 and 3.

In laboratory conditions, 144 hours after the experiment was established, in 2009 and 2010 respectively, the best biological efficacy was attained by Avaunt® 15 SC (100%), then by Alverde® 240 SC (97.72 and 99.52%), and somewhat lower by Coragen® 20 SC (96.55 and 98.99%). All caterpillars were fed with natural food.

After 216 hours, this group showed a maximum of 100% biological efficiency. Biological efficiency in the experimental group with synthetic food, was slightly lower for all insecticides (Table 2). The same conclusions were

Table 2. Biological efficacy of the tested insecticides in the suppression of the third larval instar of the gypsy moth (experiments performed in 2009 (a) and 2010 (b)).

a)

Code	Number of alive larvae per repetition								X mean		E by Abbott, %	
	I		II		III		IV					
	a	b	a	b	a	b	a	b	a	b	a	b
72 hours after the test establishment												
1	0.0	8.3	0.0	9.3	0.0	6.3	0.0	6.3	0.0	7.6	100	47.40
2	3.0	14.0	2.7	12.7	2.0	12.0	6.0	11.7	3.4	12.6	76.41	12.76
3	3.0	11.0	1.3	14.7	0.3	14.0	3.7	12.7	2.1	13.1	85.65	9.29
4	15.0	14.7	15.0	13.7	15.0	14.3	13.0	15.0	14.5	14.4		
144 hours after the test establishment												
1	0.0	1.7	0.0	6.7	0.0	2.7	0.0	2.0	0.0	3.2	100	77.19
2	0.3	0.7	0.3	1.0	0.0	1.7	1.3	0.0	0.5	0.8	96.55	94.24
3	0.0	4.7	0.0	4.0	0.0	2.7	1.3	3.3	0.3	3.2	97.72	78.02
4	15.0	14.7	15.0	13.7	15.0	14.3	13.0	15.0	14.5	14.4		
216 hours after the test establishment												
1	0.0	0.0	0.0	1.7	0.0	0.3	0.0	0.3	0.0	0.6	100	95.93
2	0.0	0.0	0.0	1.0	0.0	1.0	0.0	0.0	0.0	0.5	100	96.53
3	0.0	0.7	0.0	1.0	0.0	0.0	0.0	0.3	0.0	0.5	100	96.53
4	15.0	14.7	15.0	13.7	15.0	14.3	13.0	15.0	14.5	14.4		

b)

Code	Number of alive larvae per repetition								X mean		E by Abbott, %	
	I		II		III		IV					
	a	b	a	b	a	b	a	b	a	b	a	b
72 hours after the test establishment												
1	2.7	14.0	3.0	13.7	2.7	14.0	1.3	13.7	2.42	13.85	83.87	7.67
2	2.7	13.3	3.0	12.3	2.0	14.3	3.7	15.0	2.85	13.72	81.00	8.53
3	6.0	14.0	1.3	15.0	1.3	13.0	2.0	14.3	2.65	14.08	82.33	6.13
4	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0		
144 hours after the test establishment												
1	0.0	13.0	0.0	11.0	0.0	14.0	0.0	11.3	0.00	12.32	100	17.04
2	0.3	1.7	0.0	4.0	0.0	6.7	0.3	5.7	0.15	4.52	98.99	69.56
3	0.0	7.0	0.0	9.7	0.0	9.7	0.3	10.3	0.07	9.17	99.52	38.25
4	15.0	14.7	15.0	14.7	15.0	15.0	14.7	15.0	14.92	14.85		
216 hours after the test establishment												
1	0.0	1.3	0.0	1.7	0.0	1.3	0.0	1.3	0.00	1.40	100	90.52
2	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.00	0.07	100	99.53
3	0.0	0.7	0.0	0.0	0.0	0.7	0.0	0.3	0.00	0.42	100	97.16
4	15.0	14.7	15.0	14.7	15.0	15.0	14.7	14.7	14.92	14.77		

1 – Avaunt® 15 SC; 2 – Coragen® 20 SC; 3 – Alverde® 240 SC; 4 – distilled water;
a – natural food; b – synthetic food.

made when the mean values for the period 2009-2010 were compared, since there were no great deviations in the values of the biological efficacy attained in the individual years per experimental groups, tested preparations, blocks and repetitions (Table 3).

