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SHRUB AND TREE SPECIES USED FOR IMPROVEMENT BY AFFORESTATION OF DEGRADED LANDS IN ROMANIA

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Abstract

Forests represent a veritable barrier against land degradation, being the best choice for soil erosion control. Romanian woody flora consists of more than 300 species of forestry interest. Among them, approximately 50 shrub and tree species are often used in afforestation of degraded terrains. The aim of this review paper was to highlight the most important species used in forest land reclamation. Aspects regarding the ecological requirements and the results obtained by using these species in afforestation of the degraded lands were also presented. In conclusion, it could be stated that the Romanian experience acquired in this sphere of activity is very important considering the global warming, and afforestation of degraded terrains being the best way to counteract degradation phenomena and to mitigate the effects of climate change.

Key words: degraded terrains, forest species, forest land rehabilitation, land reclamation.

Introduction

Due to their multiple productive and protective roles, the forests are among the most important elements in human evolution (Bohateret 2012). It is well known that forests represent a veritable barrier against land degradation (Greavu and Mănescu 2010, Păcurar 2012, Constandache and Nistor 2014), being the best choice for soil erosion control (Andraş et al. 2012). As a consequence of decreasing the forest area, land degradation phenomena (i.e. water and wind erosion, landslides, etc.) are amplified (Lupu et al. 2010) and active morphodynamic processes are triggered (Nedelea and Comănescu 2011).

Nowadays, the main factors responsible for land degradation are the human activities, deforestation and inappropriate agricultural techniques being the ones with the highest impact (Păcurar 2012). In addition, grazing, urbanization and industry on the one hand, and natural processes such as water and/or wind erosion, landslides, on the other hand, have a significant contribution to land degradation as well. Moreover, the abandonment or lack of land reclamation activities contributed significantly to land degradation (Mărioara 2013).

In the last decades, special attention is given to the more and more frequent effect of climate change on forest sites (Pienaru et al. 2009, Donită and Radu 2013). Actually, climate change and land degradation processes generated by the human activities represent nowadays a certainty (Tărău et al. 2012). By taking into consideration the values of De Martonne Aridity Index recorded during the past three decades in southwestern Romania, it was concluded that the global warming is responsible for the transformation of the local climate, which is the major cause of the aridization in that region (Pravalie et al. 2014). Moreover, it is predicted that the global warming will cause changes in the water cycle (Păcurar 2007) and will determine the migration of plants to higher elevations (Târziu 2010). In Romania, it is estimated that the effects of climate change will increase also due to splitting the forest land into small and tiny areas (Giurgiu 2010), which will generate an inappropriate forest management. According to latest statistics, there are more than 0.85 million forest land owners in Romania (Behr et al. 2014). The present situation regarding the ownership status of the forest lands was caused by the processes of forest restitution, initiated shortly after the fall of communism. Under Law 18/1991, more than 0.35 million ha of forest lands were restituted, up to 1 ha per owner. Later on, according to Law 1/2000, all community, town or communal forests were restituted to their former owners, but with limitations (10 ha for individuals and 30 ha for churches, respectively). Forest restitution processes were finalized from the legislative point of view by Law 247/2005 (Abrudan et al. 2009). As a result, according to the preliminary data provided by National Forest Inventory, the state-owned forest land accounted only for 54 % in 2012 (National Forest Inventory Report 2012).

Taking into consideration that the Romania's forest cover per capita is lower than the EU average, the afforestation of degraded lands represents one of the most important Governmental policies in Romania (Abrudan et al. 2009). By 2020, a forest shelterbelt protection system of about 300,000 ha is expected to be installed (Mateoc-Sîrb et al. 2014). In this context, Romania's task, received from the EU, is to increase the forest area to a percentage higher than 30 % in the next years (Barnoaiea et al. 2010). Moreover, in the present context of global warming, the forests should be regarded as a key sector for mitigating climate change, being the most important deposit of greenhouse gases (Popa et al. 2014).

In Romania, it is estimated that an area of more than two million hectares are affected by land degradation processes (Şerbănescu 2007, Crăciunescu et al. 2014), the steppe and forest steppe regions being the most affected ones, especially due to the lowest afforestation rates from the country (Ungurean et al. 2013). Actually, the southern and eastern parts of the country face the consequences of the aridification process (Peptenatu et al. 2013). In this context, the issue of erosion control is a very important problem, therefore urgent action is needed in order to prevent and control this process (Hort et al. 2013). The main way to counteract the above mentioned degradation phenomena is land reclamation through afforestation (Colişar et al. 2009, Constandache et al. 2010), forests having a very important role in preventing land degradation (Costea 2013).

The aim of this review paper was to highlight the most important shrub and tree species used in Romania in forest land reclamation. Aspects regarding the ecological requirements and the results obtained in Romania by using these species in afforestation of the degraded lands were also presented.

Shrub and Tree Species Used in Forest Land Reclamation in Romania

Romanian woody flora of forestry interest, together with the most important introduced exotic trees accounts for approximately 300 species. Among them, around 50 shrub and tree species are commonly used in afforestation of degraded terrains. The most frequently used are the following ones: Scots pine (*Pinus sylvestris* L.), Black pine (*Pinus nigra* Arn.), European larch (*Larix decidua* Mill.), Black locust (*Robinia pseudoacacia* L.), Thorny locust (*Gleditsia triacanthos* L.), Alder species (*Alnus* spp.), Ash species (*Fraxinus* spp.), Oaks (*Quercus* spp.), Cherry species (*Prunus* spp.), Common hawthorn (*Crataegus monogyna* Jacq.), Common walnut (*Juglans regia* L.), Dog-rose (*Rosa canina* L.), White mulberry (*Morus alba* L.), Elms (*Ulmus* spp.), Oleaster (*Elaeagnus angustifolia* L.), Common sea-buckthorn (*Hippophaë rhamnoides* L.), Wild privet (*Ligustrum vulgare* L.), Common lilac (*Syringa vulgaris* L.), Desert false indigo (Amorpha fruticosa L.), and some others (Ciortuz and Păcurar 2004).

This brief review presents below the tree and shrub species that were and still are of great interest for the rehabilitation by afforestation of degraded terrains from Romania. The species are presented in alphabetical order of their scientific names, and the origin of each species is indicated.

***Acer tataricum* L. (Tatar maple; native)**
Tatar maple has great demands for high temperatures in the growing season, being also resistant to drought. Similar to Turkey oak (*Quercus cerris* L.) and Hungarian oak (*Q. frainetto* Ten.), this species can tolerate heavy and compact soils. Moreover, it can grow on soils with low salty content (Şofletea and Curtu 2007).

In Romania, Tatar maple exhibited good results in establishing the protective forest shelter belts from Dobrogea and Bărăgan regions on different soil types (Mănescu 2002, Greavu et al. 2012).

***Ailanthus altissima* (Mill.) Swingle. (Tree-of-Heaven; Eastern Asia)**
Tree-of-Heaven tolerates a wide variety of climatic and edaphic conditions. It grows on a broad range of degraded terrains, being tolerant even to saline and alkaline soils. *A. altissima* demands a warm climate and a long growing season, being resistant to drought and pollution (Clinovschi 2005, Şofletea and Curtu 2007), which tend to become among the most important pressure factors on forests nowadays (Chiriac et al. 2005).

This species represents a good option for afforestation of the degraded lands, especially in arid and poor sites (Enescu 2014a). Good results were obtained especially in the steppe region on carbonate-rich soils (Constandache et al. 2001, Mănescu 2002), on high eroded and skeletal soils (Greavu and Mănescu 2001) and also on terrains affected by gully erosion (Constandache et al. 2002). As a drawback, this species is highly invasive and poses a serious threat to biological diversity. Therefore, it should be used with great caution.

***Alnus glutinosa* (L.) Gaertn. (Black alder) and *A. incana* (L.) Moench. (Grey alder) (both native)**
Black alder is a light-demanding species, the maximum altitude for its natural occurrence in Romania being around 800–900 (1300) m a.s.l. (Clinovschi 2005). It rarely occurs in the Danube Plain (Netoiu et al. 2008). This species prefers a moderate

to cold climate and grows best in soils with low calcium content, being very resistant to flooding and stagnant water (Şofletea and Curtu 2007). It is sensitive to soil depth less than 40 cm and also to loose-textured loamy soils (Lato 2012). *A. glutinosa* is highly resistant to frost, but is sensitive to drought (Şofletea and Curtu 2007). This species is highly appreciated for its nitrogen-fixing ability and for its capability to grow in excessive moisture site conditions (Şofletea and Curtu 2007).

Grey alder represents a very important species for protection of the riversides, by fixing the degraded terrains within the torrential watersheds (Şofletea and Curtu 2007). It is also suitable for afforestation of the terrains affected by gully erosion and landslides (Constandache et al. 2002). Moreover, *A. incana* has a high annual production of fast-decomposing leaves which generate a high quantity organic matter, in the form of raw humus. According to the results obtained 15–20 years after planting, the organic matter content in the first 15–20 cm of the soil profile ranged between 2.5 and 5.1 % (Traci et al. 1981).

***Amorpha fruticosa* L. (Desert false indigo; North America)**

Desert false indigo is known to be a rustic species which has low demands regarding the soil and climatic conditions, being able to grow on degraded, poor, sandy or dry soils and to survive in adverse climatic conditions (Şofletea and Curtu 2007, Sărăteanu 2010). It is also resistant to dryness and floods (Clinovschi 2005, Şofletea and Curtu 2007).

This species was planted in Romania mainly in steppe region, on soils rich in calcium carbonates, on degraded and extremely degraded terrains, such as slopes of gullies and torrents (Untaru et al. 2003). For example, the survival rate of Desert false indigo individuals 18 years after planting was approximately 80 % and the average height was 2.8 m (Greavu and Mănescu 2001). It was also used with success in wood-steppe regions on lands affected by high-intensity sheet erosion (Constandache et al. 2001). Like Tree-of-Heaven, desert false indigo is highly invasive and represents a serious threat for biological diversity. Hence, this species should be used with great caution and only if no other option is available.

***Cornus mas* L. (Cornelian cherry) and *C. sanguinea* L. (Common dogwood) (both native)**

Both species are recommended to be used in soils with moderate humus content and with low to moderate carbonate-soils (Constandache et al. 2006). *C. sanguinea* was successfully used to control soil erosion on degraded lands in the Transylvanian Plain (Vlasin et al. 2013).

***Cotinus coggygria* Scop. (Smoke tree; native)**

Cotinus coggygria can grow in full sun, at high elevations, or in partial shade, in plains or hilly regions. This species tolerates a broad range of soils, but it prefers the dry ones, located on south-facing limestone slopes (Şofletea and Curtu 2007, Netoiu et al. 2008).

In Romania, good results were obtained in steppe region on limestone substrate, with moderately to highly eroded soils. For example, after 18 years of establishing the forest plantation, the seedling survival rate was almost 75 % (Greavu and Mănescu 2001). Satisfactory results were recorded also by using smoke tree individuals in mixed-hardwood forests corresponding to the vegetation layer dominated by sessile oak (Untaru et al. 1988) and in the composition of the protective forest shelter belts from Dobrogea and Bărăgan regions (Greavu et al. 2012).

***Elaeagnus angustifolia* L. (Oleaster; Middle Asia)**

Due to its well-developed root system, Oleaster is known as a shrub species suitable for planting in terrains with landslides phenomena (Şofletea and Curtu 2007). It grows very well in terrains affected by sheet erosion from steppe wood region, with skeleton and carbonate-rich soils (Constandache et al. 2001). According to a research conducted in a mixed plantation in steppe region, on highly eroded lands, the average height of the young individuals 18 years after planting was 2.9 m, while the survival rate accounted for 84 % (Greavu and Mănescu 2001). Moreover, *E. angustifolia* can grow also on alluvial or moderate salty soils (Negrea et al. 2013, Constandache and Nistor 2014), or even on tailing dumps, like it was the case in Moldova Nouă (Cântar et al. 2014).

It is commonly used in Romania on marginal rows of the mixed plantations because it forms a barrier against grazing, thanks to its well-developed thorns (Mihăilă et al. 2010).

***Fraxinus* spp. (Ash species)**

Common ash (*Fraxinus excelsior* L.; native), Pennsylvania ash (*F. pennsylvanica* Marsch.; North America) and Manna ash (*F. ornus* L.; native) are the most important ash species for afforestation of degraded lands in Romania. Both Common ash and Pennsylvania ash are used as main species in the afforestation compositions on soils with excess water, located in meadows of the forest and forest steppe regions (Constandache and Nistor 2014).

F. excelsior provided good results in wood-steppe region (Mănescu 2002) and in degraded lands of Transylvanian Plain (Vlasin et al. 2013).

Manna ash was used in different site conditions corresponding to wood-steppe or steppe regions, in moderate and highly eroded soils (Greavu and Mănescu 2001, Mănescu 2002). It can be used also in mixed plantations established in the vegetation layer corresponding to sessile oak (Untaru et al. 1988). It is also suitable for afforestation of the terrains affected by gully erosion and landslides (Constandache et al. 2002). In all cases, *F. ornus* should be planted only on sunny slopes (Constandache et al. 2006).

***Gleditsia triacanthos* L. (Thorny locust; North America)**

Thorny locust has a poor resistance to drought conditions (Negrea et al 2013) and it requires moderate deep soils with moderate humus content (Constandache et al. 2006).

Good results were obtained in carbonated soils, such as chernozem (Constandache and Nistor 2014) or even in regosol type (Hernea et al. 2008). In Dobrogea (southern-eastern Romania), this species was planted on heavy soils, where Black locust failed (Discuteanu 1954). Thanks to its thorns, which can be up to 10 cm long, this species is often used in mixed plantations in the marginal rows in order to provide additional protection against grazing and human impacts (Mihăilă et al. 2010).

***Hippophaë rhamnoides* L. (Common sea-buckthorn; native)**

Common sea-buckthorn is regarded as a very useful multi-purpose shrub species with a high potential for forest land reclamation (Enescu 2014b). This is mainly due to its modest ecological requirements, compared with the rest of the shrub and tree species from Romania, being able to grow in a broad range of lands, even in the most degraded ones (Frangu et al. 1991).

It has a very well-developed root system, which significantly contribute to fixing the lands. Moreover, this species is able to assimilate atmospherically nitrogen directly by roots, having an important ameliorative effect to the soil (Proorocu 2013). Last but not least, Common sea-buckthorn could be used in the composition of the protective forest shelterbelts that are surrounding the big and polluted cities (Bica et al. 2014).

***Juglans regia* L. (Common walnut; native)**

Common walnut is a species with special demands regarding the site conditions. In Romania, it prefers the regions with high temperatures and mild winters, without frosts (Netoiu et al. 2008). It is also restrictive in regard with the soil conditions, preferring the types with pH values ranging between 6.5 and 7.5 (Şofletea and Curtu 2007). *J. regia* can be easily propagated in both generative and vegetative ways (Netoiu et al. 2008). It is also a fast-growing tree species, being able to grow up to 1 meter in height in the first years of its life (Şofletea and Curtu 2007).

In order to obtain good results, it is recommended to use this species in lands with additional supply of moisture, on soils with moderately or high humus content, from steppe to forest area (Constandache et al. 2006). Exceptionally, Common walnut was used to fix the sandy soils from southern-western part of Romania (Nută 2005).

***Ligustrum vulgare* L. (Wild privet; native)**

Wild privet is generally known as a shade-tolerant species (Şofletea and Curtu 2007). Due to its high ecological amplitude (Clinovschi 2005, Şofletea and Curtu 2007, Constandache et al. 2012), *L. vulgare* is able to tolerate a wide spectrum of soil conditions, ranging from salinized soils in the southern part of the Danube Delta (Strat 2013) to soils with extremely low trophicity in the steppe region (Neşu 1999). It is also resistant to drought (Neşu 1999). This species is suitable for several categories of degraded lands (eroded soils, landslides, etc.). In the hilly regions, corresponding to the vegetation layer dominated by beech-sessile oak mixtures, it can provide good results on soils with medium to heavy textures, on lands with high to very high levels of soil erosion, on terrains with land slide or crumbling phenomena and on slopes with different inclinations. In steppe regions, it can be used on skeletal soils, on lands with shallow soils and on soils with sandy or fine textures (Bălănică et al. 1955).

L. vulgare can also play an important role in sand dune fixation, as it was demonstrated in the South-Eastern Romania (Nută 2005). Moreover, Wild privet is suitable for land reclamation and ecological restoration in coastline areas, providing a better protection of the rehabilitated area and an improvement of biodiversity (Zagas et al. 2010).

***Picea abies* (L.) Karst. (Norway spruce; native)**

Norway spruce is the most common softwood species in Romania, reason for which it is intensively planted on several types of terrains. According to some specialists (Nicolescu et al. 2003), the planting density in the case of a pure spruce culture should be reduced from 5000 to 2000–2500 saplings per hectare. By doing this, the young saplings benefit of more space for their development. Like in the case of pines, higher sapling survival and growing rates were obtained by using saplings grown in polypropylene pouches (Geambaşu 1980).

***Pinus sylvestris* L. (Scots pine) and *Pinus nigra* Arn. (Black pine) (both native)**

Both Scots pine and Black pine are suitable for afforestation of the terrains affected by gully erosion and landslides

(Constandache et al. 2002). Moreover, mixed cultures with Scots pine/Black pine and Common sea-buckthorn proved their efficiency in stabilizing lands with gully erosion on slopes with an inclination lower than 25 degrees (Untaru et al. 2003). In some cases, at the age of 15–20, due to the reducing of stand density, the high amounts of heavy snow caused damages to pine trees, by breaking their branches (Constandache 2004).

Scots pine is a veritable rustic and a pioneer tree species, being used for afforestation on degraded terrains within the sites with the lowest quality (Clinovschi 2005, Şofletea and Curtu 2007). It was successfully used on sandy soils from Oltenia region, located in southern-western Romania (Nută 2005). By decomposing of the needles, *P. sylvestris* is contributing to raw humus formation. For example, according to the results obtained 18 years after planting, the organic matter content in the first 5 cm of the soil profile was 1.4 % (Traci et al. 1981). In extremely degraded terrains, cultures with pine saplings grown in polypropylene pouches produced higher technical efficiency (Untaru et al 1980).

Black pine is an excellent species for afforestation of the degraded lands with sunny and steep slopes and carbonate-rich, skeletal and undeveloped soils (Clinovschi 2005, Şofletea and Curtu 2007, Lato 2012). Very good results were obtained by planting Black pine in degraded lands situated in the steppe region, on limestone (Greavu and Mănescu 2001) or on chernozem soils, with medium to high edaphic potential, but with severe moisture deficit (Ungurean et al. 2012). In the first situation (steppe with limestone), after 21 years from planting the maintenance percent of the seedlings was 92 % (Mănescu 2002). It was used also on alluvial soils (Dragomir and Jianu 2010) or on sterile dumps (Cărăbiş et al. 2011).

***Prunus* spp. (Cherry species; all native)**

In Romania, the most common Cherry species used in forest land reclamation are: Wild cherry (*Prunus avium* (L.) Moench.), Cherry plum (*Prunus cerasifera* Ehrh.) and Mahaleb cherry (*Prunus mahaleb* L.).

Wild cherry is a light demanding species, which needs a long growing season (Clinovschi 2005). It is recommended to use this species in terrains with additional supply of moisture, with deep and humus-rich soils (Constandache et al. 2006). *P. avium* provided good results in mixed plantations with pines (Scots pine and Black pine) on eroded soils. For example, in Vrancea County, on highly eroded soils, 25 years after planting, the average height of the wild cherry trees was 11 m (Frangu et al 1991).

In the case of *P. cerasifera*, due to its high ecological amplitude, this species is used in steppe and wood-steppe regions to establish protective forest shelterbelts on degraded terrains (Şofletea and Curtu 2007). It is recommended to use cherry plum on sunny slopes (Constandache et al. 2006).

P. mahaleb is regarded as an excellent species for controlling soil erosion within degraded terrains, in areas with warmth and high dryness (Şofletea and Curtu 2007). Due to its light demanding character, it is recommended to use this species only in sunny slopes (Constandache et al. 2006). Its culture provided good results in steppe region on limestone (Mănescu 2002), and satisfactory and good results in steppe-wood regions, on different substrates (Untaru et al. 1988, Mănescu 2002). For example, in steppe region, on limestone substrate, the survival rate of Mahaleb cherry trees 18 years after planting was approximately 60 % and the average height of the young individuals was 2.7 m (Greavu and Mănescu 2001).

***Quercus* spp. (Oaks; all native)**

Among the seven Oak species from Romania (Şofletea and Curtu 2007), Greyish oak (*Quercus peduculiflora* K. Koch), Pubescent oak (*Q. pubescens* Willd.), Pedunculate oak (*Q. robur* L.) and Sessile oak (*Q. petraea* (Mattuschka) Liebl.) are of interest to afforestation of degraded lands.

Q. pedunculiflora and *Q. pubescens* are regarded nowadays as valuable resources, in the current context of global warming, already reported in several regions across Romania (Păcurar 2014). Greyish oak was used as main species in the composition of field and communication paths protective forest shelterbelts from southern and southern-eastern parts of the country (Greavu et al. 2012). It was also planted in the lowlands forest steppe on chernozem soils (Constandache and Nistor 2014) or on limestone, granite or schist substrates (Mănescu 2002).

Pubescent oak is regarded as a peerless species in terms of its contribution to rehabilitation of degraded lands, often representing among the few solutions for installation of woody vegetation in areas deficient in rainfall and with soil and atmospheric dryness from extremely warm sites (Şofletea and Curtu 2007).

Pedunculate oak is suitable for plains and low hills region, being able to grow on different soil types such as: cambisols, chernozem or fertile alluvial without salts soils. Sessile oak prefers almost the same soil types, but it requires wetter areas (Constandache and Nistor 2014). Recently, it was reported that *Q. robur* can grow on sterile dumps, like it was the case in Jilt Basin (Cărăbiş et al. 2011).

***Robinia pseudacacia* L. (Black locust; North America)**

Black locust is a very shade intolerant and a thermophilous species, with an optimum average temperature ranging from 9 to 11 °C in Romania (Şofletea and Curtu 2007). It grows well in warm regions with long vegetation period, on sandy soils (Ciuvăt et al. 2013b), the best site conditions for its culture being Oltenia region (southern-western Romania) (Ivanschi et al. 1969). Due to its fast growing rate, exceptional vegetative propagation by sprouting capacity, this species represents an ideal one for degraded land reclamation (Ciuvăt et al. 2013b).

In Romania, Black locust was used for establishing both productive and protective (especially control of wind erosion, reclamation of disturbed sites and site improvement) plantations (Enescu and Dănescu 2013). It was planted mainly in sandy soils from southern-western part of the country (Stringer et al. 2009, Ciuvăt et al. 2013a), but also in chernozem soils with low carbon content and medium to high edaphic potential (Ungurean et al. 2012, Constandache and Nistor 2014). Exceptionally, Black locust was used in the mountain region, up to 800 m altitude, in order to control soil erosion (Traci 1960).

This species is suitable also for rehabilitation by afforestation of the sterile dumps, like in Jilt Basin case (Cărăbiş et al. 2011), and also for establishing protective shelterbelts near big and polluted cities, such as Timişoara (Bica et al. 2014).

***Syringa vulgaris* L. (Common lilac; native)**

Common lilac is an important species for fixing skeletal limestone slopes (Şofletea and Curtu 2007). It satisfactory grew in mixed plantations established in wood-steppe region and Sessile oak vegetation layer (Untaru et al. 1988). Moreover, good results were obtained in steppe region, on limestone substrate, on highly eroded soils. In one case, after 18 years of setting the forest culture, the survival rate of Common lilac individuals was 80 % (Greavu and Mănescu 2001).

***Ulmus pumila* L. (Siberian elm; Asia)**

U. pumila is a species with a high potential to adapt to different site conditions (Şofletea and Curtu 2007). Siberian elm grows on a broad range of degraded lands, being tolerant to different types of soils, even to regosoils or erodisoils (Constandache et al. 2006). It can tolerate also the very compact, rocky, salty or calcareous soils (Clinovschi 2005).

It is a thermophilous species (Netoiu et al. 2008) and it is characterized by a high resistance to drought stress (Constandache et al. 2006) and it prefers to grow in full sun (Şofletea and Curtu 2007), but it is also tolerant to the semi-shade conditions (Netoiu et al. 2008). Except its high ecological amplitude, Siberian elm is also appreciated for its fast-growing rate and its well-developed root system (Discuteanu 1954), being a suitable species for establishment of protective forest shelterbelts in arid and poor lands.

In Romania, it was mainly used in the composition of the communication paths and field protective shelterbelts from the southern-eastern (Dobrogea) and southern (Bărăgan) parts of the country (Greavu et al. 2012). Siberian elm was planted also on salty or alluvial soils (Constandache and Nistor 2014).

Conclusions

In the last decades, the issue of land degradation became a very important problem in Romania, especially due to dividing the forest stands into many very small parts, which makes impossible their proper management. Several other factors contributed significantly to the increasing of the degraded lands surface, such as deforestation, inappropriate agricultural techniques, grazing, urbanization, and industry.

Special attention should continue to be given to the very fragile forest stands located in the southern part of the country, known as the most arid area of Romania. Counties such as Teleorman, Dolj, Olt, Giurgiu, Călăraşi, Ialomita and Galati have the lowest percentage of forest cover, below 10 % (Vasilescu 2003).

As a general conclusion, it could be stated that the Romanian experience in land reclamation is extensive and very important. The information obtained could be considered in the perspective of global warming. The afforestation of lands affected by the different frequent degradation phenomena represents the best way to control soil degradation processes and to mitigate the consequences of climate change.

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VOLUME TABLES FOR THE ALEPPO PINE OF THE FOREST TATOI OF PARNITHA ATTICA (GREECE)

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Abstract

For the Aleppo pine of Tatoi forest (Parnitha, Attica, Greece), regression models that estimate the volume using diameter at breast height and/or total height were fitted. A random sample of 100 trees was taken. Eighty of these trees were used for fitting and 20 for validation. The selected model is $V = 0.267 \cdot D^2 \cdot H$ with $R^2 = 0.93$ and standard error of estimate 0.199. Comparison with a pre-existing model of 2004, revealed the similarity of the old model for the estimation of the volume of *Pinus halepensis* for the specific area.

Key words: forest management, *Pinus halepensis*, tree volume estimation.

Introduction

Pinus halepensis Mill. has a significant function in the ecology and landscape of the Mediterranean basin. This pioneer and lenient species is easily regenerated and able to colonize very low quality and degraded lands. It is an essential factor in reforestation schemes for limy soils in the arid or semi-arid climates of the Mediterranean basin, favouring the growth of broadleaf Mediterranean species (*Quercus ilex* L., *Q. coccifera* L.) within its stands (Quezel 1986). Commonly, it forms mixed stands consisting of an upperstorey of *P. halepensis* and an understorey of *Q. ilex* or *Q. coccifera* (Senni 1931, Pavari 1954, Gandullo 1972, Plaisance 1978).

The forest of Tatoi is situated on the slopes of Mount Parnitha (Attica, Greece). This area is characterized by fir forests at higher altitudes and, to a smaller extent, by Aleppo pine and evergreen broadleaves, which spread throughout the area. In the forest there are also natural grassland of extreme importance to wildlife and agricultural crops. This area was part of the former royal estate "Tatoi", which is now public land. The undulating terrain combined with the rich and varied vegetation, as well as the springs and the palace buildings, provide a landscape of exceptional aesthetic value.

The forest has occasionally been exploited as a part of the royal estate, so the human presence in Tatoi until 1973 was very intense (Management Plan of Parnitha National Park 1997). Nowadays, forest management has declined greatly. For both scientific and economic purposes, it is necessary to measure the existing woody material of the forest. The determination of the quantity of the produced wood is directly related to the volume estimation of logs. Accurate volume measurement methods are not widely used due to the lengthy process and financial costs; however, sampling methods and statistical analyses are commonly used to estimate the volume of trees (Van Laar and Akça 2007).

The purpose of this research was to develop regression models to estimate the volume of Aleppo pine trees, for the Tatoi forest, with independent variables the breast height diameter and the total height, which will be compared with the model (1):

$$V_{ob}=0.01969779+1.195396\cdot V_{ib}, \qquad (1),$$

where V_{ob} is the volume outside bark (m^3) and V_{ib} the volume inside bark (m^3), with V_{ib} = $3.3041044\cdot 10^{-5}\cdot D^{1.790332}\cdot H^{1.181907}$. D is the breast height diameter (cm) and H the total tree height (m). This model was taken from the Logging table of the forest, developed for the year 2004, from the Forest Service of Parnitha.

Materials and Methods

Study area

The forest of Tatoi is located 15 km north of Athens. The altitude range is 240–1033 m, with the largest part of the area having elevations ranging from 400–600 m. Land uses are given in Table 1 (Management Plan of Parnitha National Park 1997).

Table 1. Distribution of land uses in Tatoi area.

Land uses	Area, ha
Forest	3930
Cultivated area	88
Bare area	95
Moorland	110
Total area	**4223**

The forest of Tatoi has a long history starting from ancient Dhekelia, where the last phase of the Peloponnesian war was fought. Then, during the Ottoman period, it was a hideout for bandits and rebels. In 1872, King George I of Greece bought a part of the land, and with subsequent markets he obtained the whole forest. This area was royal property for many years, a fact that upgraded the area. The forest was under management by Danish foresters from 1884–1904, then until 1910 by Germans and then by Greeks. The forest became public three times in 1924, 1973 and 1994, always after a regime change action. Eventually, after lengthy litigations, Tatoi became property of the state in March 2003. Since then, several land uses were phased out, and currently treatments are limited to the removal of dead and decumbent trees. Grazing has been forbidden throughout the area and certain agricultural areas are strictly bordered (Management Plan of Parnitha National Park 1997).

The biggest problem for the forest of Tatoi is fires. This problem is as old as the woods of the region. The prolonged period of dry and hot weather in Attica, in addition to the prevailing flammable vegetation, intense human activity and strong winds, create favourable conditions for causing and rapidly spreading fires. Indicatively, during the period 1913–1996 there were 373 fires, with most incidents occurring July and August. For this period, the burned area reached 985.74 km^2 (Management Plan of Parnitha National Park 1997).

Forest vegetation at higher altitudes of the forest consists mostly of pure stands of *Abies cephalonica* Loud., while at lower altitudes pure or mixed stands of *Pinus halepensis* Mill. mainly occur, as well as other species such as *Arbutus unedo* L., *Quercus coccifera* L., *Quercus ilex* L., *Phillyrea latifolia* L., *Platanus orientalis* L. and others (Management Plan of Parnitha National Park 1997).

Data collection

Raw data were collected from the entire forest of Tatoi, for the Aleppo pine species (*Pinus halepensis*), in the framework of a Master thesis prepared at the Department of Forestry and Management of the Environment and Natural Resources, of Democritus University of Thrace, in Greece (Angelaki 2013).

Data were collected during the spring of 2012, based on random sampling and GPS use. Coordinates of each Aleppo pine tree were recorded and labelled on a map of the forest. One hundred trees were randomly selected and measured as follows:

- Breast height diameter *D* (m) was measured with a calliper;
- Total height *H* (m) was estimated with a Blume-Leiss altimeter;
- Form factor *f* was estimated with a relaskop.

Total volume *V* (m^3) of each tree was calculated using the formula (2) (Van Laar and Akça 2007):

$$V = \pi \cdot 4^{-1} \cdot D^2 \cdot f \cdot H \qquad (2).$$

Regression models

For regression models fitting to data, 32 models were tested (Kitikidou 2008, see Appendix). The first 14 are single-entry models that estimate V in relation to D, while the remaining 18 models are double-entry models that estimate V in relation to D and H.

Models' fitting was performed using the statistical software SPSS V.19 (Kitikidou 2005, IBM 2010). Initially, significance of the analysis of variance (Sig. F), of the Variance Inflation Factors – VIFs, of the tolerances and of the t-test for the regression coefficients (Sig. *t*), were examined. The models that remained for further comparison were those that had: Sig. $F < 0.05$; VIFs < 10; Tolerances > 0.0001; Sig. t for regression coefficients < 0.05.

Then, the comparison criteria of Table 2 were calculated.

The same criteria used for fitting data were used for validation data. When the selected regression model, based on the values of the above criteria from fitting data, gives similar values for the validation data, then the model is valid, i.e. it will have similar values for the regression coefficients, if we take a new sample (Draper and Smith 1997). The method of separation of the data (split data) mimics the validation of a model by taking a new sample, separating the data that were originally collected in two parts, fitting and validation data (Ezekiel and Fox 1959, Marquardt and Snee 1975). In this study, 80 % of the data (80 trees) were used for fitting and the remaining 20 % (20 trees) for validation.

Results

1. Exploratory data analysis

Before applying statistical approaches aimed at testing specific hypotheses, we applied an Exploratory Data Analysis as a quantitative approach to help understand data. Descriptive statistics of the sampled trees are given in Table 3.

Table 2. Criteria for regression models comparison.

No	Criterion	Formula	Optimum value	References
1	Absolute mean error	$\sum_{i=1}^{n} \frac{\left\lvert V_i - \hat{V}_i \right\rvert}{n}$	0	Mayer and Butler (1993) Janssen and Heuberger (1995) Wackerly et al. (2008)
2	Standard error of the estimate	$\sqrt{\frac{\sum_{i=1}^{n}\left(V_i - \hat{V}_i\right)^2}{n-p}}$	min	Ezekiel and Fox (1959) Mathews (1987) Draper and Smith (1997) Wackerly et al. (2008)
3	Coefficient of determination R^2	$1-\frac{\sum_{i=1}^{n}\left(V_i - \hat{V}_i\right)^2}{\sum_{i=1}^{n}\left(V_i - \bar{V}\right)^2}$	1	Ezekiel and Fox (1959) Kvålseth (1985) Mathews (1987) Mayer and Butler (1993) Janssen and Heuberger (1995) Draper and Smith (1997) Everitt and Skrondal (2010)
4	Root of the mean squared error	$\sqrt{\frac{\sum_{i=1}^{n}\left(V_i - \hat{V}_i\right)^2}{n}}$	min	Anderson and Woessner (1992) Draper and Smith (1997)
5	Sum of squared errors	$\sum_{i=1}^{n}\left(V_i - \hat{V}_i\right)^2$	0	Mayer and Butler (1993)
6	Sum of relative squared errors	$\frac{\sum_{i=1}^{n}\left(\frac{V_i - \hat{V}_i}{V_i}\right)^2}{n} 100$	0	Draper and Smith (1997)
7	Relative mean squared error, %	$\sum_{i=1}^{n}\left(\frac{V_i - \hat{V}_i}{V_i}\right)^2$	0	Draper and Smith (1997)
8	Average deviation	$\frac{\sum_{i=1}^{n}\left\lvert V_i - \hat{V}_i \right\rvert}{\sum_{i=1}^{n}\hat{V}_i} 100$	0	Kenney and Keeping (1962) Snee (1977) Havil (2003)
9	Variance ratio	$\frac{\sum_{i=1}^{n}\left(\hat{V}_i - \bar{\hat{V}}\right)}{\sum_{i=1}^{n}\left(V_i - \bar{V}\right)}$	1	Mayer and Butler (1993) Janssen and Heuberger (1995) Chen and Deo (2006)
10	Regression coefficients *α*, *β*	$V_i = \alpha + \beta \hat{V}_i + \text{error}$	*α*=0 *β*=1	Mayer and Butler (1993) Janssen and Heuberger (1995)

Notation: V_i – observed values of volume; $\hat{V}_i$ – estimated values of volume from the regression model; n – number of observations; p – number of regression coefficients; $\overline{Y}$ – average of observed volumes; $\bar{\hat{V}}$ – average of estimated volumes.

Boxplots of the measured variables (Figure 1) show a few outliers and some extremes for form factor and volume. All distributions are symmetric, except that the volume appears to be slightly positively skewed.

Table 3. Descriptive statistics of the sampled trees.

Variable	Count	Average	Standard deviation	min	max
D, m	100	0.32	0.09	0.13	0.62
H, m	100	17.42	4.51	8.00	32.00
f	100	0.32	0.08	0.18	0.87
V, m^3	100	0.53	0.47	0.04	2.88

2. Model selection

At first, the problematic models against Sig. F, tolerances, VIFs and Sig. t for regression coefficients were found and excluded from further comparison. For the remaining models, the comparison criteria of Table 2 were calculated. According to Table 4, the models that had the best values (highlighted) and were selected are the following:

$V = b_1 \cdot D^2 \cdot H$ (model No 15);

$V = b_0 + b_1 \cdot D^2 \cdot H$ (model No 16).

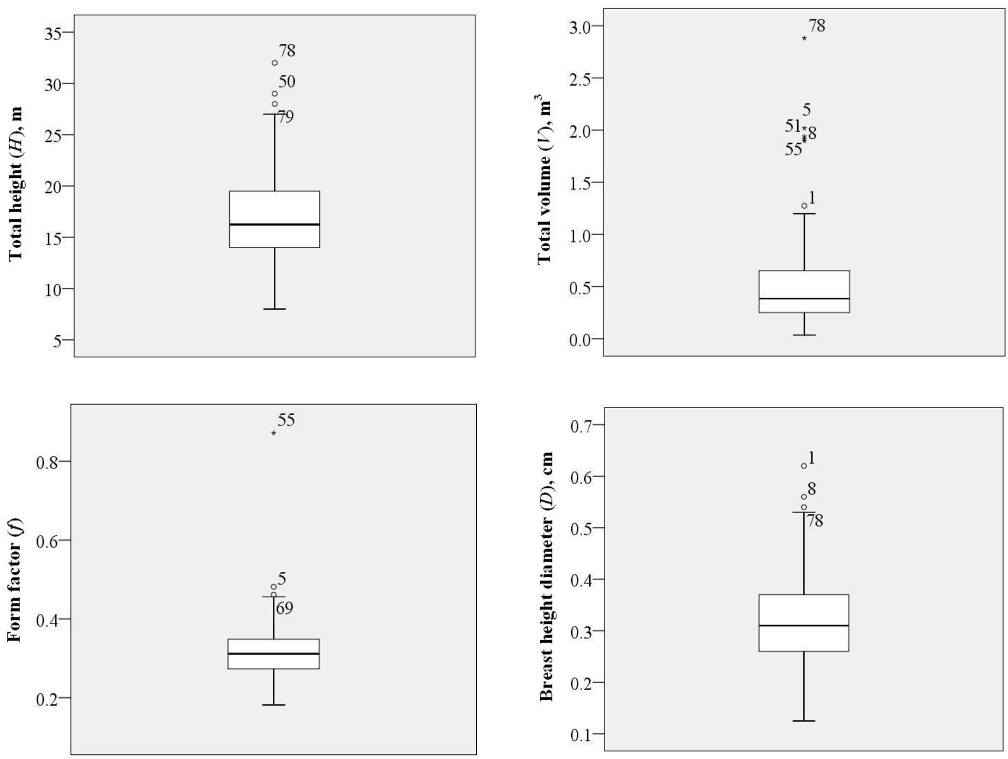

Fig. 1. Boxplots of measured variables.

Table 4. Criteria for regression models comparison – fitting data.

Criterion	Absolute mean error	Standard error of the estimate	Coefficient of determination R^2	Root of the mean squared error	Sum of squared errors	Sum of relative squared errors	Relative mean squared error, %	Average deviation	Variance ratio	Regression coefficients α, β	
Optimum value	0	min	1	min	0	0	0	0	1	0	1
1	0.192	0.299	0.798	0.297	7.041	28.282	35.352	32.486	0.754	-0.142	1.174
9	3046.931	3191.360	<0	3151.215	794412593.7	5215565824.9	6519457281.1	100.018	13476785.51	1.999	0.001
11	3046.931	3191.3560	<0	3151.215	794412593.7	5215565824.9	6519457281.1	100.018	13476785.51	1.999	0.001
15	0.106	0.199	0.910	0.198	3.141	5.132	6.415	18.653	0.894	-0.034	1.037
16	0.101	0.200	0.911	0.197	3.104	4.852	6.066	18.244	0.912	-0.00005	1.000
17	0.144	0.231	0.882	0.227	4.105	121.086	151.358	25.955	0.883	-0.0004	1.000
22	1.501	1.611	<0	1.591	202.514	18966.929	23708.661	158.443	2.780	1.018	0.490
23	1.502	1.607	<0	1.587	201.510	18921.432	23651.790	158.431	2.745	1.027	0.499
24	1.501	1.621	<0	1.590	202.284	19012.550	23765.687	158.470	2.779	1.019	0.491
26	1.501	1.611	<0	1.591	202.514	18966.929	23708.661	158.443	2.780	1.018	0.490

3. Model validation

For the 20 trees used for validation, we followed the same procedure. According to Table 5, comparison of the models that remained after the initial exclusion of models based on Sig. *F*, tolerances, VIFs and Sig. *t* for regression coefficients, lead to the selection of the following:

$V = b_1 \cdot D^2$ (model No 1);

$V = b_1 \cdot D^2 \cdot H$ (model No 15).

4. Final model selection and comparison with the existing one

From the regression models comparison, for the 80 trees (fitting data), we concluded that the best models were nos 15 and 16. For the 20 trees (validation data), the best models were nos 1 and 15. The model that was finally selected and compared with the previous Logging table of the year 2004, was the one that had optimum results in both processes (fitting and validation), i.e. the model no 15.

Table 5. Criteria for regression models comparison – validation data.

Criterion	Absolute mean error	Standard error of the estimate	Coefficient of determination R^2	Root of the mean squared error	Sum of squared errors	Sum of relative squared errors	Relative mean squared error, %	Average deviation	Variance ratio	Regression coefficients α, β	
Optimum value	0	min	1	min	0	0	0	0	1	0	1
1	0.054	0.076	0.988	0.074	0.109	1.798	8.989	12.836	0.996	-0.019	1.037
9	1.439	1.560	<0	1.480	43.780	1287.181	6435.905	141.003	5.946	0.802	0.376
11	1.439	1.560	<0	1.498	43.780	1287.181	6435.905	141.003	5.946	0.802	0.376
15	0.055	0.072	0.989	0.070	0.098	0.716	9.580	13.371	0.980	0.024	0.953
22	1.439	1.564	<0	1.483	44.009	1401.695	7008.473	141.015	5.983	0.789	0.363
23	1.440	1.565	<0	1.485	44.091	1379.696	66898.478	140.970	5.957	0.780	0.354
26	1.439	1.564	<0	1.483	44.009	1401.695	7008.473	141.015	5.983	0.789	0.363

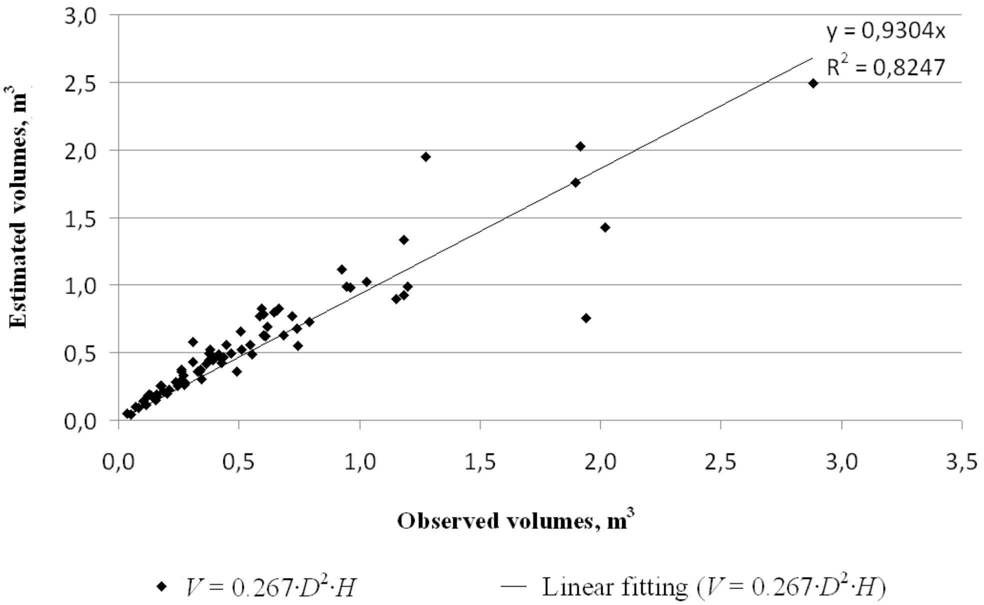

Fig. 2. Linear relationship between observed volumes and estimated from the selected model of the present study.

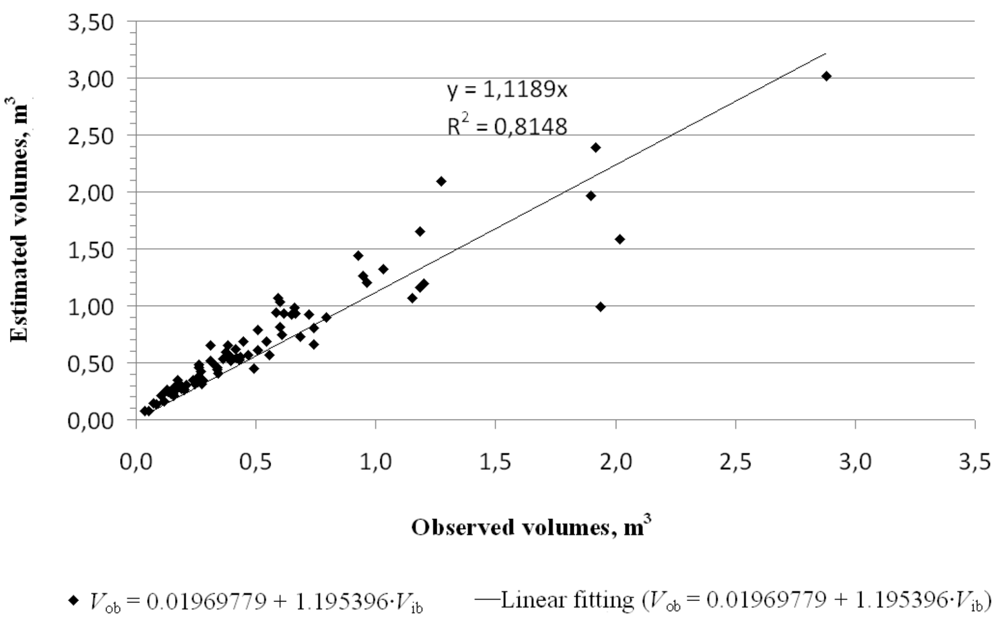

Fig. 3. Linear relationship between observed volumes and estimated from the previous logging table.