In the third evaluation (216 hours after exposure), the analysis of variance showed statistically significant differences between all three tested preparations from the experimental group fed with the foliage and Coragen from the group fed with the synthetic food on the one hand , and both control groups, and other experiments with synthetic food on the other hand. LSD test showed that, at the end of the experiment, the study preparations formed four homogeneous groups within which there were no statistically significant differences (Table 4) of biological efficacy in the suppression the gypsy moth larvae.

Conclusion

The results of the laboratory studies of the biological efficacy of three chemical insecticides of the third generation (Avaunt®, Alverde®, Coragen®) in the suppression of gypsy moth (*Lymantria dispar*), showed that their biological efficacy was maximal. They have all

Table 3. Biological efficacy of the tested insecticides in the suppression of the third larval instar of the gypsy moth (in the period 2009–2010).

Code*	Number of alive larvae per repetition								X mean		E by Abbott, %	
	I		II		III		IV					
	a	b	a	b	a	b	a	b	a	b	a	b
72 hours after the test establishment												
1	1.35	11.15	1.50	11.50	1.35	10.15	0.65	10.00	1.21	10.70	91.80	27.26
2	2.85	13.65	2.85	12.50	2.00	13.15	4.85	13.35	3.14	13.16	78.71	10.54
3	4.50	12.50	1.30	14.85	0.80	13.50	2.85	13.50	2.36	13.59	84.00	7,61
4	15.00	14.85	15.00	14.35	15.00	14.65	14.00	15.00	14.75	14.71		
144 hours after the test establishment												
1	0.00	7.35	0.00	8.85	0.00	8.35	0.00	6.65	0.00	7.80	100	46.39
2	0.30	1.20	0.15	2.50	0.00	4.20	0.80	2.85	0.31	2.69	97.89	81.51
3	0.00	5.85	0.00	6.85	0.00	6.20	0.80	6.80	0.20	6.42	98.64	55.88
4	15.00	14.70	15.0	13.85	15.00	14.65	13.85	15.00	14.71	14.55		
216 hours after the test establishment												
1	0.00	0.65	0.00	1.70	0.00	0.80	0.00	0.80	0.00	0.99	100	93.18
2	0.00	0.00	0.00	0,65	0.00	0.50	0.00	0.00	0.00	0,29	100	98.00
3	0.00	0.70	0.00	0.50	0.00	0.60	0.00	0.30	0.00	0.52	100	96.42
4	15.00	14.7	15.00	13.85	15.00	14.65	13.85	14.85	14.71	14.51		

* Legend in Table 2

the necessary properties (high biological efficacy, mechanism of action, resistance to watering and a small amount of application) for use in forest ecosystems.

In addition, these insecticides may be used as part of an Integrated Pest Management program to control pest which can include biological, cultural, and genetic practices aimed at preventing economic pest damage.

Table 4. Test of the least significant differences per experiment groups.

Code*	Homogeneous groups						Code	Homogeneous groups					Code	Homogen. groups			
period 2009-2010, X mean																	
After 72 hours							After 144 hours						After 216 hours				
1a	▓						1a	▓					3a	▓			
3a	▓	▓					3a	▓					1a	▓			
2a		▓					2a	▓					2a	▓			
1b			▓				2b		▓				2b	▓	▓		
2b				▓			3b			▓			3b		▓	▓	
3b					▓	▓	1b				▓		1b			▓	
4b						▓	4b					▓	4b				▓
4a						▓	4a					▓	4a				▓

* Legend in Table 2

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