Two models were compared:

$V = 0.267 \cdot D^2 \cdot H$ (selection of the present study);

$V_{ob} = 0.01969779 + 1.195396 \cdot V_{ib}$, where $V_{ib} = 3.3041044 \cdot 10^{-5} \cdot D^{1.790332} \cdot H^{1.181907}$ (selection of the previous logging table).

In the following figures, on the horizontal axis the observed volumes are displayed, while on the vertical the volumes estimated using the model chosen in this study (Figure 2) and the Logging table of 2004 (Figure 3). Ideally, the points of the graphic should form a straight line with a slope of 45º, starting from the origin. One can easily notice that the selected model of this research has a very good fit, like the previous model of the year 2004.

Discussion

There are numerous studies as regards Aleppo pine volume estimation models. Indicatively, we mention Smiris et al. (1998), Montero et al. (2000), Montero et al. (2001), Trasobares et al. (2004), Condés and Sterba (2008). Comparing our model with the one developed by Martínez-Millán et al. (1993) and used in Montero et al. (2001) (mean breast height diameter between inventories in Montero et al. (2001) ranged between 4.39 and 5.46 cm, and mean total tree height between 10.5 and 13.9 m), we can demonstrate that the forest studied in the present research gives slightly smaller volumes per tree. Montero's et al. model is:

$V = 0.077186 \cdot D^{1.84818} \cdot H^{0.88012}$,

where total volume V is in dm^3, breast height diameter D in cm and total tree height H in m.

Estimated volume ranges between 0.009 and 0.018 m^3, while in the forest of Tatoi, the estimated volume, within the same range of breast height diameter and height as Montero et al. (2001), extends between 0.005 and 0.011 m^3.

With rapidly decreasing forest cover due to human activities, afforestation and reforestation have become a necessity and is being avidly pursued. For Mediterranean, semi-arid and dry climatic zones, *Pinus halepensis spp.* has become a good choice for afforestation and reforestation, due to its drought resistance and good growth potential (Maestre and Cortina 2004). Due to past wildfire cases and from perspective of forest dynamics study, its allometry has recently been emphasized to devise better afforestation and reforestation strategies. Volume and biomass are visual measures of plant's photosynthetic activity and is significant in terms of wildfire control measures, while their patterns give insights into plant physiology and growth (Badía and Martí 2008). Allometric models are the most used tool to assess volume or biomass from forest inventory data (e.g. tree diameter and height) (Picard et al. 2012). Smiris et al. (2000) analyze the aboveground biomass of Aleppo pine forests in Kassandra Peninsula in Chalkidiki in Greece and refer that the knowledge of amount of biomass (flammable material) and its distribution helps in the prevention of forest fires.

In this frame allometry (volume and biomass estimation through diameter and height) should be studied. Prudent volume estimation of *P. halepensis* can greatly enhance success in afforestation and reforestation techniques and better ecological control as well as can help in planning of forest fire prevention.

Conclusion

In this study, a double-entry volume table model was developed and validated, using a random sample of 100 Aleppo pine trees, from the Tatoi forest of Parnitha, Attica. Comparison of the selected model with the pre-existing model of 2004 showed that they are similar, regarding volume estimation. When compared to a study in Spain (Montero et al. 2001), we noticed that the Tatoi forest gives slightly smaller volumes per tree.

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Appendix

Compared regression models

$V = b_1 \cdot D^2$ (1)

$V = b_0 + b_1 \cdot D^2$ (2)

$V = b_0 + b_1 \cdot \pi \cdot 4^{-1} \cdot D^2$ (3)

$V = b_1 \cdot D + b_2 \cdot D^2$ (4)

$V = b_0 + b_1 \cdot D + b_2 \cdot D^2$ (5)

$V = b_0 + b_1 \cdot D + b_2 \cdot D^2 + b_2 \cdot D^3$ (6)

$V = b_0 + b_1 \cdot D + b_2 \cdot D^2 + b_3 \cdot D^3 + b_4 \cdot D^4$ (7)

V = function $[D, D^2, D^{-1}, D^{-2}, \ln D, \ln(D^2)]$ (8)

$\ln v = b_0 + b_1 \cdot \ln D$ (9)

$\ln v = b_0 + b_1 \cdot \ln D + b_2 \cdot D^{-1}$ (10)

$\ln v$ = function $[D, D^2, D^{-1}, D^{-2}, \ln D, \ln(D^2)]$ (11)

$v \cdot D^{-2} = b_0 + b_1 \cdot D^{-1}$ (12)

$v \cdot D^{-2} = b_0 + b_1 \cdot D^{-2}$ (13)

$v \cdot D^{-2} = b_0 + b_1 \cdot D^{-1} + b_2 \cdot D^{-2}$ (14)

$V = b_1 \cdot D^2 \cdot H$ (15)

$V = b_0 + b_1 \cdot D^2 \cdot H$ (16)

$V = b_0 + b_1 \cdot D^2 + b_2 \cdot H$ (17)

$V = b_0 + b_1 \cdot D^2 + b_2 \cdot H + b_3 \cdot D^2 \cdot H$ (18)

$V = b_0 + b_1 \cdot D + b_2 \cdot D^2 + b_3 \cdot D \cdot H + b_4 \cdot D^2 \cdot H$ (19)

$V = b_0 + b_1 \cdot D^2 + b_2 \cdot H^2 + b_3 \cdot D^2 \cdot H + b_4 \cdot D \cdot H^2$ (20)

V = function $[D, H, D^2, H^2, D \cdot H, D^2 \cdot H, D \cdot H^2, D^2 \cdot H^2, D^{-1}, H^{-1}, D^{-2}, (D \cdot H)^{-1}, D^{-2} \cdot H^{-1}, H \cdot D^{-1}, H \cdot D^{-2}, \ln D, (\ln D)^2, \ln H, (\ln h)^2, \ln(D^2), \ln(D^2 H)]$ (21)

$\ln v = b_0 + b_1 \cdot \ln(D^2 \cdot H)$ (22)

$\ln v = b_0 + b_1 \cdot \ln(D \cdot H)$ (23)

$\ln v = b_0 + b_1 \cdot \ln D + b_2 \cdot \ln H$ (24)

$\ln v = b_0 + b_1 \cdot \ln D + b_2 \cdot \ln H + b_3 \cdot (\ln D)^2 + b_4 \cdot (\ln H)^2$ (25)

$\ln v$ = function $[D, H, D^2, H^2, D \cdot H, D^2 \cdot H, D \cdot H^2, D^2 \cdot H^2, D^{-1}, H^{-1}, D^{-2}, (D \cdot H)^{-1}, D^{-2} \cdot H^{-1}, H \cdot D^{-1}, H \cdot D^{-2}, \ln D, (\ln D)^2, \ln H, (\ln H)^2, \ln(D^2), \ln(D^2 \cdot H)]$ (26)

$v \cdot D^{-2} \cdot H^{-1} = b_0 + b_1 \cdot D^{-2} \cdot H^{-1}$ (27)

$\frac{v}{D^2 H} = b_0 + b_1 \cdot D^{-2} + b_2 \cdot H^{-1} + b_3 \cdot D^{-2} \cdot H^{-1}$ (28)

$\frac{v}{D^2 H} = b_0 + b_1 \cdot (D \cdot H)^{-1} \cdot b_2 \cdot H^{-1} + b_3 \cdot D^{-1} + b_4 \cdot D^{-2} + b_5 \cdot D^{-2} \cdot H^{-1}$ (29)

$v \cdot D^{-2} \cdot H^{-1} = b_0 + b_1 \cdot H^{-1} + b_2 \cdot (D \cdot H)^{-1} + b_3 \cdot H \cdot D^{-2} + b_4 \cdot D^{-2} \cdot H^{-1}$ (30)

$v \cdot D^{-2} = b_0 + b_1 \cdot H$ (31)

$D^2 \cdot H \cdot v^{-1} = b_0 + b_1 \cdot D$ (32)

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TESTING OF AMERICAN, GERMAN AND BULGARIAN DOUGLAS-FIR PROVENANCES ON TWO TEST SITES IN BULGARIA SIX YEARS AFTER OUTPLANTING

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Abstract

Two provenance tests with 29 German, American and Bulgarian Douglas-fir provenances were established in Bulgaria with 2-year-old plants in 2006. The trials were established in the forest enterprises "Berkovitsa" (Western Balkan Range) and "Borima" (Central Balkan Range). The spacing was 2 x 2 m, with 49 and 25 plants per plot, respectively, with three replications. The trials were evaluated for survival, mean growth and current annual height increment in the last (the sixth) year after outplanting. The results show that the trees in "Berkovitsa", which grow on more suitable site according to climate, had less mortality and better growth compared to these on the site "Borima". Statistical analysis shows the beginning of differentiation between the provenances. Differences between the provenance regions are insignificant. Indications are given for the procurement of Douglas-fir seed for Bulgaria.

Key words: current height increment, Douglas-fir, height growth, provenance trials, survival.

Introduction

Douglas-fir is one of the most frequently introduced coniferous tree species in Europe. One of the main prerequisites for profitable management of Douglas-fir plantations is their high productivity. Numerous provenance tests were carried out in Europe to establish the most appropriate and most productive origins (Hermann and Lavender 1999, Govedar et al. 2003, Zas et al. 2004, Isajev and Lavadinović 2007, Weller 2012, Bastien et al. 2013, Lavender and Hermann, 2014). In one of the first provenance tests, carried out in 1910, 10 provenances of the three varieties (subspecies) of Douglas-fir were introduced, namely var. *viridis* ("green" coastal Douglas-fir), var. *caesia* ("gray" Douglas-fir) and var. *glauca* ("blue"

Douglas-fir). The results of 90 years of research confirmed the better growth of green Douglas-fir (var. *viridis*) compared to the other two varieties (Stimm and Dong 2001).

During the period 1923–1930 the first Douglas-fir provenance tests were carried out in the Netherlands. Studies on growth in height and volume also show the better opportunities of coastal versus continental provenances (Veen 1951).

In 1969 a Douglas-fir provenance test (Perić et al. 2009) was made in Istria (Croatia). The experiment covered 10 provenances – seven from the United States (five from Washington and two from Oregon) and three from Denmark, Bulgaria and Croatia. The average survival rate at the age of 40 of all provenances amounted to 64.8 %. The best growth in height was found in the SHELTON provenance from Washington, and the lowest – in SHADE COVE from higher altitude in Oregon. All provenances were vital and in good health condition. In 1982 the Institute of Forestry in Belgrade carried out two provenance tests in Central and Eastern Serbia with 18 Northamerican provenances from Oregon to British Columbia (Lavadinović et al. 2009). Growth in height in the 15-th year of age varied from 43 to 89 cm with an average of 75.2 cm.

In 1987, seeds from 55 American Douglas-fir provenances (6 from the coast of Washington and Oregon, 8 continental, 38 from the Cascade Mountains and 3 from the states Arizona, Montana and New Mexico) were imported in Bulgaria. They were used for germination tests and early diagnosis of seedlings in nursery (Popov 1990, Petkova and Popov 1998). Several provenance tests were established in Bulgaria with seedlings from these provenances. The coastal provenances and these from Cascade Mountains from the states of Washington and Oregon are characterized with the best growth. The continental provenances lag behind in growth and show a strong sensitivity to fungal pathogens *Rhabdocline pseudotsugae* Syd. and *Phaeocryptopus gaeumanii* (T. Rohde) (Popov 1995, Popov 2001, Petkova 2011).

To provide high quality seeds of Douglas-fir, particularly in forecasted climate change, two provenance tests with seeds from German, Bulgarian and American stands were established in 2006. The first results of this experiment have been published (Petkova et al. 2008).

The purpose of this work was to present the results of studies on the survival rate and height growth of Douglas-fir of the sixth year after the planting.

Objects and Methods

Objects of this study were two Douglas-fir provenance tests, established in the spring of 2006 in Forest Enterprises Berkovitsa (Western Balkan Range) and Borima (Central Balkan Range). The experiment covers 29 provenances (Table 1), of which 20 from older stands in Germany (8 from Bavaria, 7 from Rhineland Palatinate and 5 from Lower Saxony), 6 from selected Douglas-fir stands in Washington (USA) and 3 from older Douglas-fir plantations in Bulgaria.

The provenance tests were established in a 2×2 m plot design in three replications, in Berkovitsa with 49 (7×7 m) plants on each plot, in Borima – 25 (5×5 m) plants on each plot.

The trial in Berkovitsa was established at the top of the northeast facing

Table 1. Data for Douglas-fir provenances included in the provenance tests.

No	Country or province	Provenance region, code	Register, No	Forest enterprises	Altitude, m	Geographic coordinates	
						Longitude	Latitude
1	Bavaria	853 04	91853041851	Münnerstadt	300–310	10°12’	50°13’
2	Bavaria	853 04	91853040941	Lohr	440–455	9°30’	50°0’
3	Bavaria	853 04	91853041161	Schweinfurt	380	12°14’	50°3’
4	Bavaria	853 06	91853060171	Stadtsteinach	490–600	11°34’	50°13’
5	Bavaria	853 06	91853060381	Schnaittenbach	480–500	11°58’	49°30’
6	Bavaria	853 05	9185304222	Griesbach	460–510	13°13’	48°27’
7	Bavaria	853 05	9185304043	Freising	420	11°45’	48°24’
8	Bavaria	853 06	91853060211	Stadtsteinach	550–650	11°31’	50°10’
9	USA	422-2.0	EC-20 (1995)	Mineral, Wa	533	122°10’	46°42’
10	USA	403-1.5	EC-13 (1998)	Darrington, Wa	381	121°35’	48°22’
11	USA	403-1.0-1.5	EC-11 (2001)	Darrington, Wa	381	121°45’	48°20’
12	USA	403-1.0-1.5	EC-41 (2001)	Darrington, Wa	381	121°33’	48°20’
13	USA	403-1.0-1.5	EC-13 (2001)	Darrington, Wa	381	121°35’	48°22’
14	USA	012-3.0	no data	Forks, Wa	838	124°13’	48°05’
15	Rhineland Palatinate	853 04	72853040014	Kaiserslautern	280–310	7°46’	49°25’
16	Rhineland Palatinate	853 05	73853050162	Osburg	565–590	6°45’	49°45’
17	Rhineland Palatinate	853 04	71853040432	Mayen	410–500	7°00’	50°17’
18	Rhineland Palatinate	853 05	73853050232	Daun	560–620	6°41’	50°10’
19	Rhineland Palatinate	853 05	73853050182	Schneifel	530	6°30’	50°15’
20	Rhineland Palatinate	853 05	73853050102	Gerolstein	520–550	6°30’	50°15’
21	Rhineland Palatinate	853 04	72853040013	Kaiserslautern W	280–310	7°46’	49°25’
22	Lower Saxony	853 01	31853040012	Oldendorf	180	9°27’	51°56’
23	Lower Saxony	853 04	34853040013	Münden	300	9°46’	51°19’
24	Lower Saxony	853 01	31853040013	Oldendorf (SPl)	290	9°25’	51°51’
25	Lower Saxony	853 01	35853010212	Ahlhorn	30	7°58’	52°54’
26	Lower Saxony	853 01	11853010091	Segeberg	40	10°07’	53°56’
27	Bulgaria	PME135	no data	Smiljan	950	24°46’	41°38’
28	Bulgaria	PME095	C02PME09500132	Kazanlak	750	25°26’	42°38’
29	Bulgaria	PME064	no data	Newestino	650	22°46’	42°12’

slope with gradient 8 ° and altitude a.s.l. 850 m, latitude 43°13'9.78'' N and longitude 23°05'25.02'' E. The soil is Cambisols (FAO), mixed sandy and clay, slightly stony, friable, deep, fresh to moist, on subsoils schists. The habitat is medium rich to rich. The reaction of the soil is acidic (pH = 4.67). The climate in the region is temperate with average annual temperature 10.4 °C and average temperature in the growing period (April–September) 17.4 °C. The absolute maximum temperature reaches up to 40.6 °C, and the absolute minimum temperature drops to –23.2 °C. The average annual precipitation amount is 825 mm. More than half of the rainfall (488 mm) comes during the growing period (April–September).

The provenance test in Borima was located on the sunny south-facing slope with 14 ° and altitude 1067 m a.s.l., latitude 42°54'52.2'' N and longitude 24°53'41.2'' E. The soil is Cambisols (FAO), mixed sandy and clay, slightly stony, densified, medium deep, fresh to moist. The habitat is medium rich to rich. The climate is temperate, but more continental compared to Berkovitsa. The average annual temperature is 9.8 °C and the average temperature during the growing period (April–September) is 17.7 °C. The absolute maximum temperature reaches 39.8 °C, and the absolute minimum temperature drops to –28.2 °C. The average annual precipitation amount is 767 mm. More than half of the rainfall (446 mm) comes during the growing period (April–September).

In a trial in Forest Enterprise Berkovitsa the height and current annual height increment of 20 plants for each provenance and each replication were measured of the last (the sixth) year after planting 20 plants for each provenance and each replication. The values of average height and average survival rate were processed statistically with program Statistika 8.0. The Duncan multiple range test was used to establish the influence of the provenance on the mean height and current height increment, and the statistical significance of differences.

The height and last height increment of all trees were measured only for 12 provenances of the first replication at trial plantation in Borima. Because of large losses in the majority of provenances in that plantation there was not sufficient number of surviving trees. Data have not been processed statistically.

Results and Discussion

During the sixth year after the planting the survival rate of Douglas-fir in Berkovitsa is very high – an average of 94.4 % (Fig. 1).

The highest survival rate – 98.6 % was observed in provenances 25 Ahlhorn (Lower Saxony), 8 Stadtsteinach (Bavaria) and 13 Darrington (USA). With a very good survival of 98 % are characterized provenances 16 Osburg and 17 Mayen from Rhineland Palatine, 26 Segeberg from Lower Saxony and American provenance 11 Darrington, Wa. Survival below 90 % was demonstrated by American provenance 12 Darrington and two Bavarian provenances, 5 Schnaittenbach and 2 Lohr. Provenance 25 Ahlhorn was characterized with the fewest losses and provenances 2 Lohr (Bavaria) and 12 Darrington (USA) – with the most losses also of the first year after the planting (Petkova et al. 2008).

The data about the single groups showed (Table 2), that the highest survival rate in the test site in Berkovitsa had a group of Rhineland Palatinate – 96.4 %, followed by the group of Lower Saxony –

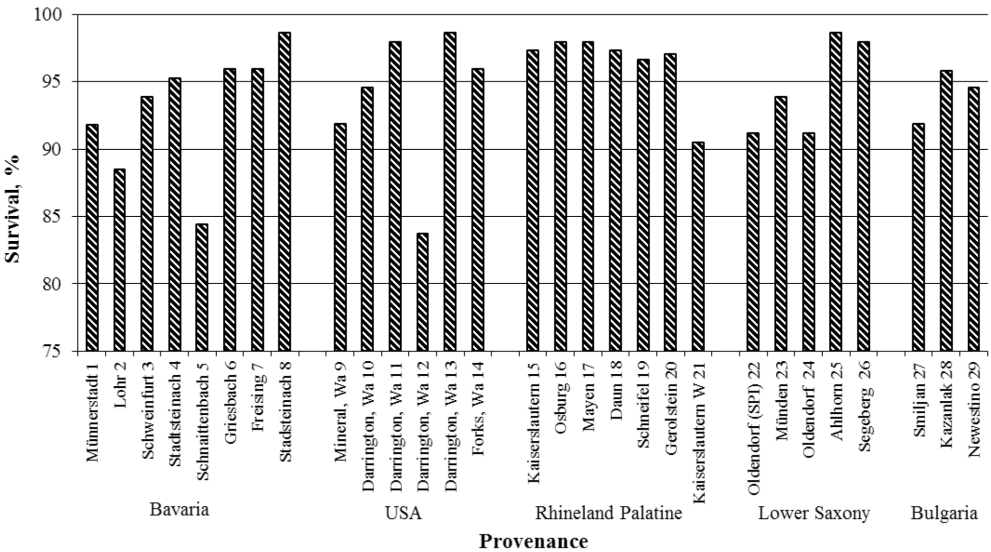

Fig. 1. Survival of Douglas-fir provenances on the test site Berkovitsa.

94.6 %. The Bavarian group had the lowest survival – 93.1 %. Average position was occupied by Bulgarian (94.1 %) and USA (93.8 %) groups. Overall losses in the test site in Berkovitsa were negligible.

The mean height of Douglas-fir in Berkovitsa in the sixth year after planting was 3.4 m. The differences between the provenances were minimal (Table 2).

One way analysis of variances (ANOVA) was used to establish the influence of the provenance, replication and interaction between them on the mean height of Douglas-fir in the test site in Berkovitsa by significance level of $p < 0.01$ (Table 3).

The results of the Duncan test (Table 4) showed that the mean height of provenances could be arranged in 13 homogeneous groups. Provenance 3 Schweinfurt (Bavaria) was characterized with the most

Table 2. Survival, mean height and current annual height increment of the last year after the planting of the provenance regions on the two test sites.

Provenance group	Survival, %		Height (H), m		Height increment (Zh), cm	
	Berkovitsa	Borima	Berkovitsa	Borima	Berkovitsa	Borima
Bavaria	93.1	64.0	3.5	2.6	94.4	86.7
USA	93.8	40.0	3.4	1.9	96.9	78.5
Rhineland Palatine	96.4	52.0	3.3	2.0	89.7	70.2
Lower Saxony	94.6	36.0	3.5	2.6	95.6	82.2
Bulgaria	94.1	64.0	3.3	2.4	86.1	77.8

Table 3. Significance of factors on the mean height in the provenance test in Berkovitsa.

Indicator	Degree of freedom	Mean square	Fisher's criterion	Significance level
Provenance	24	6.295	11.551	0.000
Replication	2	10.270	18.852	0.000
Provenance * Replication	48	3.224	5.916	0.000

rapid growth and had a mean height of 3.9 m, followed by provenances 23 Münden (Lower Saxony), 18 Daun (Rhineland Palatinate) and 11 Darrington (USA) with a height of 3.8 m, 26 Segeberg (Lower Saxony), 1 Münnerstadt and 4 Stadtsteinach (Bavaria) with a mean height of about 3.7 m. Provenance 18 Daun from Rhineland Palatinate showed rapid growth in height also in the first year after the planting (Petkova et al. 2008). Provenance Darrington was characterized by very good growth in 14 provenance trials and was recommended for planting in North-Western Germany (Weller 2010).

Provenance 19 Schneifel (Rhineland Palatinate) was characterized as the slowest growing with a mean height of 2.6 m, followed by provenance 24 Oldendorf (Lower Saxony) and 5 Schnaittenbach (Bavaria) with a mean height of about 2.8 m. Genetic studies have shown that provenance 5 Schnaittenbach belongs to the subspecies gray Douglas-fir (Konnert and Ruetz 2006, Konnert et al. 2010, Konnert and Fussi 2012). Other provenances showed an average growth in height.

A comparison of the mean height of 12 provenances of the experimental trial in Borima with the same in Berkovitsa showed that these in Borima had less growth (Table 4). An exception was the provenance 6 Griesbach, which in both areas had similar mean height. This provenance could be described as highly adaptive to different conditions of the habitat.

The current annual height increment of the last (the sixth) year after the planting in Berkovitsa was 93.3 cm. The best height increment was found in American provenances – 96.9 cm, and the lowest – in Bulgarian ones – 86.1 cm (Table 2). The Bulgarian provenances showed the poorest height increment also in the first year after the planting (Petkova et al. 2008).

The performed one way analysis of variances (ANOVA) showed the importance of the provenance, replication and combination of provenance and replication on the height increment of Douglas-fir experimental trial Berkovitsa at significance level $p < 0.01$ (Table 5).

The results of the Duncan test showed that height increments of all provenances were arranged in 10 homogeneous groups (Table 6). The largest height increment was measured in provenance 11 Darrington (USA) – 106.9 cm, followed by provenances Münden 23 and 26 Segeberg (Lower Saxony), 3 Schweinfurt (Bavaria), 22 Oldendorf (SPI) from Lower Saxony, 12 and 10 Darrington (USA) and 6 Griesbach (Bavaria), which also have a considerable current height increment from 101.1 to 104.7 cm.

With the poorest height increment were characterized provenances 5 Schnaittenbach (Bavaria), 19 Schneifel (Rhineland Palatinate) and 24 Oldendorf (Lower Saxony). These provenances also had the lowest height. Provenance 5 (Schnaittenbach) had the poorest height increment also in the first year after planting (Petkova et al. 2008). The

Table 4. Mean heights (H) of the Douglas-fir provenances six years after planting and Duncan multiple range test results for provenances in Berkovitsa.

Provenance	Borima	Berkovitsa													
	H±S.E., m	H±S.E., m	Homogeneous groups												
Schneifel 19	1.85±0.25	2.62±0.11	a												
Oldendorf 24		2.76±0.11	a	b											
Schnaittenbach 5		2.83±0.12	a	b	c										
Mineral, Wa 9		3.05±0.11		b	c	d									
Lohr 2		3.06±0.09		b	c	d	e								
Smiljan 27		3.11±0.12			c	d	e								
Mayen 17	1.52±0.15	3.18±0.11				d	e	f							
Forks, Wa 14		3.23±0.08				d	e	f	g						
Kaiserslautern W 21		3.35±0.09				d	e	f	g	h					
Gerolstein 20	1.66±0.10	3.36±0.11				d	e	f	g	h	i				
Newestino 29		3.39±0.13					e	f	g	h	i				
Osburg 16	2.54±0.26	3.45±0.12						f	g	h	i	j			
Kazanlak 28	2.38±0.19	3.46±0.11						f	g	h	i	j			
Kaiserslautern 15		3.47±0.10						f	g	h	i	j	k		
Darrington, Wa 13		3.48±0.10						f	g	h	i	j	k	l	
Ahlhorn 25	2.63±0.20	3.49±0.10						f	g	h	i	j	k	l	
Darrington, Wa 10		3.49±0.10						f	g	h	i	j	k	l	
Oldendorf (SPI) 22		3.54±0.10							g	h	i	j	k	l	
Griesbach 6	3.17±0.13	3.55±0.09							g	h	i	j	k	l	
Stadsteinach 8		3.55±0.10							g	h	i	j	k	l	
Darrington, Wa 12		3.58±0.10								h	i	j	k	l	m
Freising 7	2.06±0.22	3.63±0.10								h	i	j	k	l	m
Stadtsteinach 4		3.67±0.10								h	i	j	k	l	m
Münnerstadt 1	2.27±0.24	3.68±0.08								h	i	j	k	l	m
Segeberg 26		3.71±0.07									i	j	k	l	m
Darrington, Wa 11	1.93±0.14	3.78±0.11										j	k	l	m
Daun 18	2.52±0.21	3.81±0.10											k	l	m
Münden 23		3.82±0.10												l	m
Schweinfurt 3	2.72±0.22	3.90±0.09													m

Note: S.E. – standard error; different letters unit homogenous groups of provenances without significant difference between mean height by tested provenances according to Duncan's multiple range test at the 0.05 significant level.

Table 5. Significance of factors on current annual height increment.

Indicator	Degree of freedom	Mean square	Fisher's criterion	Significance level
Provenance	24	4149.593	6.910	0.000
Replication	2	3973.095	6.616	0.001
Provenance * Replication	48	3431.374	5.714	0.000

Table 6. Current height increment of the Douglas-fir provenances of the last (the sixth) year after the planting (Zh) and Duncan multiple range test results for provenances in Berkovitsa.

Provenance	Borima	Berkovitsa										
	Zh±S.E., cm	Zh±S.E., cm	Homogeneous groups									
Schnaittenbach 5		76.0±4.9	a									
Schneifel 19	62.0±10.2	76.4±3.7	a									
Oldendorf 24		77.2±3.0	a									
Smiljan 27		82.8±3.8	a	b								
Mayen 17	48.4±7.4	85.4±2.9	a	b	c							
Mineral, Wa 9		86.1±3.6	a	b	c							
Lohr 2		86.7±4.1	a	b	c	d						
Newestino 29		88.1±4.3		b	c	d	e					
Kazanlak 28	77.8±6.4	88.3±3.8		b	c	d	e					
Gerolstein 20	60.8±4.3	88.5±3.1		b	c	d	e					
Osburg 16	91.5±8.9	89.7±3.8		b	c	d	e					
Ahlhorn 25	82.2±8.3	91.0±2.8		b	c	d	e	f				
Forks, Wa 14		91.7±2.9		b	c	d	e	f	g			
Stadtsteinach 4		92.4±2.9		b	c	d	e	f	g	h		
Kaiserslautern W 21		94.2±2.9			c	d	e	f	g	h	i	
Kaiserslautern 15		95.1±3.4			c	d	e	f	g	h	i	
Stadsteinach 8		95.9±2.9			c	d	e	f	g	h	i	j
Münnerstadt 1	75.0±8.8	97.7±4.1				d	e	f	g	h	i	j
Daun 18	88.5±7.9	97.9±2.9				d	e	f	g	h	i	j
Darrington, Wa 13		98.7±3.2					e	f	g	h	i	j
Freising 7	68.0±6.2	99.2±3.4					e	f	g	h	i	j
Griesbach 6	105.2±3.4	101.1±3.3						f	g	h	i	j
Darrington, Wa 10		101.8±3.3						f	g	h	i	j
Darrington, Wa 12		101.8±2.5						f	g	h	i	j
Oldendorf (SPI) 22		102.9±3.4							g	h	i	j
Schweinfurt 3	98.8±10.4	103.0±3.2							g	h	i	j
Segeberg 26		103.4±2.4								h	i	j
Münden 23		104.7±2.5									i	j
Darrington, Wa 11	78.5±7.7	106.9±2.9										j

Note: S.E. – standard error; different letters unit homogenous groups of provenances without significant difference between total height by tested provenances according to Duncan's multiple range test at the 0.05 significant level.

other provenances occupied a middle position.

The average height increment of the 12 surviving provenances in the test site in Borima was 78.1 cm. This value was significantly lower than that of the same provenances in Berkovitsa. Impressively high height increment was observed in provenance 6 Griesbach (Bavaria). The provenances 16 Osburg (Rhineland Palatinate), 3 Schweinfurt (Bavaria) and 18 Daun (Rhineland Palatinate) also showed very good height increment.

The high survival of Douglas-fir in Berkovitsa (94.4 %) was a sign of the very good conditions in this experimental trial. In some plants yellowing of the needles was observed, which could be explained by aggravated deteriorating microenvironment (waterlogging). Early formation of cones were found by some individuals, which indicates a weakening of the physiological condition.

In some plants, damages by game were observed – bites on the upper parts of the shoot, broken shoots and debarking. Affected plants were located most often near the bordering of the trial plantations.

Conclusions

The provenances from Germany and from the State of Washington (USA) had negligible losses and good growth at afforestation in Bulgaria.

Seeds from the stands of “gray” Douglas-fir, as well as seeds from the stand Schnaittenbach (Bavaria) should not be used. Whether this is the case with the stands of Oldendorf (Lower Saxony) and Schneifel (Rhineland Palatinate), should be checked genetically, because these provenances have a poor growth.

The great adaptability of Douglas-fir in different habitats, and its successful moving with 10° latitude on south of the original location were confirmed with negligible losses and good growth of the provenance test “Berkovitsa”. The larger losses and poor growth of the provenance test “Borima” are primarily due to inadequate care and lack of fence.

In the future, also in the context of climate change, the provenances of “green” Douglas-fir from the USA, as well as the genetically tested German stands, will be the only suitable ones. For the conditions in Bulgaria, regardless of somewhat poorer growth, they could rely on the seeds from older plantations in the country.

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APPLYING CHILL DAYS MODEL IN PHENOLOGICAL OBSERVATIONS OF *FAGUS SYLVATICA* L. IN NATURAL FORESTS

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Abstract

Dormancy is a period when temperate species are not active. Their physiological processes are delayed until the appearance of stimuli interrupt the dormancy. This period is commonly separated in two stages. The first stage is a rest period, when the buds remain dormant due to growth-arresting physiological conditions and the second is a quiescent period when buds are waiting favourable environmental conditions to burst. The Chill Days Model is a sequential model that uses the accumulation of chill days to break the rest and accumulation of anti-chill days to overcome quiescence. Predicting the beginning of the growing season and the duration of cooling is important for the analysis of forest ecosystems. The Chill Days Model gives a good prediction of the bud burst. The site-specific temperature threshold for *Fagus sylvatica* L. with this model was established as 7.3 °C and chill requirements were defined as 124 chill days in Balkan Range.

Key words: bud burst models, dormancy, phenology.

Introduction

Common beech is widespread deciduous species in Europe. Spring phenologycal phases are the subject of many discussions and research (Donnely et al. 2006, Bertin 2008, Vitasse et al. 2009). There are many models for predicting the budburst of different species. Some of them use chilling units or chilling hours (Utah, North Carolina, Low chilling), other – the temperature above 5 °C for accumulation (Slavov and Kazandjiev 2006). The first group weighted the temperature for the effectiveness of breaking dormancy with different scales. The last one uses different temperature sums for predicting the phase of phenologycal development. All of these models do not give good or proper results.

The temperature and light are of the most popular factors which are studied for the impact on the phenology of trees (Donnely et al. 2006, Bertin 2008, Caffarra and Donnelly 2011, Menzel et al. 2011). There have been various attempts to predict the beginning of growing season mostly in this connection (Falusi and Calamassi 1990, Schaber and Badeck 2003, Jeong et al. 2013). The model that has been applied in this research gives very good results on fruit and deciduous trees in Italy: Kiwifruit (*Actinidia deliciosa* C.F. Liang & A.R. Ferguson), Wild cherry (*Prunus avium* L.), Common pear (*Pyrus* *communis* L.), Evergreen oak (*Quercus ilex* L.), Common aspen (*Populus tremula* L.) (Cesaraccio et al. 2004, Linkosalo et al. 2006, Kamali et al. 2007).

Chill Days Model is based on the idea that chilling accumulates to break rest and heating accumulates to overcome quiescence (Cesaraccio et al. 2004). The model uses degree day calculations to determine chill days (units for chilling) and anti-chill days (units for heating). The model was tested in Balkan Range on Common beech and site-specific temperature threshold and chill requirements were established.

Chill Day Model is described for predicting bud-burst based on accumulation of negative chill days during rest until reaching a chilling requirement and then accumulation of positive anti-chill days during quiescence until reaching zero, which corresponds to release of dormancy. Cooling factors range from 0 when the temperatures do not help to meet their cooling requirements to 1.0 when the temperature is most effective to meet the requirements.

Fig. 1. The sample plot.

Threshold temperatures are important because some temperatures contribute to the cooling requirements while others do not. High temperatures do not contribute to meet the cooling requirements and in most cases, below a lower temperature threshold (0 °C) are not considered effective for cooling.

Site Description and Plant Material

Chill Days Model (C_D) was applied to phenologycal observations made on *Fagus sylvatica* L. in Balkan Range, near Vitinya pass. The stand is dominated by Common beech, aged between 120 and 140 years, at an altitude of 950 m west, at 23°55´48" latitude and 42°55´39" longitude (Kolarov et al. 2002). The soil is Distric-Eutric Cambisols, (Fig. 1) (Kolarov et al. 2002, ICP-Forest Manual 2000).

The climate is one of the most important factors for phenology. Figure 2 shows that maximum and minimum temperatures are at the same range among the years at the sample plot. The minimum temperatures in April started exceeding 0 °C and maximum temperatures are round 13 °C at the same month. This is the time of buds burst and the time which is marked in the model. There is a pick in minimum temperatures in October which is observed every year. This is the time of the end of autumn colouration of leaves which is the part of calculations for the beginning of next vegetation season. In the model there are introduced minimum and

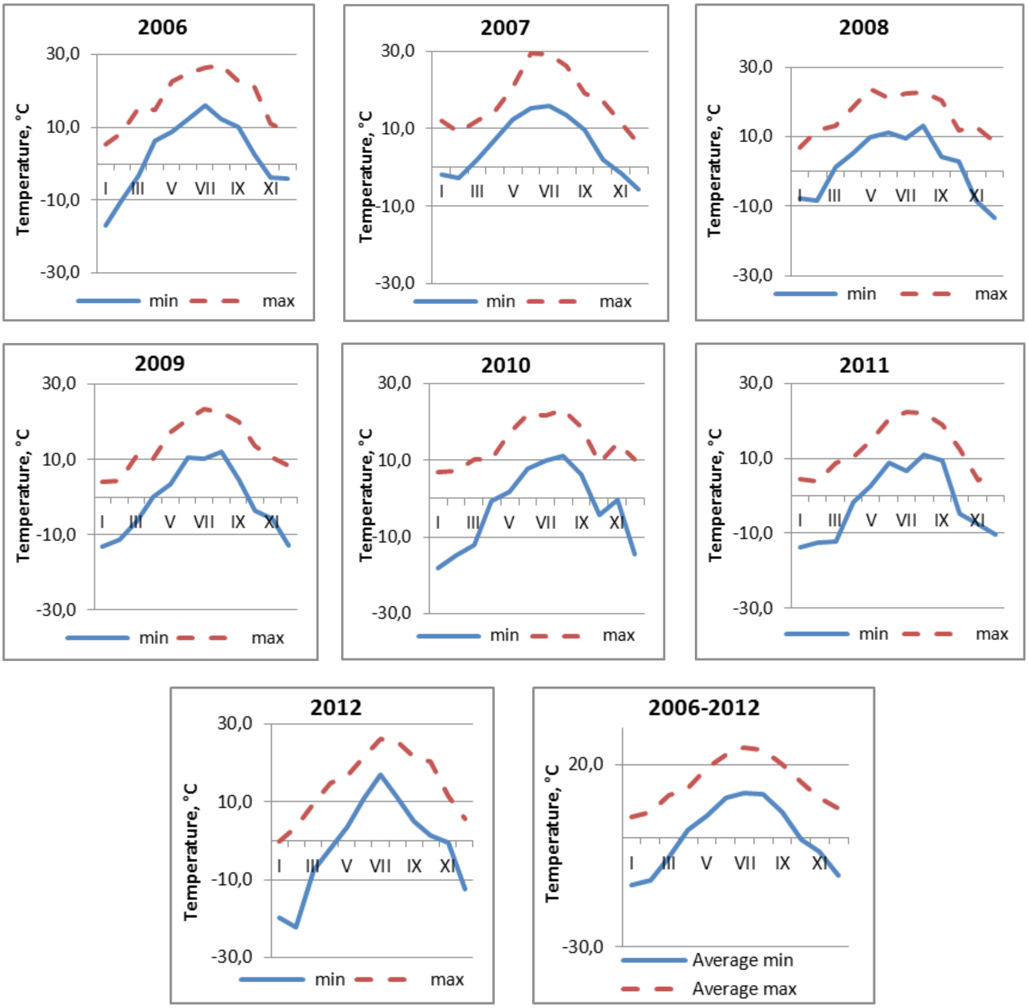

Fig. 2. Maximum and minimum air temperature for the period 2006–2012.
Note: Abscissae of all graphics are inflicted months of the years.

maximum daily temperatures for the period. The plot is situated in temperate forest vegetation zone (Georgiev 1991).

Methods

Chill days (C_D) Model is a sequential model that uses the accumulation of chill days (C_d) to break the rest and accumulation of anti-chill days (C_a) to overcoming quiescence. Negative C_d values are accumulated until they reach a pre-selected value that is identified as the chilling requirement (C_R). The chilling requirement is met on the day when $\sum C_d \leq C_{R,}$ which corresponds to breaking rest. On the following day, the model begins to add anti-chill days on each day starting at C_R, until $C_R + \sum C_a \geq 0$ at the predicted bud-burst. The chill days and anti-chill days both depend on the selection of a temperature threshold (T_C) and C_R, so these parameters are iterated to find the combination that best predicts the bud-burst dates. Chill days and anti-chill days are calculated from daily maximum (T_x) and minimum (T_n) temperature data and T_C. For calculating C_d and C_a, there are five possible cases that depend on the relationship between T_x and T_n relative to T_C and 0 °C (Table 1 according to Cesaraccio et al. 2004).

The accuracy of bud-burst estimation was evaluated by comparing observed and predicted days of chilling.

Results and Discussion

Start, end and duration of cooling periods during the reference period were calculated by using the model of Chill Days (Cesaraccio et al. 2004). The values are recorded in DOY (day of year). They are based on the entered values of temperature in the model. There were no observations during 2010 and that is shown on Figure 3 as a line.

Table 1. Chill days (C_d) and anti-chill days (C_a) equations for the five cases that relate the maximum (T_x) and minimum (T_n) temperature to the threshold temperatures T_c and 0 °C.

Number	Temperature cases	Chill Days	Anti-chill days
1	$0 \leq T_C \leq T_n \leq T_x$	$C_d = 0$	$C_a = T_M - T_C$
2	$0 \leq T_n \leq T_C < T_x$	$C_d = -\left[(T_M - T_n) - \left(\frac{T_x - T_C}{2}\right)\right]\left[(T_M - T_n) - \left(\frac{T_x - T_C}{2}\right)\right]$	$C_a = \frac{T_x - T_C T_x - T_C}{2 \quad 2}$
3	$0 \leq T_n \leq T_x \leq T_C$	$C_d = -(T_M - T_n)$	$C_a = 0$
4	$T_n < 0 < T_x \leq T_C$	$C_d = -\left(\frac{T_x}{T_x - T_n}\right)\left(\frac{T_x}{2}\right)\left(\frac{T_x}{T_x - T_n}\right)\left(\frac{T_x}{2}\right)$	$C_a = 0$
5	$T_n < 0 < T_C < T_x$	$C_d = -\left[\left(\frac{T_x}{T_x - T_n}\right)\left(\frac{T_x}{2}\right) - \left(\frac{T_x - T_C}{2}\right)\right]\left[\left(\frac{T_x}{T_x - T_n}\right)\left(\frac{T_x}{2}\right) - \left(\frac{T_x - T_C}{2}\right)\right]$	$C_a = \frac{T_x - T_C T_x - T_C}{2 \quad 2}$

Note: T_M is the mean daily temperature.

In order for the model to calculate the values for chilling requirements and temperature threshold, it is necessary to set start and end dates of the growing season. The starting date should be considered when 50 % of the organs are at observed stage. The selected dates correspond to full phenologycal stage. The observations are made by one and the same observer, once a week.

Figure 3 reports the dates of onset of cooling. The cooling begins with the completion of the growing season during the year and continues until the swelling of the buds begins, which marks the start of the next growing season. The period between two dates shows the time of buds' cooling in that particular year. These are the values of the graph, which have a negative meaning and almost in all years, reach

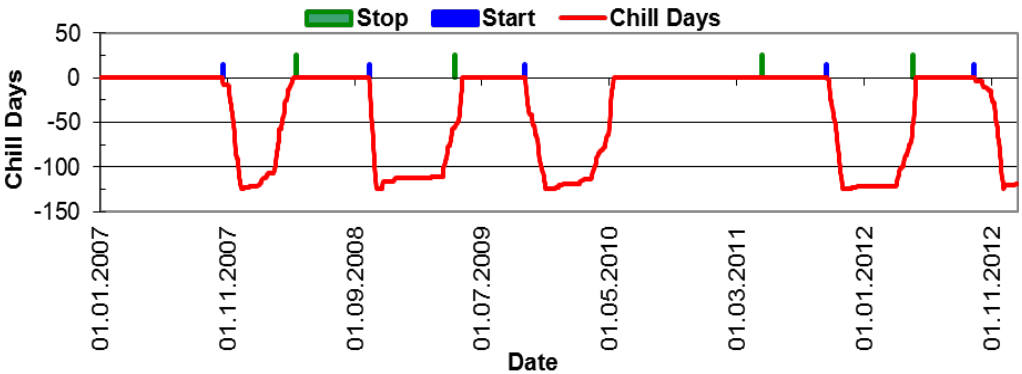

Fig. 3. Start and end dates of growing season.

Note: There were no observations in 2010.

up to 124. So are the days reported in the model for cooling as Chill Days. On Figure 3 is given the visible length of the days of cooling. The most extended period of detention of these values is in the range 21.10.2008 to 17.03.2009 and 9.11.2011 to 17.03.2012. The first date shows reaching cooling requirements, and the second one – beginning of accumulation of stimulating temperatures.

According to the applied model for Common beech were obtained 124 days required for cooling and 7.3 °C threshold temperature in Vitinya. It is possible to understand the difference between beginnings of the vegetation period in different altitudes, and different ages for the distinct species. With more analysis (of the researched species), it is possible to find the important meanings of the varied exposure at the same altitudes and diverse dates of beginning of the vegetation season.

The accuracy of the Model is evaluated with root mean square error between predicted and observed dates (RMSE = 12, where: RMSE is the smallest set mean square error between the observed and predicted values).

Chill Days Model predicted pretty good the days of leaf fall to the beginning of the leafing (Fig. 3). Cooling and warming of buds are the important periods of phenologycal development of beech trees. They are one of the most important external signals that determine the rate and intensity of bud burst.

The cooling factor as a function of the temperature could be developed and used to evaluate the effectiveness of cooling and the temperature at the exit from the dormancy.

Conclusions

The good performance of the Model may attribute to the fact, that it approximates the rest and quiescent of the rest by accounting the temperatures according to the phenologycal date, not according to the weather changes.

Chill Days Model can be tested on Common beech at different places and altitude to find the requirements of observed species, especially in cases with difficulties in establishment of the key factor of bud-burst. It will improve the accuracy of the model.

The advantage of this model is that beginning of foliage definition is necessary; it is bound by the previous growing season, which shows the plasticity of the species to changes in abiotic factors. This binds the beginning and end of the vegetation to the changes in temperature during the autumn, winter and spring.

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PRELIMINARY TREATMENT OF MINING WASTE FOR THE PURPOSES OF ITS FUTURE UTILIZATION

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Abstract

The extraction of minerals in an open way inevitably leads to generation of large amounts of mining waste for storage and disposal. The last require vast areas. And although the present priority continues to be prevention of already generated waste it is important to try to cease pollution by achieving the requirement of getting to the process of ’end of waste‘. Typically, this conversion of waste into materials is matched with the process stage of pre-treatment ensuring reliable recovery. Mining waste contains valuable and often searched substances that can be used as an alternative to natural materials.

Key words: classification of mining waste, clay minerals, pre-treatment of mining waste, separation of clay materials.

Introduction

European Union has a very ambitious policy and legislation for waste management by seeking to change the traditional thinking of waste as an unwanted burden in perspective thinking about waste as a raw material and energy resources, especially in the last five years. The long-term aim of EU is to become a ’recycling society‘, which seek to improve efficiency of resource use to reduce the overall use of non-renewable natural resources, respectire environment (Anonimous 2004, 2006).

Methods for treatment of mining waste from different minerals aiming production of one or several products are known (Fall et al. 2005, Kyoseva et al. 2009, Li et al. 2006, Li at al. 2010, Pelovski et al. 2003, Yossifova 2007). These methods correspond to different degrees of Reference Document of Best Available Techniques for Management of Tailings and Waste Rock in Mining Activities. To achieve compliance with EU objectives for recovery of mining waste (Anonimous 2004, 2011), it is necessary to investigate the properties and possibilities for utilization of coal mining wastes.

According to the National Statistical Institute, of the total hazardous wastes, generated in 2011 in Bulgaria, 92.7 % are generated by mining industry. The amounts of hazardous waste are 174,692,154 t, of which less than 0.1 % are delivered for recovery. The remaining part is a subject of disposal operations on the territory of enterprises, which generated the waste (Anonimous 2012).

In our previous studies are discussed the chemical properties of mine waste generated in some of the fields of Pernik Province. These studies are related to classification of mining waste and methods of its utilization. The current article is a continuation of this development in the utilization (Kostadinova and Todorova 2010, 2014).

Mining waste mining in an open way, for the most part contains clay material that this rate of production in foreseeable future will prove demand, insufficient material. Conditional provision with kaolin raw material in the country, based on objects in operation is 20 years in these rates of production and not more than 80 years on the basis of all the stocks in the country (Anonimous 2012).

In order to replace more natural materials with waste, method for separation of water aiming to get a second material – clay minerals and rocks, were developed.

The aim of this paper is to develop a process for separation of different minerals from mining waste. To achieve the objective the following has been done: sampling from the same field in different time periods of mine operation; mineralogical analysis of samples; waste classification; developing a methodology for water separation and quantification of separated fractions.

Table 1. Distribution of samples by year of formation.

Sample	Year of formation
Sample 1	2012
Sample 2	2010
Sample 3	1990
Sample 4	2000
Sample 5	1965

Materials and Methods

Object of analysis and characterization of the waste is given in Table 1. The purpose of sampling is to identify changes in the qualitative composition of geological substrates in different periods of time.

Samples were taken by criteria 'during formation', regardless of how coal mining was performed.

The method of water separation has been developed with mining waste taken from Mini Otkrit Vagledobiv JCS, section Teva (Fig. 1), which previous studies have shown that contain clay minerals (Fig. 2).

Fig. 1. Location of sampling points in Mini Otkrit Vagledobiv JCS, section Teva.

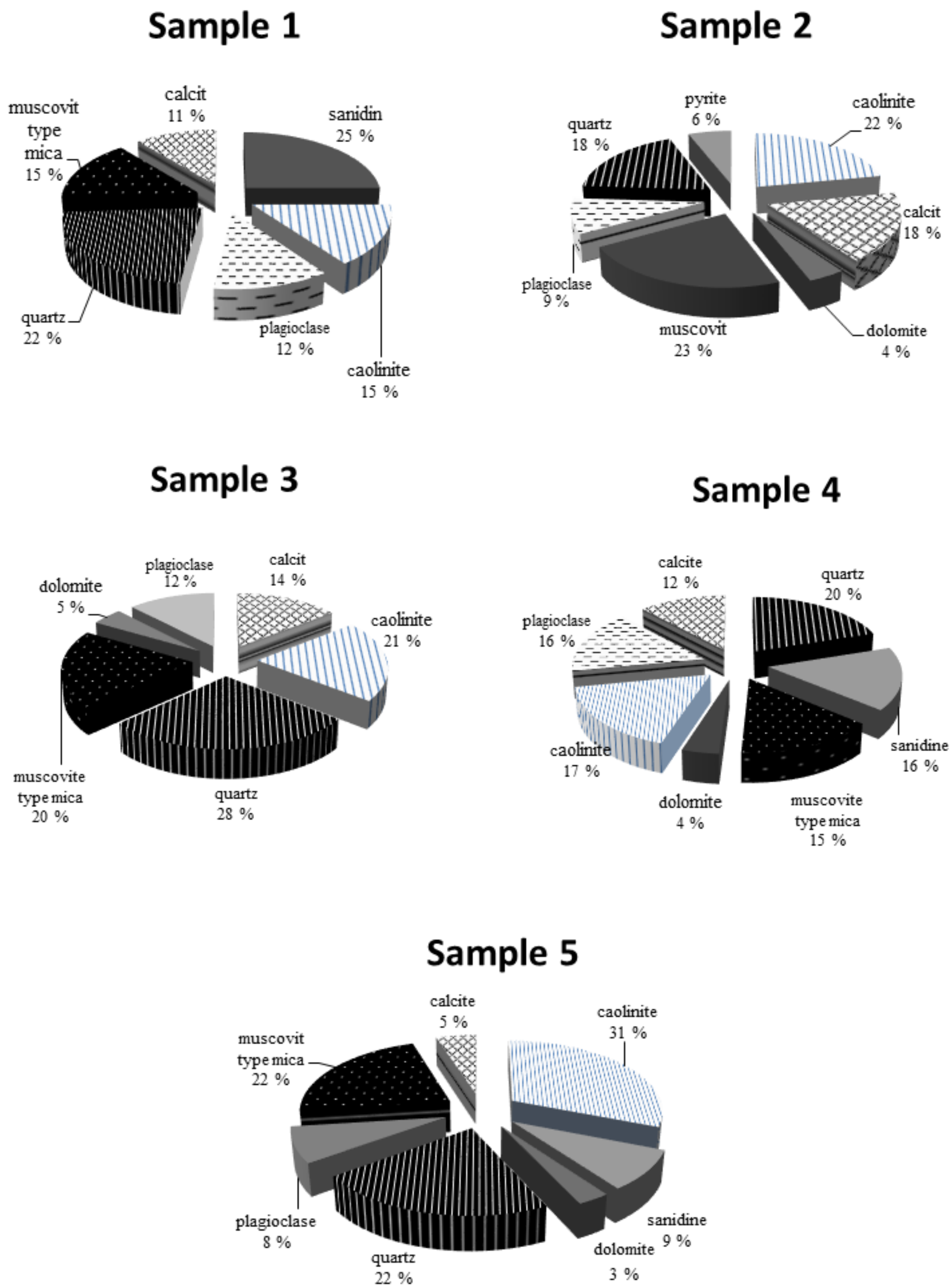

Fig. 2. Content of minerals in the samples.

When one talks about waste utilization and reaching 'end of waste', it is important to know the content of harmful, toxic and other elements and substances which would give them hazardous properties. In order to determine the waste properties characterization and classification was made by analyzing five samples generated during different periods of mine operation.

The waste is classified based on legal documents:

• Regulation for specific requirements for management of mining waste (Anonimous 2009);

• Regulation 2/2014 on waste classification (Anonimous 2014b).

The mining waste generated by Mini Otkrit Vagledobiv JCS, section Teva is classified according to legal documents as: 01 01 02 – wastes from exploration and extraction of non-metallic minerals.

The waste complies with the requirements of the National Regulation for specific requirements for management of mining waste category 'inert waste' and though it has been shown that in the analyzed samples there are coal impurities, which are flammable waste thus allowing its classification as non-hazardous non-inert waste (Anonimous 2009, 2013, 2014a, 2014b).

Methods of water separation

A method has been developed with the main aim of separation of clay minerals and rock mass in mining waste using the characteristics of the clay to absorb water and this way to achieve destruction of the particles resulting from the conjugation/adhesion of clay minerals in dry condition (Fig. 3), and to investigate the possibility of their separation (Anonimous 1987, Kovachev et al. 1991).

Necessary equipment for testing the methodology for water separation consists of:

- electronic scale, with 1 g accuracy;
- sieve in size 0.045 mm;
- measuring cylinder with accuracy 2 cm;
- drying oven with the temperature range 60–80 °C;
- suitable container of 10 l;
- hose for decanting.

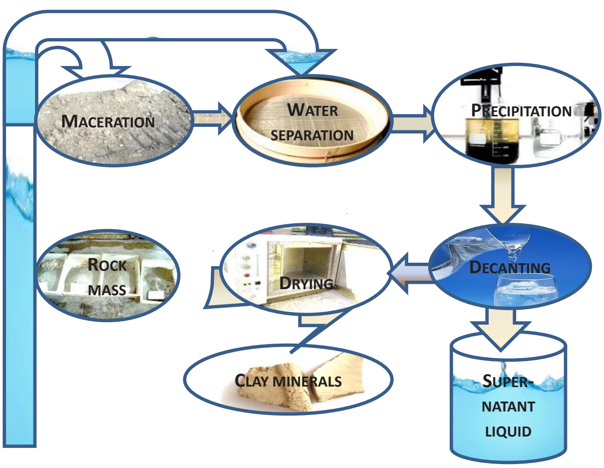

Fig. 3. Methodology for water separation.

Approximately 150–200 g of geological substrates was used depending on the type of sample and the content of clay minerals in it. The sample is placed into a suitable container and 1.5 l of water was added on top of it. Incubation for about 1–2 h followed with frequent stirring. This assures breaking of the already formed coarse particle clays.

The resulting mixture after water addition is transferred

Table 2. Results from the developed methodology for water separation.

Sample	Sample weight, g	Water consumption for separation, l	Quantity decanted water, l	Quantity over sieved fraction, after drying, g	Quantity under sieved fraction after drying, g
Sample 1	200	12.0	10.8	28	168
Sample 2	202	5.5	4.7	187	11
Sample 3	200	9.5	9.1	28	167
Sample 4	158	8.0	7.1	40	114
Average	190	8.75	7.92	70.75	115

to a sieve (0.045 mm) for the process of separation and water is added gradually. The process continues as long as clear water starts passing. The final separation is reported based on the color of the effluent on a white background. The amount of liquid used depends on the content of clay minerals in the sample and varies widely (5 to 15 l sample of 200 g). Liquid passed through the sieve, together with the clay minerals, is collected in a suitable container and allowed to precipitate within 12–24 h. After it, the supernatant liquid is decanted, and thus about 80–90 % of the input to flush water is recovered and is available for re-use in the process of separation.

Thus the divided fractions – above and below 0.045 mm after decanting are carried in a suitable container, placed in a drying oven to complete drying

Results

In that methodology are processed four composite laboratory samples of mining waste from open pit mining. The test results are presented in Table 2.

In accordance with The Opinion of the European Economic and Social Committee on ’Processing and exploitation, for economic and environmental objectives of waste industrial and mining waste in the European Union‘ to avoid disposal of mining waste, developed methodology trying to turn mining waste into raw material is applicable in various economic fields.

The results show significant abnormalities due to inhomogeneity of the samples. To implement the experiments laboratory samples taken were not subjected to a pretreatment to modify the particles’ size.

For separation finely dust particles and clay particles in samples of mine waste with total weight of 760 g, 35 l of water were used, which gives an amoun
t of approximately 42 l/kg. Suitable for reused liquid in the process of water separation is 31.7 l, which is 90 % of the original quantity.

Conclusion

From the experiments on the proposed methodology following conclusions could be drawn:

1. Wastes generated in Mini Pernik, in different periods of its activity, have similar mineral content regardless the method of mining. They are classified as waste from exploration and extraction of non-metallic minerals, which is non-hazardous non-inert.

2. Mineralogical analysis suggests the presence of pyrite in only one of the tested samples. Due to the fact that mining waste from coal industry is expected content of coal impurities, it is an advantage for their future utilization, for example for production of bricks.

3. A methodology for waste utilization has been developed on the basis of water separation to clay minerals. It was found that:

- clay minerals in mining waste from Mini Otkrit Vagledobiv JCS, section Teva reach 31 % with an average content of 21 %;
- the quantity of clay minerals and particulate matter is diverse and ranges from 5.4 % to 84 % by weight of individual samples in water separation;
- the under sieved fraction can be utilized as raw material for production of bricks and tiles;
- the over sieved fraction is suitable for back backfill underground workings, concrete additives, etc.;
- for separation of fine dust particles and clay particles in samples of mine waste with total weight of 760 g, 35 l of water are used, which gives an amount of approximately 42 l/kg;
- liquid suitable for reusing in the process of water separation is 31.7 l – 90 % of the original quantity.

Pre-treatment of mining waste in the developed methodology allows utilization of clay as an alternative to natural resources and utilization of rock mass as an alternative to crushed stone as raw materials.

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ENZYME ACTIVITY OF FOREST FIRE-INFLUENCED SOILS FROM NORTH SLOPES OF RILA MOUNTAIN (REGION OF DOLNA BANIA)

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Abstract

Enzyme activity of forest soils affected by forest fire under conifers (Pinus sylvestris L., Larix desidua Mill.) and mixed (Pinus sylvestris L., Quercus cerris L.) forests from north slopes of Rila Mountain (region of Dolna Bania) have been analysed in samples, collected 7 days after wildfire occurrence. Results on catalase, cellulase and protease enzyme activities in fire-affected soils show a considerable increase in comparison with the same enzyme activities of the control (fire-unaffected) site. The activities of these three enzymes can be used as indicators of the on-going biological processes in post fire-affected soils. The values of catalase and cellulase were lower in the forest litter, and higher in the subsequent layers of soil. Protease activity increases in depth in three of analyzed fire-influenced soils. In the control soil the activity of this enzyme remains constant in depth of the soil profile. Higher pH values and lower soil humidity were established in fire-affected soils compared to the control soil. A higher content of organic carbon and nitrogen in the soil of surface fire as compared to those with crown fire and control soil was established.

Key words: fire-influenced forest soils, catalase activity, cellulase activity, protease activity.

Introduction

The fires are a major cause of forests degradation. They cause transformation of habitats, which affects their natural biological functions. The impact of fire on soil microorganisms is direct through heating and/or indirect by modifying the properties of the soil.

The most important factor that determines the number, composition and activity of microorganisms in soil after a fire is the severity of burning, which is determined by its intensity and duration. The adverse effects of fire on the soil depend on its intensity and the properties of impacted soil. The combustion of organic matter leads to an increase in the content of mineral compounds in the soil. According to the studies of Bogdanov (2008) magnesium and calcium are the elements, which amounts are changed most significantly. The content of the total nitrogen is an important indicator of forest recovery processes after fire. Changes are found in the particle size distribution of fire-influenced soils, which are mainly in the sand/silt ratio. The clay fraction is changed to a relatively less extent (Bogdanov 2012). The changes which occur in composition, physico-chemical properties and structure of the soil after fire usually lead to a decrease in the number of specific microbial groups (Wells et al. 1979). The activity of soil microbiota also decreases due the changes in content and composition of soil organic matter. In a short term, the number of heterotrophic bacteria and their activity increases, mainly due to an increase in dissolved carbon content and availability of nutrients in the fire-influenced soils. After depletion of easily mineralized organic compounds, the initial increase of microbes count is reduced and the remaining carbon and nitrogen forms become more resistant to microbial degradation (Mataix-Solera 2009).

Studies of Hernández et al. (1997) show that the activity of the enzymes dehydrogenase, urease, protease, arylsulfatase, phosphatase, β-glucosidase are inhibited in soils for 9 months after the fire is extinguished. In such soils they established a high content of nitrate, ammonium ions, digestible forms of phosphorus and potassium, whereas the organic carbon content was lower than that in unaffected soils. A reduction of nitrogen mineralization and nitrification for the first year after the fire and increase during the third year in fire-influenced soils compared to the control were registered by Miesel et al. (2011). They documented a consistent reduction in the activities of acidic phosphatase, chitinase and phenoloxidase. The reduction of chitinase activity reveals a decrease in the bacterial abundance, reduction of the importance of chitin as a source of C and N or an increase in the availability of more labile forms of organic matter (Hanzlikova and Jandera 1993). The reduction in enzyme activities after a fire can be a consequence of a high intensive fire (Fioretto et al. 2005), increase in soil pH (Acosta-Martínez and Tabatabai 2000) and reduction of soil microbial biomass (Kandeler and Eder 1993). Reduction of nitrogen mineralization in soils after fire is also noted by other authors (Monleon et al. 1997, DeLuca and Zouhar 2000, Durán et al. 2009). Phosphatases (acidic and alkaline) can clearly demonstrate the extent of fire impact on the soil, even one year after it (Pourezza et al. 2014). Staddon et al. (1998) found that the acidic phosphatase activity remains suppressed four years after the fire. The above mentioned and other studies demonstrate that soil biochemical properties, and particularly soil enzymatic activity are sensitive to stress on ecosystems and have the potential to serve as an indicator of the sustainability of managed ecosystems (Bergstrom et al. 1998, Dick 1994). Thus, the activity of soil enzymes can be used as a bio-indicator of ongoing biological processes in fire-influenced soils and the recovery process occurring in them.

The aim of this study was to investigate the soil protease (actual), cellulase and catalase (potential) activities in order to identify the most powerful enzyme activity as an indicator of ongoing biological processes in burned soils.

Materials and Methods

Representative sampling sites (RSS) have been chosen within the coniferous forests on the territory of Dolna Bania Government Forestry Enterprises (GFE). They were situated on the north slopes of Rila Mountain, affected by a forest fire in August 2012. The soil samples were collected 7 days after wildfire occurrence in tree replications at each RSS and at both depths – 0–5 and 5–20 cm. The altitude of the studied area is 650 m a.s.l. The plantations studied are twenty-five years old. Their main characteristics are presented in Table 1.

The soil from the studied region is Haplic Luvisols, LVh (WRBSR 2006). The control sampling site was established at a 20 m distance from the fire-affected ones. The samples were taken with a sterile instrument from 0–5 cm and 5–20 cm depths of the soil profile, as well as forest litter from fire-affected sampling sites, in sterile paper bags. Four more soil samples from the surface mineral soil have been collected randomly at each RSS in order to ensure homogenous and representative samples for analyses. Sampling and sample preparation processing have been performed following the contemporary methodological approach, described in details in ICP Manual (Cools and De Vos 2010). The samples were transported and analyzed within 48 h. Prior the incubation, they were stored in a refrigerator at 4–10 °C.

The enzymatic activities of fire-affected soils were studied through measuring the activity of three enzymes – cellulase, catalase (potential activity) and protease (actual activity).

The catalase activity was determined in triplicate by manganese-metric method (Khaziev 1976).

To determine the cellulose-degradation activity the laboratory method of Khaziev (1976) was used. The three sterilised filter paper strips were applied to the soil samples in Petri dishes. The incubation was performed at 60 % humidity of soil. Every 15th day the percentage of the degraded paper area was accounted.

The actual protease activity was investigated through setting-up of three

Table 1. Studied areas parameters.

RSS, No	Studied cases	Dominant tree species	Area, ha	Geographical positions, latitude and longitude
1	Crown fire	*Pinus sylvestris* L.	0.2	42°19’23.78” N 23°46’37.09” E
2	Crown fire	*Larix desidua* Mill.	9.3	42°19’23.77” N 23°46’37.10” E
3	Surface fire	*Pinus sylvestris* L.	4.4	42°19’23.75” N 23°46’37.11” E
4	Surface fire	*Pinus sylvestris* L. *Quercus cerris* L.	0.9	42°19’23.58” N 23°46’36.89” E
5	Unburned	*Pinus sylvestris* L.	0.6	42°19’25.25” N 23°46’36.40” E

Table 2. Chemical characteristics of studied soils.

RSS, No	Soil depth, cm/forest litter	Soil moisture content* (W, %) Mean	pH (H_2O) Mean ± SD	C, % Mean ± SD	N, % Mean ± SD
1	FL	4.8	7.44±0.22	41.55±2.15	0.4485±0.10
	0–5	8.7	6.06±0.13	1.656±0.25	0.0417±0.02
	5–20	10.7	5.53±0.11	1.380±0.19	0.0313±0.01
2	FL	4.1	8.01±0.16	30.60±2.17	0.3691±0.08
	0–5	8.4	6.42±0.10	1.932±0.19	0.0849±0.02
	5–20	8.5	6.12±0.08	1.242±0.08	0.0700±0.02
3	FL	3.8	7.28±0.20	54.45±3.14	0.6578±0.11
	0–5	8.6	5.81±0.09	2.346±0.21	0.0954±0.03
	5–20	7.7	5.40±0.08	1.794±0.09	0.0819±0.02
4	FL	7.1	6.80±0.10	40.65±3.55	0.2915±0.06
	0–5	9.3	6.12±0.11	3.450±0.13	0.1298±0.04
	5–20	9.3	5.85±0.07	2.208±0.11	0.0981±0.02
5	FL–L	6.14	5.06±0.08	59.70±3.45	1.5647±0.06
	FL–F	7.67	5.08±0.06	45.90±2.21	0.6877±0.03
	FL–H	9.4	5.37±0.07	22.20±2.03	0.1495±0.03
	0–5	16.1	5.47±0.10	2.070±0.11	0.0932±0.02
	5–20	23.13	5.26±0.11	1.104±0.06	0.0521± 0.02

undeveloped film reel strips with size 5×3.5 cm in each soil layer from RSS and subsequent extraction and recognition the percent of decomposed area (30 days after the setting) (Khaziev 1976). Main soil characteristics are given in Table 2.

Statistical data processing includes calculation of the mean values of triplicated analyses for each parameter and standard deviation (± SD), bivariate correlation (Pearson) to analyse correlation among soil enzyme activities and soil chemical properties. Statistica 7 program for Windows and Excel 2010 were used for calculations.

Results and Discussion

Soil physicochemical properties differed between fire affected and control RSS (Table 2). Soil moisture content (W, %) was lower in forest litter layer from fire affected variants. Compared to the control sites, pH after forest fire was increased in all experimental variants especially in that, affected by crown fire. Soil organic carbon content was lower in soil affected by crown fire at 0–5 cm, compared to the unburned control, while the surface fire, provoked an increase in soil organic carbon amount.

Changes in enzyme activities between fire affected and unburned variants are

Table 3. Catalase activity of soil microorganisms.

RSS, No	Variants	Forest litter layer (*FL*)/Soil depth, cm	Catalase, mL O_2·30 min^{-1} Mean ± SD
1	Crown fire	Forest litter	0.47±0.06
		0–5	1.85±0.09
		5–20	1.87±0.06
2	Crown fire	Forest litter	1.10±0.10
		0–5	1.87±0.06
		5–20	1.97±0.06
3	Surface fire	Forest litter	0.73±0.03
		0–5	1.73±0.06
		5–20	1.62±0.06
4	Surface fire	Forest litter	1.33±0.03
		0–5	1.73±0.08
		5–20	1.63±0.06
5	Control sampling site	*L* – layer of forest litter (*FL – L*)	1.63±0.08
		F – fermentative layer of forest litter (*FL – F*)	0.63±0.03
		H – humified layer of forest litter (*FL – H*)	0.98±0.03
		0–5	1.65±0.05
		5–20	1.82±0.03

presented in Table 3, 4 and Fig. 1. The correlation matrices of the physicochemical indexes of forest litter/soil and catalase and protease enzymes activities is presented in Table 5. The activity of cellulase was assayed in relation to time course changes.

Catalase enzyme activity plays an important role in soil solution chemistry and can change oxidation-reduction reactions in soil (Wang et al. 2012). Results show that low catalase activity was observed in the litter of the fire-influenced coniferous forest (RSS 1, 2 and 3) and in *F* and *H* layers of the control site (Table 3). The activity of litter enzyme was higher for mixed forests (RSS4) and lowest for that of the Scots pine (RSS1). For control site forest litter the catalase activity had its highest value in *L* layer, followed by that in humified *H* layer and was lowest in the fermentation *F* layer.

The metabolism of microbes, including synthesis of enzymes is activated mainly by external factors, one of which is the forest fire. Higher catalase activities with approximately equal values were found in the

Table 4. Protease activity of soil microorganisms.

RSS, No	Soil depth, cm	Protease, % degraded area 30th day Mean ± SD
1	0–5	2.87±0.06
	5–20	1.42±0.03
2	0–5	8.57±0.08
	5–20	25.67±0.06
3	0–5	7.10±0.10
	5–20	42.85±0.09
4	0–5	8.57±0.06
	5–20	11.67±0.06
5	0–5	1.41±0.05
	5–20	1.44±0.05

fire-influenced soils compared to those in the control one. The catalase activity and physicochemical properties of forest litter varied considerably within studied variants. However, positive correlation between catalase activity and moisture content of the forest litter was found (Table 5). The lowest moisture in forest litter from fire affected sites is probably due to greater losses of water through transpiration from disturbed stands (Gömöryová et al. 2008). The enzyme activity of the mineral soil from RSS, affected by surface fire (No 3 and 4) and that in the soil from control sampling site (No 5) is lower in comparison with that influenced by crown fire. The established values for surface 0–5 cm soil layer of the RSS3, RSS4 and RSS5 were equal to 1.7 ml $O_2 \cdot 30\ min^{-1}$. The similar values 1.6 ml $O_2 \cdot 30\ min^{-1}$ were found for catalase activity in soil from 5–20 cm layer of RSS3 and RSS4. This result is consistent with those of Eivazi and Bayan (1996) who studied the effects of long-term prescribed burning on enzyme activities in a forest ecosystem and found that burning greatly reduced enzyme activities, mainly due to the destruction of forest floor vegetation.

The catalase activity in soil influenced by crown fire (RSS1 and RSS2) is higher 1.9–2.0. In both burned and unburned forests, the distribution trend of enzymes activities of spruce (*Picea balfouriana* var. *rubescens* Rehder & E.H.Wilson) forest was similar (Yong-Mei et al. 2005). The increased activity of catalase in the top 20 cm soil of the crown fire affected forests may be due to the changed forms of organic compounds caused by burning. In our study, the catalase activity showed rather strong positive correlation to soil pH, whereas it correlates negatively with soil moisture, organic matter and total nitrogen content (Table 5). Gömöryová (2004) found that catalase activity exhibits highly significant correlations ($P > 0.999$) with soil reaction. The negative correlation of catalase with the soil moisture

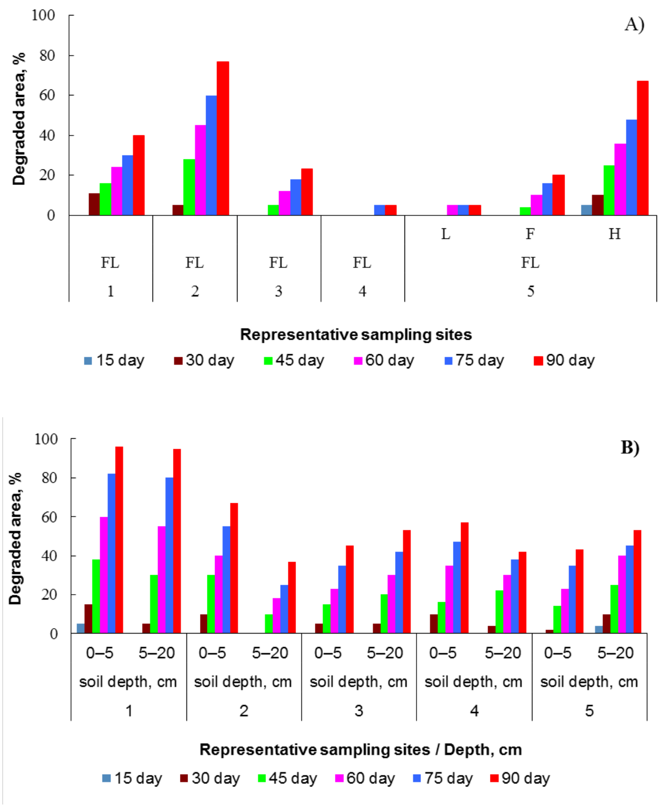

Fig. 1. Cellulase activity of soil microorganisms: A) in forest litter; B) in mineral soil layer 0–5 cm and 5–20 cm.

content (W, %) in 0–5 cm of mineral soil could be interpreted in terms of differences in soil oxidation status (Gömöryová et al. 2008). High significant negative correlations occurred between catalase and organic matter of soil. Because of increased soil enzyme activities in surface soil horizons, the transformation of organic matter was faster than that in deeper horizons and the rapid release of inorganic nutrients in soil during this process could be the reason for negative correlation.

The slight catalase increase in depth of the soil under European larch (RSS2), and in the 5–20 cm soil layer of the control site (Scots pine – RSS5) could be probably in result of its presence of vegetation origin. The negative correlation of catalase activity with organic matter and soil nitrogen content was kept within 20 cm soil depth.

Cellulase is an enzyme that breaks down cellulose. Through its activity in the soil, the direction and the rate of mineralization processes could be characterized. It was found that the activity of cellulase is increased after day 30

Table 5. Correlation coefficients (*R*-value) between enzyme activities and chemical properties of forest litter and mineral soil at 0–5 cm and 5–20 cm depth (significance at *P* < 0.05 level).

Variable	W, %	pH (H_2O)	C, %	N, %	Enzymes
Forest litter					
W, %	1.00	-0.82	0.34	0.71	0.66
pH		1.00	-0.78	-0.88	-0.3
C, %			1.00	0.82	-0.14
N, %				1.00	0.05
Catalase					1.00
Soil depth 0–5 cm					
W, %	1.00	-0.35	0.98	0.86	-0.89
pH		1.00	-0.14	-0.02	0.69*
C,%			1.00	0.92	-0.79*
N, %				1.00	-0.73*
Catalase					1.00
Soil depth 5–20 cm					
W, %	1.00	-0.53	-0.39	-0.16	0.02
pH		1.00	0.19	0.39	0.35
C, %			1.00	0.77	-0.82*
N, %				1.00	-0.64*
Catalase					1.00
Soil depth 0–5 cm					
W, %	1.00	-0.35	0.98	0.86	0.48
pH		1.00	-0.14	-0.02	0.33
C, %			1.00	0.92	0.62*
N, %				1.00	0.86*
Protease					1.00
Soil depth 5–20 cm					
W, %	1.00	-0.53	-0.39	-0.16	-0.50
pH		1.00	0.19	0.39	0.18
C, %			1.00	0.77	0.30
N, %				1.00	0.56
Protease					1.00

Note: *correlation is significant $p < 0.05$.

of fire occurrence in the soil from all fire-affected sites. In the forest litter of the control site, cellulase activity increases gradually from *L* through *F* and reaches maximum values in *H* layer, which is determined by higher mineralization rate in the humified layer (Fig. 1).

In the sites affected by the fire, the enzyme activity of forest litter is lower than that in the soil depths, except European larch (RSS2). In general, cellulose activity is lower in recently burned soils than in non-burned ones (Fioretto et al. 2002) due to the high heat sensitivity of fungi which are the main cellulose degradators in soil. Moreover, cellulose is one of the most heat-affected organic fractions in soil during forest fire occurrence (Fernández et al. 2001).

The highest cellulase activity was observed in both mineral soil and litter of RSS1 site at 90th day of observation, whereas the lowest one was recorded for the surface (0–5 cm) and deeper (5–20 cm) soil layers of the control (RSS5) and RSS3, respectively. All these are covered with Scots pine forest. An increased cellulase activity, especially in the surface soil layer, was observed in the soils affected by the fire in comparison to that of the control.

The crown fire affects more significantly the cellulase activity in comparison with a surface fire, probably, as a result of organic matter and ash accumulation after fire. Higher levels of cellulase activity were found in variants of crown fire in comparison to those in variants of surface fire. Wang et al. (2012) showed that the increase in organic matter accumulation improved cellulase activity, thus increasing the decomposition rate of cellulose in plant litter.

The cellulase activity decreases in depth of the fire-influenced soils (except RSS3) in a contrast to the control soil (RSS5), where the opposite trend is recorded. Cellulose activity in RSS5 is relatively lower as that in RSS3.

The protease activity of the soil microorganisms is much higher in the fire-influenced soils in comparison with that of the control soil (Table 4).

The proteolytic enzymes in soil catalyze the hydrolysis of proteins to peptides and amino acids, thus affecting soil N cycling. The protease activity of fire-affected surface soil layer (0–5 m) was suppressed in a comparison to the activity of the enzyme in the deeper soil layer (except RSS1). The highest protease activity in the 5–20 cm soil layer was observed in RSS3 site (Scots pine), and the lowest one – in RSS1 and RSS5.

In the surface soil layer, the highest protease activity was found in both – RSS2 (crown fire) with European larch and RSS4 (surface fire) with a mixed forest of Scots pine and Turkey oak. The lowest protease activity was observed in the soil of RSS1 (crown fire) and in the control soil (RSS5), both covered with Scots pine.

The reason for this trend may be indirect effect of fire (especially of the crown one) on the enzymes' activities from the surface soil layers, enhancing migration and accumulation of proteins (highly mobile) along soil profile to the deeper soil layers. Soil proteins are more mobile in soil than other carbon sources (Buyer et al. 2001).

In our opinion, there is a relation of the variation of enzyme activity to soil moisture, pH, organic carbon and nitrogen contents – the most important soil characteristics controlling survival and growth of many microbes (Table 5). In our study, catalase activity showed a strong positive correlation to soil pH, whereas the correlation with soil moisture, organic matter and nitrogen content were negative. Neutral-alkaline pH optima have been reported for soil proteases (Kamimura and Hayano 2000). In many studies, the strong dependence of catalase activity on organic C and pH was observed (Müllebner 1984, Gömöryová 2004, Shi et al. 2008). The increase in soil catalase activity affects soil solution chemistry trough changes of soil redox conditions. This change of soil solution has the potential to dissociate bonds within complexes of organic matter and therefore alter soil carbon storage. Hence, the high value of catalase activity in mineral soil indicated an accelerating transformation of organic matter.

High positive correlation of the protease enzyme activity with nitrogen and carbon content in the surface 5 cm of the mineral soil layer indicated the activation of soil microorganisms by addition organic materials in result of burning (Dick 1994, Nannipieri et al. 1983). Organic residues added to soil promote microbial and soil enzyme activities (Masciandaro et al. 1997).

The processes of soil organic matter decomposition/mineralization result in the release of NH_4^+ and CO_2 as end-products. Factors governing organic matter decomposition also influence on N mineralization. Among them, soil moisture and pH are here mentioned as important factors affecting soil organic matter decomposition, and therefore also for N mineralization.

Among the processes of soil organic matter decomposition/mineralization resulting in the release of NH_4^+ and CO_2 as end-products are soil moisture and pH. Our results show low values of correlation coefficients for protease activity and soil moisture and pH, which suppose stable enzyme activity at soil pH range from 5.06 to 6.42 and soil moisture from 6.1 % to 7.7 %. In dry soil, enzymes may have become adsorbed to clays and tannins, rendering them less susceptible to proteolytic breakdown but able to maintain reactivity (Ensminger and Gieseking 1942, Kandeler 1990).

Conclusions

The activities of catalase, cellulase and protease are higher in the fire-influenced soils compared to the control ones, which indicate the high sensitivity of microorganisms to fire. Higher temperature reduces enzymes' activities compare with that in the mineral deeper soil layers.

Catalase activity was highest in *L* layer of the forest litter from the control sampling site in comparison to the other two layers of forest litter, while cellulase activity reached maximum in both studied mineral soil layers of soil from RSS 1. There was no changes in catalase activity along soil profile of control sampling site. The protease activity depends on fire intensity and the lowest in the topsoil layer, which is the one most affected by the fire. Along the soil depth of fire affected soils the protease activity increases in the soil of the control sampling site. The activity of this enzyme remains low and constant in depth of the soil profile.

There is no clear relationship between the enzyme activities and the type of vegetation. The crown fire provokes the higher catalase and cellulase activity in comparison to those, influenced by surface fire. This trend was not observed for the protease activity.

The catalase activity showed a strong positive correlation to soil pH, whereas the correlation with soil moisture, organic matter and nitrogen content were negative. High positive correlation of protease activity was found with nitrogen and carbon content in the surface 5 cm of the mineral soil layer indicated.

The activity of the investigated enzymes can be used as a bioindicator of ongoing biological processes in fire-influenced soils and the recovery processes occurring therein.

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HABITAT PREFERED AND HOME RANGE OF HAND-REARED AND RELEASED CHUKAR PARTRIDGE (*ALECTORIS CHUKAR* GRAY, 1830) IN SE BULGARIA

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Abstract

Between August 2009 and May 2010 74 Chukar Partridge (25 August to November 2009 and 49 from February to May 2010) were released. Chukar Partridges inhabit different size areas between 31.5 and 65.7 ha, but about 10 % of the area of the habitat. Birds prefer thorn bushes during the day to the other areas (χ^2 = 13.185, *df* = 4, p < 0.05). Data show low efficiency of the release and the need to change the practices of resettlement of Chukar Partridges in Bulgaria.

Key words: Chukar Partridge, bird habitat preferences, bird reintroduction, bird habitat selection.

Introduction

In Bulgaria, Chukar Partridge (*Alectoris chukar* Gray, 1830) has a decreasing population trend after 1990 (Stoychev et al. 2007, Delov 2011, Gruychev 2014). The species is missing completely from the suitable habitats of Sakar Mountain (Stoychev et al. 2008, Gruychev 2014) and has disappeared in Western Strandzha Mnt. too where it was nesting in the early 90s of the last century (Milchev 1991, Milchev 2010).

Recovery programs of wild Galliformes with released birds are common hunting practice in Europe (Gortázar 1998, Gortázar et al. 2006). In Bulgaria, the recovery of Chukar by release of farm birds suffered major setbacks over the last decade. After settling the majority of the birds died within the first two weeks (Gruychev 2012). Setting the preferences of birds to certain parts of the habitats brings important knowledge that would determine measures of the recovery of the species as well as the reasons for the losses.

Studies of Chukar habitat in the Kardzhali region associate its distribution with humus-carbonate soils or heavily eroded soils. There are growing many drought resistant grasses and shrubs providing food and shelter for the birds (Ivanov 1985). In other places where Chukar has been successfully acclimatized the main requirements are the presence of cliffs and rocky talus (Christensen 1970). Grazing intensity, the human presence, the burning of the habitat and the lack of permanent water sources are some of the factors determining the distribution, dispersal and surviving of

Chukars (Harper et al. 1958, Kirby and Grosz 1995, Robbins et al. 2002, Bhattacharya et al. 2007, Alexis and Powell 2008, Larsen et al. 2010).

The aim of this study was to determine dispersion and habitat preferences of Chukar Partridge in Bulgaria. This can lead to optimization of the release and improve the results of the recovery programs for the Chukar.

Material and Methods

Study area

The study area is located in Southeastern Bulgaria and occupies parts of Thracian lowland and Sakar Mountain. We investigated three habitats near the villages Polyanovo (habitat P), Bulgarin (habitat B) and Levka (habitat L) (Fig. 1). The landscape is hilly with gently slopes and single small cliffs and rock formations in the ravines. The soils are sandy. The area falls within the of Trans-Mediterranean climatic zone (Bondev 1991). The average rainfall is 600–995 mm with a maximum in November and December. The driest period is from August to September.

The Chukar's habitats are characterized by a prolonged drought in the summer, but there are many evenly distributed micro-reservoirs over the entire territory. Maritsa River flows nearby one of the studied areas (Fig. 1). The average annual temperature during the study period was 13.3 °C. The average rainfall was 615 mm (data from nearest weather station Svilengrad). The average altitude near (B) is about 159 m, (P) – 193 m, (L) – 199 m.

The vegetation consists in: trees, single trees from Oaks (*Quercus* spp.), Pear (*Pyrus* spp.) and small mixed groups of Oak and Hornbeam (*Carpinus orientalis* Mill.); shrub, dominant shrub species is Jerusalem Thorn, (*Paliurus spina-christi* Mill.), but there are Roses (*Rosa*), Blackthorn (*Prunus spinosa* L.) and Hawthorn (*Crataegus* spp.); grass, the dominant grass species is *Bothriochloa ischaemum* (L.) Keng, Black beard (*Chrysopogon gryllus* L.) and Bulbous bluegrass (*Poa bul-*

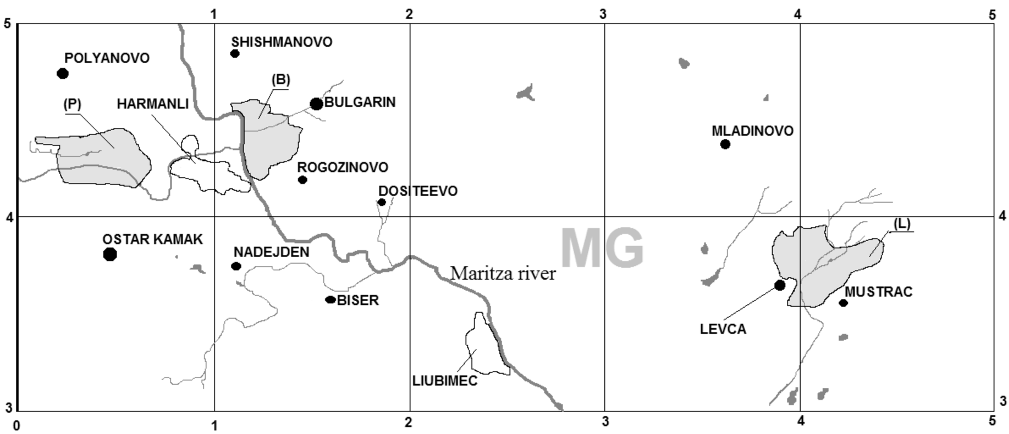

Fig. 1. The location of study site in SE Bulgaria: (P) – study habitat Polyanovo; (B) – study habitat Bulgarin and (L) – study habitat Levka.

bosa L.). Less frequently are found species like hawksbeard (*Crepis sancta* (L.) Babc.), Prickly juniper (*Juniperus oxycedrus* L.), Holy grass (*Anthoxantum odoratum* L.), Duch clover (*Trifolium repens* L.), Volga fescue (*Festuca valesiaca* Schleich ex. Gaudin).

Field methods

The survey was conducted from February 2009 to November 2010. 74 Chukar Partridge (25 August to November 2009 and 49 from February to May 2010) were studied. The birds were adapted in the aviary, adaptation cages and directly released in three habitats within the land borders of the villages Bulgarin (B), Polyanovo (P) and Levka (L) (Fig. 1). In each habitat were released the following numbers of birds: (P) – 27 birds (22 in spring and 5 in autumn); (B) – 20 birds (10 in spring, 10 in autumn); (L) – 27 birds (17 in spring and 10 in autumn).

All Chukars were marked with radio-telemetry transmitters RI-2B (Holohil system Ltd) and located every 7 days with precise GPS coordinates after eye contact. The localization is done at least one hour after sunrise and before sunset, thus avoiding differences in habitat use in terms of time of the day (Swihart and Slade 1985).

The habitats were classified as: 1) Wetlands; 2) Rocks; 3) Grassland; 4) Deciduous forests; 5) Thorn (*Paliurus*) bushes; 6) Acacia forest; 7) Black pine plantations and 8) Farmland.

Data analysis

The bird home range was determined using minimum convex polygon (MCP) (Mohr 1947). Only birds that were seen more than 25 times were included in the determination of MCP (Kenward 2001). There were 5 Chukar Partridge in (L) and 2 in (P). The birds were seen 27 times in (L) and 28 in (P). The total home range was determined using the average home range of each bird in each habitat with Calhome Software (Kie et al*.* 1996). The area of MCP in (B) was determined as the area of MCP of all localized birds because there were too few sights. It is only used to determine the preferences of habitat and not collated with other MCP studied regions.

The preferred habitats of the birds were determined by dividing the area of the habitat into different categories in MCP 100 % (Table 1) and by calculating the total number of localities of each bird in the respective habitat category. After that a Chi-squared test was used to determine whether the number of locations in one area was significantly higher than the number of locations in the other areas (Fowler and Cohen 1995).

The obtained results were compared and tested for statistically significant differences with nonparametric (Kruskal-Wallis and Mann-Whitney Tests) and Chi-square test. In all statistical tests the significance level was $P<0.05$. All statistical analyzes were performed with PAST (Hammer et al*.* 2001).

Results

Distribution of areas in the study regions showed significant occupation of territory habitat of *Paliurus* (Table 1).

Chukars occupy different sized areas (average ± stdev (min-max) ha). In (P), the area of the MCP (100 %) is 31.5 ± 7.6 (26.2–36.9), in (B) 62.5,

Table 1. Percentage distribution of habitat types in the three study areas and number of localities of Chukar Partridge (*Alectoris chukar*) in each habitat

Type of habitat area	Polyanovo (P)			Bulgarin (B)			Levka (L)		
	habitat, %	100% MCP, %	locations, number	habitat, %	100% MCP, %	locations, number	habitat, %	100% MCP, %	locations, number
Wethlands	9.27	6.65	9	18.82	4.38	2	1.54	0.15	0
Rocks	2.95	5.98	7	0	0	11	7.89	17.75	13
Grasslands	10.65	21.98	22	4.24	11.03	10	0	0	0
Deciduous forest	8.00	1.01	1	3.27	15.56	0	5.08	0	0
Thorn (*Paliurus* spp.) bushes	68.56	64.38	25	59.22	51.51	16	45.29	45.24	18
Acacia forest	0.49	0	0	0	0	0	0	0	0
Black pine plantation	0.00	0	0	6.28	0	0	25.1	32.9	0
Farmland	0.08	0	2	8.17	17.52	9	15.1	3.96	0

in (L) 65.7 ± 32.7 (24.6–116.2). It was possible calculated MCP (90 %) in (P) and (L) – 20.5 ± 0.01 (20.48–20.5) and 39.6 ± 19.7 (20.2–60.2) ha respectively. There are no significant difference in the average area of MCP (90 %) in both study areas (Hc = 1.35, p = 0.245). In (P) Chukars occupy 10.1 % of the area of habitat, in (B) 10.7 % and in (L) 9.11 %. The density of the Chukar Partridge in study habitats is different. During the first weeks after release it is 9.3 ind./100 ha in (P), 32 ind./100 ha in (B) and 23.2 ind./100 ha in (L). Spring density of birds in the MCP (100 %) during the first week after the release is: (P) 7.6 ind./100 ha; (B) 16 ind./100 ha; (L) 14.6 ind./100 ha. It decreases in the following 9 weeks, and them remains constant (P) and (L) and in (B) all birds were died after 4 weeks (Fig. 2).

Chukar Partridge in (P) prefer *Paliurus* bushes during the day compared to the other areas (χ^2 = 13.185, df = 4, p < 0.05). Chukars in habitat in (B) analysis cannot highlight significant differences (χ^2 = 9.22, df = 4, ns). In habitat (L), Chukars prefer from Thorn bushes back to the other areas (χ^2 = 12.19, df = 4, p < 0.05).

The average distance of birds from permanent water sources in habitats was: (P) 311.03 ±227.4 (60–138 m), (B) 287.5 ± 235.1 (60–840 m), (L) 412.5 ± 322.2 (60–1120 m). There was no significant difference between the alienation of Chukars of wetlands in the three study areas (Hc = 1.62, p = 0.44).

Discussion

The results for the home ranges in this study are similar to those in other ones for Chukar Partridge (Lindbloom 1998; Walter 2000, 2002). They show that Chukars inhabit relatively small areas in spring and summer, despite their territoriality during the breeding season. It is unknown, however, whether in winter birds travel to greater distances as a result of persecution or in search of food. Therefore, the habitat remains an important factor for the release of Chukar Partridges. The results of this study indicate that farm Chukar Partridges remain around the places of release. These results showed the need for mosaic location of adaptation cages and aviaries in the habitat when introducing Chukars. This will insure the uniform bird distribution across the habitats. In (B), the spring density is greater than the other examined areas (16 ind./100 ha). Here the losses are 100 % after the fifth week of release. Although there may be a number of factors determining the amount of loss, small size of the habitat in combination with high density of birds leads to unsatisfactory results in a short period of time. In (P) and (L), the density nine weeks after the release is between 1–4.3 ind./100 ha, which again is an unsatisfactory result. With these methods of release it is impossible to create a stable group of Chukar Partridges and to ensure their successful reproduction in the future. Similar results were reported in general for many Galliformes (Sokos et al. 2008). The authors recommend switching to semi-breeding and release or capture and relocation of birds from wild populations. The latter is impossible at the current time in Bulgaria. The density of the Chukar Partridges in the three study areas is different, but they occupy almost the same area of the habitat (about 10 %). These habitats satisfy the requirements of the species, which is why Chukars do not wander or move to other areas. Similar results are also known in the USA (Nevada; Benolkin 1988). In (P) spring density of Chukars, first week after displacement, is nearly twice lower than that in (L). However, in both habitats Chukar Partridges use the same parts.

Chukar Partridges prefer *Paliurus* bushes over other parts of the habitat. In other studies for wild Chukar population in Bulgaria, breeding density increase with increase to percentage of rock outcrop and Chukar Partridge preferred two plant community (*Paliurus spina-christi - Poa bulbosa* and *Paliurus spina-christi-Eryngium campestre-Anthoxanthum odoratum*) for nesting (Gruychev et al. 2014). Although coverage of rocks, sometimes up to 50% of the habitats, followed by shrubs and grasses (Jonsgard 1973, Moreland 1950, Galbreath and Moreland 1953) in our study coverage of the rocks is relatively small. This can be predetermined preferences of Chukars into the *Paliurus* bushes. The birds seek refuge in the undergrowth of *Paliurus*, unlike the case, where 30–50 % of habitats are covered with rocks. Also little coverage of rocks can be one of the reasons for the losses after release. The characteristics of the habitats of Chukar in the studied areas are quite different from those in other parts of the species range and this is probably

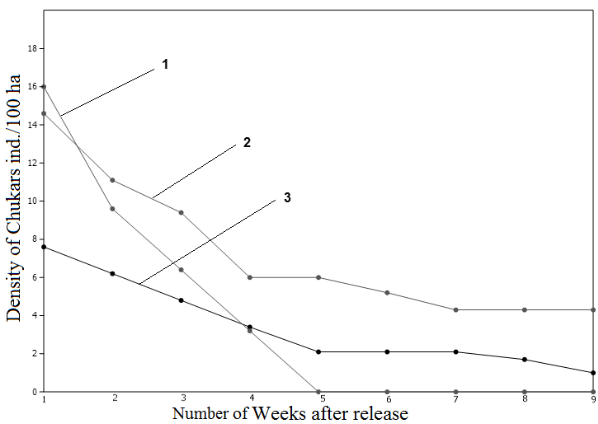

Fig. 2. Dynamics of spring density of Chukar partridge (*Alectoris chukar*) in weeks after the release of the three study areas (ind./100 ha).

Note: 1 – Bulgarin (B); 2 – Levka (L); 3 – Polyanovo (P).

caused of this preferences. This should be taken into account when selecting sites for construction of aviaries and adaptation cages in these habitats.

The average distance to permanent water sources identified by us are relatively short. The distance of birds which moved away from permanent water sources in western Utah was 625 (390–1664 m) (Larsen et al. 2010). In other studies this distance was about 280 m (Lindbloom 1998; Walter 2000, 2002). Red-legged Partridge in Spain established at medium distances from water sources (328 and 285 m) (Boralho et al. 1998). In our study areas there are numerous small permanent water sources (Fig. 1) and this is the likely cause that Chukar Partridges do not stray at long distances in the summer months. The presences of numerous small ponds are not a crucial factor in the distribution of the Chukar in the research area.

The results of this study showed low efficacy of the applied practices for recovery of the Chukar Partridge in Bulgaria. Changes are needed in production practices of farm Chukars and change to semi-breeding in restoring the local native populations of the Chukar Partridge in Bulgaria.

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CARBON STOCK IN STANDING DEAD TREES OF *PINUS ROXBURGHII* SARG. IN SUB-TROPICAL PART OF GARHWAL HIMALAYA

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Abstract

The aim of the present study was to assess the total standing dead trees of Chir pine and their potential of carbon stock in the forests. Chir pine (*Pinus roxburghii*) in Himalayan forest plays a major role in carbon sequestration. Chir pine is a very prominent forest cover in Uttarakhand, out of total 24,414.80 km^2 area under forests; it occupies 3,943.83 km^2 which is 16.15 % of total forest area of the state. In sub-tropical region of Garhwal Himalaya, large numbers of standing dead trees have been observed on southern aspect and increasing continuously last few years. Dead standing (snag) trees in the forest play equal role in carbon sequestration as live trees. Although regular inputs of dead tree components play important role in enrichment of forest floor though litter, twigs, fruits and some time entire tree. The results show that the average standing dead trees were reported 14.83 ha^{-1} of which mean volume and carbon stock values were of 23.14 m^3·ha^{-1} and 14.67 t·ha^{-1} respectively.

Key words: Chir pine, carbon stock, ecosystem services, snag, sub-tropical region.

Introduction

Biomass is a measure of forest structure and function, with both live and dead components. Live biomass through photosynthesis activity sequesters carbon from the atmosphere, whereas, dead biomass retains carbon for longer period even for decades and releasing it gradually by decomposition process (Siccama et al. 2007). Dead trees play a number of important roles in the structure and function of ecosystems (Franklin et al. 1987), provide habitat for organisms (Harmon et al. 1986), influence wildfires (Kauffman et al. 1998), and play roles in carbon and nutrient cycles (Fahey 1983, Krankina and Harmon 1995).

When forests are degraded or cleared, their stored carbon is released into the atmosphere as carbon dioxide (CO_2). Carbon is returned to the atmosphere via three of the largest fluxes: oceanic release (~90 Pg·C·yr^{-1}), plant respiration (~59 Pg·C·yr^{-1}), and soil respiration (~58 Pg·C·yr^{-1}) (IPCC 2000, Luo and Zhou 2006, Houghton 2007, Lambers et al. 2008). Carbon content in plant tissue is approximately half of the dry weight of aboveground live biomass (Malhi and Grace 2000).

Deforestation in the tropics is a major source of carbon emissions and an active contributor to global warming. The Intergovernmental Panel on Climate Change (IPCC) estimated that 1.7 billion tons of carbon is released annually due to land use change, of which the major part is tropical deforestation (IPCC 1995). Tropical deforestation is estimated to have released of the order of 1–2 billion tons of carbon per year during the 1990s, roughly 15–25 % of annual global greenhouse gas emissions (Malhi and Grace 2000, Fearnside and Laurance 2003, Houghton 2005). The largest source of greenhouse gas emissions in most tropical countries is from deforestation and forest degradation. Estimates of emissions from global deforestation range from more than 25 % of global GHG emissions (IPCC 2000), and the vast majority of these emissions are coming from developing countries in the tropics.

Standing and fallen dead trees are the major types of coarse woody residues and their relative contribution to total ecosystem biomass varies greatly in the landscape, depending on forest types, disturbance regimes, topography, and stand age (Spies et al. 1988, Harmon and Hua 1991). Among the multiple functions of coarse woody debris in forest ecosystems, the provision of habitat for many organisms and the long-term storage of carbon and other elements in the forest floor are critical for the maintenance of biodiversity and the continuity of biogeochemical processes (Bowman et al. 2000, McKenny and Kirkpatrick 1999, Harmon et al. 1990, Crowford et al. 1997, Hart 1999, Nakamura and Swanson 1994).

The studies related to soil organic carbon stock of Chir pine have been carried out (Singh et al. 2011, Sheikh et al. 2009) and particularly studies of biomass and carbon stock have also been carried out (Jina et al. 2008, Sheikh et al. 2011) but the studies related to biomass of standing dead trees of Chir pine is completely lacking, particularly in sub-tropical region of Garhwal Himalaya. Therefore, to understand the standing dead trees volumes and carbon available on Chir pine forest, the following objectives were selected: (i) to estimate the volume of standing dead trees of Chir pine forest and (2) to estimate the total carbon stock (above and below ground) of standing dead trees of Chir pine forest.

Material and Methods

Study area

The study was carried out in Chir pine forests (Fig. 1) of sub-tropical region of Garhwal Himalaya in Tehri Garhwal district of Uttarakhand. Three different sites of Chir pine forest were selected for study each of 4, 6 and 7 hectares patches. The Chir pine dead trees were observed only in southern aspect and none of them were observed in other aspects. Therefore, enumeration of trees was done from southern aspect. Chir pine dead trees in the selected sites were enumerated for volume and carbon stock assessment.

The study sites are located between 30°28.179′–30°28.189′ N latitude and 78°27.338′–78°27.700′ E longitude at 1100, 1200 and 1250 m a.s.l. The climate of the study area is quite distinct in a year and represents three different marked seasons, i.e. summer, winter and rainy. The climate is influenced by monsoon pattern of rainfall. The winter is very cold with minimum temperature dropping to 4 °C (December) and during summer temperature reaches up to 32 °C (June). The average annual rainfall reported 1000 mm and approximately 75 % rainfall is received from mid-June to mid-September. The relative humidity varies from 35 % (May) to 92 % (August).

Fig. 1. Dead standing trees in natural Chir pine forest sites: a – close view of forest; b – large number of dead trees in small patch; c – young dead trees; d– falling branches of dead trees.

Data collection

Carbon estimation of Chir pine dead standing trees in each site was carried out by complete enumeration basis. The data was collected for diameter at breast height (1.3 m) of individual tree, using tree caliper for the estimation of growing stock volume density (GSVD) following volume equations of Forest Research Institute and Forest Survey of India publications for Chir pine (FSI 1996, Sharma et al. 2010). The estimated GSVD ($m^3 \cdot ha^{-1}$) was fur-

Table 1.Volume and carbon stock in standing dead Chir pine forest at three different sites.

Site	Aspect	Altitude, m a.s.l.	Dbh, cm	Height, m	Dead trees, ha^{-1}	Volume, $m^3 \cdot ha^{-1}$	AGC, $t \cdot ha^{-1}$	BGC, $t \cdot ha^{-1}$	Carbon stock, $t \cdot ha^{-1}$
I	Southern	1100	45	22.91	11.00	16.39	7.785	2.61	10.39
II	Southern	1200	46	19.97	17.75	28.53	13.55	4.46	18.01
III	Southern	1250	45	20.97	15.75	24.51	11.64	3.97	15.61
Mean			45.3	21.28	14.83	23.14	10.99	3.68	14.67

ther converted into aboveground biomass density (AGBD) of tree components (stem, branches, twigs and leaves), which was calculated by multiplying GSVD of the Chir pine forest with appropriate biomass expansion factor (BEF) (Brown et al. 1999). The belowground biomass density (BGBD; fine and coarse roots) for Chir pine forests were estimated using Cairns et al. (1997). Above ground carbon and below ground carbon was estimated by using the co-efficient of 0.50 for conversion from biomass to carbon stocks (Brown and Lugo 1982, Roy et al. 2001, Malhi et al. 2004). The above ground and below ground carbon was added to estimate total carbon of standing dead trees of Chir pine in each site.

Results and Discussion

The carbon stock of Chir pine dead standing trees in different sites is shown in Table 1. The highest numbers of standing dead trees (17.75 trees ha^{-1}) were recorded in site II followed by site III (15.75 trees ha^{-1}) and site I (11 trees ha^{-1}). The maximum and minimum volume of Chir pine trees were 28.53 $m^3 \cdot ha^{-1}$ and 16.39 $m^3 \cdot ha^{-1}$ for site II and site I respectively (Table 1). Carmona et al. (2002) reported the volume of snags (14–924 $m^3 \cdot ha^{-1}$) for temperate forests in Chiloe Island, Chile. The ranged value of above ground carbon of standing dead Chir pine was 7.785 $t \cdot ha^{-1}$ to 13.55 $t \cdot ha^{-1}$ (Table 1). The maximum below ground carbon was 4.46 $t \cdot ha^{-1}$ followed by 3.97 $t \cdot ha^{-1}$ and 2.61 $t \cdot ha^{-1}$ for site II, site III and site I respectively. The maximum (18.01 $t \cdot ha^{-1}$) total carbon of Chir pine trees was recorded in site II followed by site III (15.61 $t \cdot ha^{-1}$) and site I (10.39 $t \cdot ha^{-1}$) (Table 1). A study carried out by Oswalt et al. (2008) in Caribbean St. John, U.S. Virgin Islands reported that dead woody materials contributed an estimated 16 percent (8.9 $mg \cdot ha^{-1}$ ± 0.8) of the total aboveground and belowground carbon, however, total standing dead tree (snag) basal area ranged from 0.0 to 1.3 $m^2 \cdot ha^{-1}$. In the present study, it was observed that southern aspect was more susceptible for increasing number of dead Chir pine trees, might be due to this aspect receiving more sun light intensity which is comparatively drier of other aspects result in it may also change the soil physico-chemical properties, which may lead to favourable environment for pathogens. Guarın and Taylor (2005) reported that the temporal patterns of tree death were reported similar on north facing and south facing slopes but, the density of dead trees was higher on north facing slopes than south slopes.

In the present study, when bark from Chir pine dead trees was removed, termite attack below tree bark was observed, which could be another reason for dead trees. A study shows that epidemic tree deaths further exacerbate global warming because the forest changes from a net carbon sink to a carbon source, as the dead trees can no longer absorb carbon dioxide and release tons of the greenhouse gas (Jim Agar 2008).

Altitude was negatively correlated with volume, above and below ground carbon of standing dead Chir pine trees. Volume of standing dead trees was significantly positive correlated with below ground carbon and total carbon stock at 5 % level. Above ground carbon of standing dead Chir pine trees were correlated perfectly positive with volume. Total carbon stock was also significantly correlated ($p < 0.05$) with above and below ground carbon of standing dead trees of Chir pine.

Deadwood plays a key role for sustaining forest productivity and environmental services such as stabilizing forests and storing carbon by its biodiversity. Volume of deadwood depends on productivity, pattern of natural disturbance, succession stages, forest history and human intervention. Deadwood type and volume vary between different forest types and management systems. Some disturbance has linked to the production standing deadwood (e.g. dry-out), or fallen deadwood (e.g., storm damage). Both downed and standing dead wood store carbon are important components of forest carbon dynamics but also release carbon via decomposition and combustion in fires. A study carried out by Domke (2011) in the U. S. for estimate changes in the standing dead tree biomass and carbon stock and the results suggest that density reductions and structural loss in standing dead trees substantially decreases estimates of standing dead tree biomass and carbon at tree, plot, and regional scales. Pugh and Gordon (2012) carried out a study in a regions of western North America with snow-dominated hydrology, the presence of forested watersheds where, widespread tree death in these watersheds can dramatically alter many ecohydrological processes including transpiration, canopy solar transmission and snow interception, sub-canopy wind regimes, soil infiltration, forest energy storage and snow surface albedo.

In Europe species associated with deadwood now make up the largest single group of threatened species. Of the 1,700 species of invertebrates in the UK dependent for at least part of their life cycle on deadwood, nearly 330 are Red Data Book-listed because they are rare, vulnerable or endangered (Smith 2004). In Sweden, one of the most densely forested countries in Europe, 805 species dependent on deadwood are on the national Red List (Sandström 2003). Major forest carbon pools include trees, understorey vegetation, deadwood, litter, and soil. Deadwood is important as it is both a store and source of carbon. This will now change because national carbon inventories are required under the Kyoto Protocol of the 1992 United Nations Framework Convention on Climate Change (Woldendorp 2002). Deadwood releases carbon to the atmosphere during microbial respiration from decomposer organisms and are the source of carbon. In cool climates, microbial activity is restricted and decomposition very slow and deadwood remain for long-term storage on site becomes a source of carbon stock for long period.

The dead standing trees, fallen components of trees helps in maintaining environmental carbon. Live biomass sequesters carbon through photosynthesis from the atmosphere, whereas dead biomass can retain carbon for decades, releasing it gradually by decomposition (Siccama et al. 2007, Kim 2009). Live and dead components affect many aspects of forest ecology. Because wildlife species require varying amounts and spatial arrangements of live and dead biomass (McCarney et al. 2008, Kim 2009). But from the study area has been observed that the branches and some time entire tree are used by the local villagers for fuelwood purposes. It has also been observed that Chir pine forest are prone to fire and damage large amount of residual of forest floor after fire and become source of environmental CO_2. It has observed from the study sites that the forests are normally affected every year by fire. These forests become extremely vulnerable to fires when they are located on dry sites (Kumar et al. 2013), which due to course of time may be affected by certain termite or beetle attack and die. It is evidence from the earlier studies (Fowler and Sieg 2004, Parker et al. 2006) that fire-damaged or drought-stressed trees are generally attacked by bark beetles. Droughts, wildfires, and insect and disease outbreaks have left large numbers of standing dead and dying trees in western forests. Forest inventories conducted by the U.S. Department of Agriculture Forest Service estimate the average annual mortality volume (i.e., the volume of wood in the trees that die or are killed each year by natural causes) to be about 2,500 million cubic feet (USDA FS 2005, 2006). Management of these dead and dying trees, in particular post fire salvage, is controversial (Peterson et al. 2009).

Thus the human interference affect the natural decomposition process of trees is forest floor and further affect nutrient release and carbon storage from the fallen and standing dead trees. Therefore, special strategies need to be done on government level, village community level to aware the people for maintaining these standing dead trees and even after fallen trees in the forest floor for long term carbon sequestration and ecosystem services.

Conclusion

The study suggests that the dead trees have potential in carbon sequestration. After falling these dead trees and their components also enrich forest floor. The process of further decomposition of dead trees and its components cannot be avoided. But some time these trees are cut and used by villagers for fuelwood and other purposes, which directly affect the natural process of enrichment of forest floor and the potential of carbon sequestration. Therefore, the awareness of villagers about its effect on forest floor and direct emission of gases through use of fuelwood can be minimized.

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TRENDS IN THE DEVELOPMENT OF FORESTED AREAS OF BULGARIA FROM 1990 TO 2012 ESTABLISHED BY THE RESULTS OF THE PROJECT „CORINE LAND COVER”

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Abstract

The main objectives of the EU project „CORINE Land Cover” are creation of a unified European land cover map through interpretation of satellite images and preparation of a digital database of land cover and the changes in it. The results are used for assessment of land cover and its state and policy formulation on protection of the environment, planning and management of agriculture, forestry, transportation, etc. at European and national level. The databases created in different stages of the project “CORINE Land Cover” – Bulgaria are analysed, some of the most typical changes in the forest territories of Bulgaria concerning the forested areas during the period 1990–2012 are identified, and from there the trends in their development are found.

Key words: satellite images, deforestation, afforestation, change detection, CLC classes, CLC changes.

Forests in the World, in Europe and Bulgaria

Today, one third of the Earth’s land surface, or a total of 34 million km^2 is covered by forests. Half of the planet’s forests are located in Russia, Brazil, Canada, the US and Congo. Most forested are some small countries like Suriname (95 %) and Gabon (84 %). Worldwide afforested areas begin to decrease dramatically over the past 150 years. A major cause of deforestation is the need for agricultural lands.

According to a study of the World Wildlife Fund (World Wide Fund for Nature – WWF) in the future the rate of deforestation in the world will fall, but if we do not initiate anything, 230 million hectares of forests will be extinct until 2050 (Galhidi 2011). Only a balance between the processes of deforestation and afforestation may change these trends.

Over 40 % (1.77 million km^2) of the land territory of the EU is covered by forests and almost 20 % (over 14 million ha) of forest habitats are included in the „Nature 2000” network. Unlike in many other parts of the world forest cover in the EU increases by 0.4 % per year. (Anonymous 2015).

Forests in Bulgaria provide about 85 % of the water flow in the country or around 3.6 billion m^3 of clear drinking water. They have a significant role in reducing greenhouse gas emissions into the atmosphere, accumulating carbon in the biomass and actually absorbing carbon dioxide. In this connection, the adaptation of forests to climate change is one of the most important objectives of forest policy.

Priceless are forests for soil protection, water and climate, and protection from natural disasters. By parameters of forest fire risk Bulgaria is usually associated to the traditionally dangerous regarding fires Mediterranean region. For the last 15 years in the forest territories of the country an annual average of about 500 fires are registered, affecting on average 11,000 ha of these territories. Direct damage is estimated an average of 13 million leva per year.

Lands and forests within the forest territories of Bulgaria store:

- over 80 % of the protected species of plants;
- over 60 % of the threatened by extinction animal species;
- over 60 % of the types of habitats of priority for conservation;
- eight of the twelve landscape complexes defined in the National Strategy for the Conservation of Biodiversity as unique and representative for the Bulgarian biodiversity;
- populations of 43 globally threatened species.

In order to protect this diversity 3 national parks, 10 nature parks, 55 reserves and 35 maintained reserves have been separated on the territory of the country (Anonymous 2006).

What changes have occurred in the forested areas of the forest territories of Bulgaria for the period 1990–2012, covering the former four stages of the project „CORINE Land Cover”? According to the methodology of the project (Bossard et al. 2000) forests and open areas in the forest territories are fully integrated in Class 3 – „Forests and semi-natural areas” as its subclasses (Anonymous 1994). This analysis tracks the evolution of these 6 CLC classes (level 3) (Anonymous 2011):

- 3.1.1. – „Broad leaved forest”;
- 3.1.2. – „Coniferous forest”;
- 3.1.3. – „Mixed forest”;
- 3.2.2. – „Moors and heathlands”;
- 3.2.4. – „Transitional woodland scrub”;
- 3.3.4. – „Burnt areas”.

Extraction of Summarized Data by Periods for the Studied Land Cover Classes and Analysis of the Results

Results from the project „Corine Land Cover 1990”

In Table 1 the data are summarized from the output database CLC1990 for the studied 6 classes, indicating the number of polygons and their total area, as well as their percentage representation for the entire mapped territory.

Results in the database CLC1990 show that in 1990 the forested areas within the forest territories (deciduous, coniferous and mixed forests) are 3,553,457 ha or 31.6 % of the territory of the country. This exceeds the official data for 1990 – 3,348,032 ha (Bogdanov 2002). The guaranteed total accuracy of the database CLC1990 is 85 %. Under this condition, the difference found of 205,425 ha (5.8 %) is negligible. The main reason for this difference is that according to the methodology of the project open areas smaller than 25 ha within the forest areas are not differentiated as separate polygons of another land cover class. Furthermore, there are self-seeded but not inventoried forest stands.

Table 1. Land cover classes in the database CLC1990 – Bulgaria.

No	CLC code (level 3)	CLC class	Number of polygons	Area	
				ha	%
1	3.1.1.	Broad leaved forest	5,369	2,385,150	21.21
2	3.1.2.	Coniferous forest	1,946	551,865	4.91
3	3.1.3.	Mixed forest	3,765	616,442	5.48
4	3.2.2.	Moors and heathlands	100	32,458	0.29
5	3.2.4.	Transitional woodland scrub	7,787	755,818	6.72
6	3.3.4.	Burnt areas	15	2,361	0.02

Changes occurred in the period 1990–2000

Finding the land cover changes in the period 1990–2000 is a necessary step for obtaining the updated database CLC2000. Change detection is a process of comparison and analysis of vector and raster data from the beginning and the end of the period. Taking the wright decisions is determined by the good knowledge of the study area as a natural region, the socio-economic conditions, the seasonal changes in the reflectance of the land cover, and by the knowledge and experience in computer assisted interpretation of multitemporal satellite images.

For the period 1990–2000, 2670 polygons of changes in the land cover of Bulgaria are registered with a total area of 122,579.7 ha, which is 1.10 % of the mapped area. As a total, the changes in the studied 6 land cover classes are 54.2 % of the area of all changes detected for the territory of Bulgaria.

The changes in class 3 – „Forests and semi-natural areas” are associated with two large-scale processes in these areas, which are described with the following CLC codes (level 3):

- Deforestation – cut down forests: „3.1.1. to 3.2.4.”, „3.1.2. – 3.2.4.” and „3.1.3. – 3.2.4.” and burned areas: „3.1.1 to 3.3.4.”, „3.1.2. – 3.3.4.” and „3.1.3. – 3.3.4.”.

The number of changes reflecting the cut forests is 701 with a total area of 29,859.4 ha, which is 24.25 % of the total area of all changes for the territory of Bulgaria. Burned areas almost do not affect the total forested area – seven in number with a total area of 948.6 ha or 0.01 % of the total area of the changes;

- Afforestation – „3.2.4. – 3.1.1.”, „3.2.4. – 3.1.2.”, „3.2.4. – 3.1.3.” and „3.3.4. – 3.1.1.”.

The number of changes related to self-seeded or planted forests is 965 with a total area of 36,767.9 ha, which amounts to 29.86 % of the total area of all changes for the territory of Bulgaria. The area of restored deciduous forests in burned areas is 206.6 ha, which is 0.001 % of the total area of all the changes.

This is the only stage of the project, within which time span afforestation process dominates the process of deforestation. Over the period 1990–2000, the forested areas in the forest territories have increased by 6,166.5 ha. The reason is mainly that a change in land cover is registered only if the area of the polygon is over 25 ha. As we will see in the next stages loggings in the forests are mostly with smaller areas and therefore many of them are not included in the database CLCchange1990–2000.

Results from the project „Corine Land Cover 2000”

In Table 2 the data for the studied 6 land cover classes are summarized, taken from the output CLC2000 database for this second stage of the project. The number of polygons is given, as well as their total area and percentage representation for the mapped territory. As a result, the wooded areas are 3,564,390 ha or 31.97 % of the territory of Bulgaria, which is 166,083 ha (4.6 %) more than that in the data of EFA 2000 – 3,398,307 ha (Bogdanov and Tonchev 2011). Again the difference is negligible, and the main reasons are the same as those for 1990.

Changes occurred in the period 2000–2006

For this second stage of the project „CORINE Land Cover” some substantial changes have been made in its methodology (Büttner and Kosztra 2007). A change of a land cover / land use class is registered in the database CLCchange2000–2006, when the following geometrical requirements are fulfilled:

- A minimum area of 5 ha of the actual and the technical changes, the same for the aggregated area of „elementary” polygons smaller than 5 ha (incl. the „island” type);
- A minimum polygon width of 100 m, keeping the area restrictions.

The total number of changes identified for the period 2000–2006 is 2,706 with an area of 58,464.6 ha, which is 0.52 % of the mapped territory (Stoimenov 2008). In general the total area of the changes in the studied 6 land cover classes is 83.5 % of the total area of all changes for the entire mapped territory. From the comparison of these data with the similar data from the previous period is seen that when the improved methodology is used, the area of the changes in forest territories compared to the area of all identified changes greatly increases – from

Table 2. Land cover classes in the database CLC2000 – Bulgaria.

No	CLC code (level 3)	CLC class	Number of polygons	Area	
				ha	%
1	3.1.1.	Broad leaved forest	5,431	2,366,779	21.05
2	3.1.2.	Coniferous forest	2,018	548,265	4.88
3	3.1.3.	Mixed forest	4,032	649,346	5.78
4	3.2.2.	Moors and heathlands	89	32,166	0.29
5	3.2.4.	Transitional woodland scrub	7,706	735,105	6.54
6	3.3.4.	Burnt areas	3	1,265	0.01

54.2 % to 83.5 %. The two processes in the forest territories that are analyzed in this case have the following dimensions:

- Deforestation – 1,659 changes with a total area of 34,810.2 ha which is 59.5 % of the total area of all changes identified on the territory of Bulgaria. Burned areas in total are 382.6 ha or 0.65 % of the total area of changes;
- Afforestation – in total for the three classes (level 3) – 3.1.1., .3.1.2. and 3.1.3. – 685 changes with a total area of 13,563.5 ha or 23.19 % of the area of all changes identified on the territory of Bulgaria.

In contrast to the period 1990–2000 in which the process of afforestation dominates, in the period 2000–2006 the process of deforestation is already dominant. In general, as a result of the two processes forested areas in the forest territories have decreased by 21,629.3 ha.

Figure 1 shows an example of the process of deforestation. This is part of the numerous cuttings east of Lukovit (their total area within the Basic Working Unit is 1,752 ha). Similar examples can be found often near settlements – illegal cutting of firewood.

Figure 2 is an example of recovery of deciduous forests.

Results from the project „Corine Land Cover 2006”

The results obtained for the studied 6 types of land cover are summarized in Table 3. They show that forested areas are 3,541,847 ha or 31.61 % of the mapped territory. According to the EFA in 2006 the forested area is 3,691,858 ha (Bogdanov and Tonchev 2011). While the results in the databases CLC1990 and CLC2000 indicated that forested areas were more than those in the official data, in 2006 according to the data of EFA these areas are with 150,011 ha (4.2 %) larger than these in the CLC2006

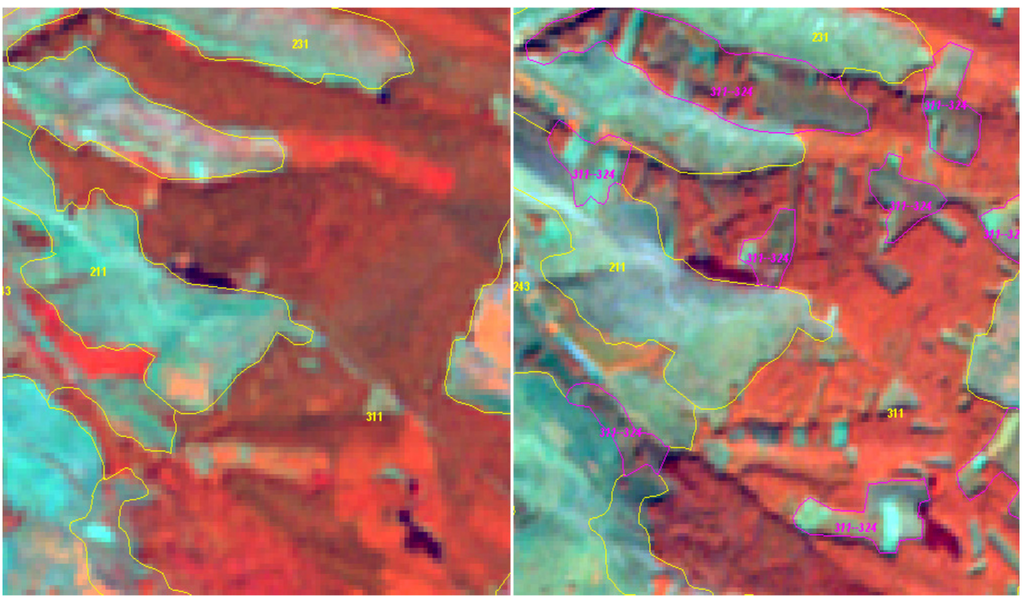

Fig. 1. Cut deciduous forests – change „3.1.1.-3.2.4.”

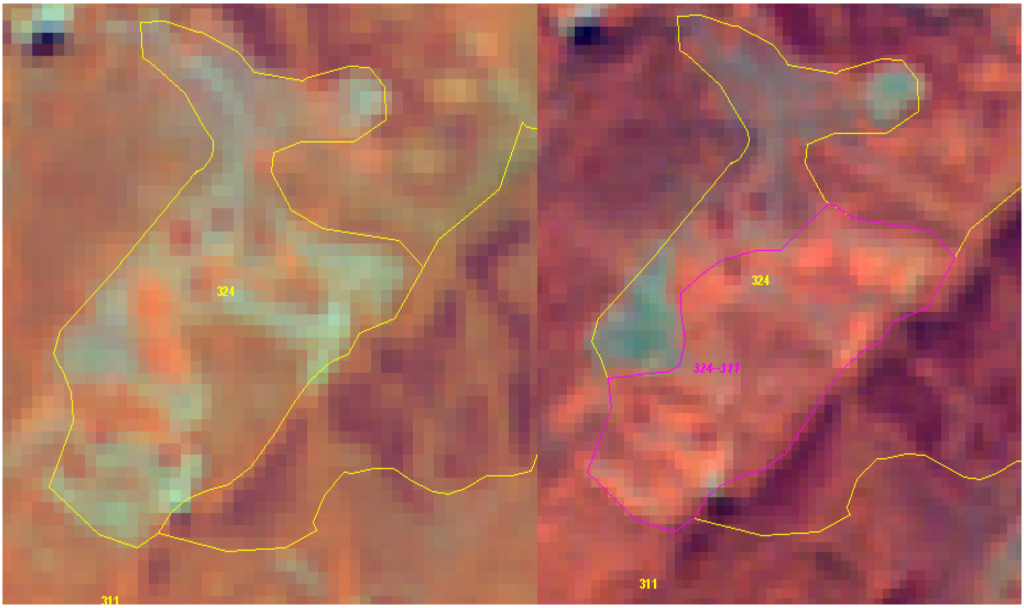

Fig. 2. Recovered deciduous forests – change „3.2.4.-3.1.1."

database. The reasons are from one side the changed methodology of the project and the mapping of all changes with an area of over 5 ha, and on the other – the domination of the process of deforestation over this of afforestation as a result of the numerous illegal cuttings identified by computer interpretation and analysis of multitemporal satellite images.

Changes occurred in the period 2006–2012

For the period of 6 years (2006–2012) 2,484 changes have been detected in the land cover of Bulgaria with a total area of 49,375.6 ha, which is 0.44 % of the mapped territory (Stoimenov 2014). More than half of them are in class „Forests and semi-natural areas". They are mostly related with the two analysed

Table 3. Land cover classes in the database CLC2006 – Bulgaria.

No	CLC code (level 3)	CLC class	Number of polygons	Area	
				ha	%
1	3.1.1.	Broad leaved forest	5,673	2,342,393	20.90
2	3.1.2.	Coniferous forest	1,955	546,247	4.88
3	3.1.3.	Mixed forest	4,117	653,207	5.83
4	3.2.2.	Moors and heathlands	82	26,620	0.24
5	3.2.4.	Transitional woodland scrub	7,986	730,756	6.52
6	3.3.4.	Burnt areas	2	409	0.00

Table 4. Changes in the 6 CLC classes (level 3) of the class „Forests and semi-natural areas" for the period 2006–2012.

Afforestation			Deforestation		
Type of change	Number	Area, ha	Type of change	Number	Area, ha
3.1.1.-3.2.4.	1,067	14,317.2	3.2.4.-3.1.1.	183	4,312.7
3.1.2.-3.2.4.	138	3,726.7	3.2.4.-3.1.2.	1	7.6
3.1.3.-3.2.4.	136	2,444.3	3.2.4.-3.1.3.	-	-
3.1.1.-3.3.4.	2	15.0	3.3.4.-3.1.1.	-	-
3.1.2.-3.3.4.	4	68.9	3.3.4.-3.1.2.	-	-
3.1.3.-3.3.4.	-	-	3.3.4.-3.1.3.	-	-
TOTAL:	**1,347**	**20,572.1**	**TOTAL:**	**184**	**4,320.3**

processes on these areas. The process of deforestation encompasses 1,347 changes with a total area of 20,752.1 ha, which is 41.01 % of the total area of all the detected changes for the period. The scope of the afforestation process is considerably smaller – 183 changes with a total area of 4,320.3 ha, which is only 8.75 % of the area of all registered changes for the period. The process of deforestation dominates. As a result of the two processes the forested areas in the forest territories have decreased by 16,251.8 ha.

Results from the project „Corine Land Cover 2012"

According to the annual report of the Executive Forest Agency (Anonimous 2013), at the end of 2012 the forest territories have a total area of 4,163,415 ha. The forested area is 3,795,338 ha, which is 91.15 % of the total area of the forest territories and 34.19 % of the territory of Bulgaria. The results from the „CORINE Land Cover 2012" project for the studied 6 types of land cover are summarized in Table 5. Forested areas are 3,531,711 ha or 31.42 % of the mapped territory. This area is with 263,596 ha smaller than it is according to

Table 5. Land cover classes in the database CLC2012 – Bulgaria.

No	CLC code (level 3)	CLC class	Number of polygons	Area	
				ha	%
1	3.1.1.	Broad leaved forest	5,697	2,337,881	20.80
2	3.1.2.	Coniferous forest	1,932	542,772	4.83
3	3.1.3.	Mixed forest	4,112	651,058	5.79
4	3.2.2.	Moors and heathlands	82	26,738	0.24
5	3.2.4.	Transitional woodland scrub	8,079	741,423	6.60
6	3.3.4.	Burnt areas	3	441	0.00

the data of the EFA for 2012. The difference is negligible again, and the main reasons are the same as those already mentioned for the previous stages of the Project.

Trends in the Development of Forested Areas Within the Forest Territories of Bulgaria from 1990 to 2012

The processes of deforestation and afforestation in the forest territories for the periods 1990–2000, 2000–2006, 2006–2012 and totally for the entire period 1990–2012 are summarized in Table 6. The number of polygons is shown within the three types of forests according to the CLC nomenclature, as well as their area and percentage share in the total mapped territory. The ratio between the areas covered by the two processes can be seen in Fig. 3. The period in which the afforestation process dominates the process of deforestation is 1990–2000. In the period 2000–2006, forest destruction is in the largest scale. Figure 4 presents the balance of forested areas in the forest territories by periods. Out of the three forest classes only the class „conif-

Table 6. Deforestation and afforestation in forested areas within the forest territories from 1990 to 2012.

Period	Deforestation				Afforestation				Balance for the period, ha
	Change	Number	Area		Change	Number	Area		
			ha	%			ha	%	
1990–2000	3.1.1. – 3.2.4.	530	20,935.6	70.11	3.2.4. – 3.1.1.	745	28,117.9	76.47	+7,182.3
	3.1.2. – 3.2.4.	77	5,288.0	17.71	3.2.4.–3.1.2.	42	1,212.7	3.30	-4,075.3
	3.1.3. – 3.2.4.	94	3,635.8	12.18	3.2.4.–3.1.3.	178	7,437.3	20.23	+3,801.5
					Balance for the period 1990–2000				**+6,908.5**
2000–2006	3.1.1. – 3.2.4.	1,273	22,006.2	64.93	3.2.4.–3.1.1.	613	11,393.2	84.60	-10,613.0
	3.1.2. – 3.2.4.	236	7,414.3	21.88	3.2.4.–3.1.2.	25	514.4	3.82	-6,899.9
	3.1.3. – 3.2.4.	149	4,471.9	13.19	3.2.4.–3.1.3.	47	1,558.8	11.58	-2,913.1
					Balance for the period 2000–2006				**-20,426.0**
2006–2012	3.1.1. – 3.2.4.	1,067	14,317.2	69.88	3.2.4.–3.1.1.	183	4,312.7	99.83	-10,004.5
	3.1.2. – 3.2.4.	138	3,726.7	18.19	3.2.4.–3.1.2.	1	7,6	0.17	-3,719.1
	3.1.3. – 3.2.4.	136	2,444.3	11.93	3.2.4.–3.1.3.	0	0	0	-2,444.3
					Balance for the period 2006–20112				**-16,167.9**
Total for the period 1990–2012	3.1.1. – 3.2.4.	2,870	57,259.0	67.98	3.2.4.–3.1.1.	1,541	43,823.8	80.33	-13,435.2
	3.1.2. – 3.2.4.	451	16,429.0	19.49	3.2.4.–3.1.2.	68	1,734.7	3.18	-14,694.3
	3.1.3. – 3.2.4.	379	10,552.0	12.53	3.2.4.–3.1.3.	225	8,996.1	16.49	-1,555.9
					Total balance for the period 1990–2012				**-29,685.4**

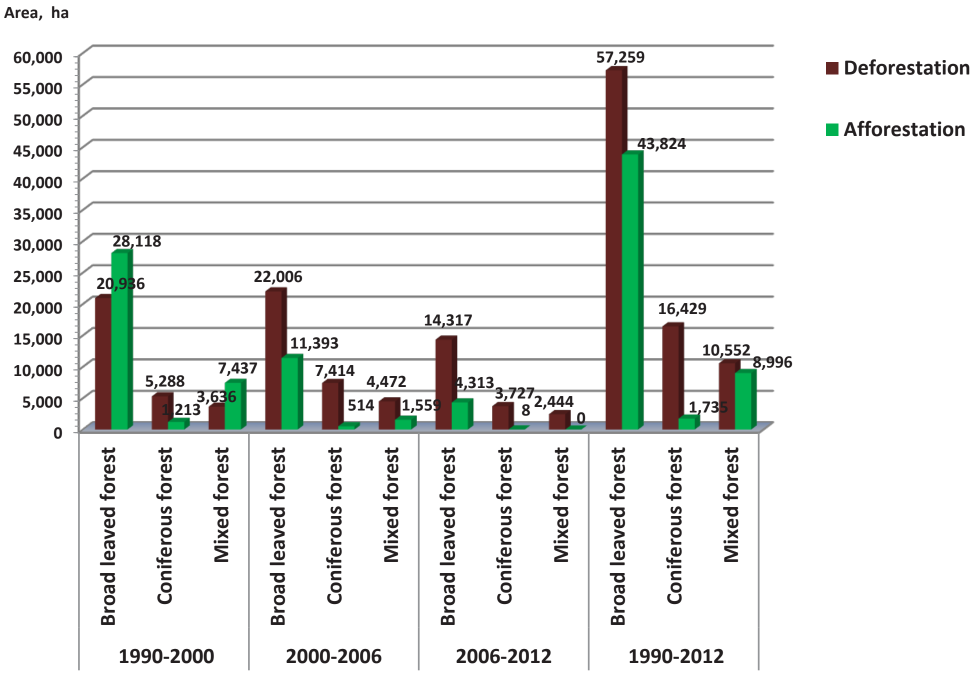

Fig. 3. Processes of deforestation and afforestation in the forest territories from 1990 to 2012.

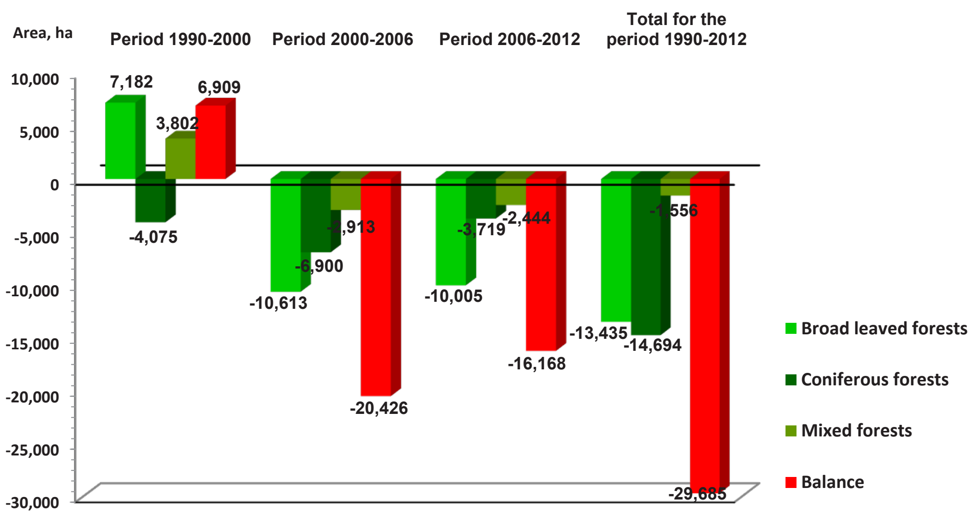

Fig. 4. Balance of wooded areas in the forest territories from 1990 to 2012.

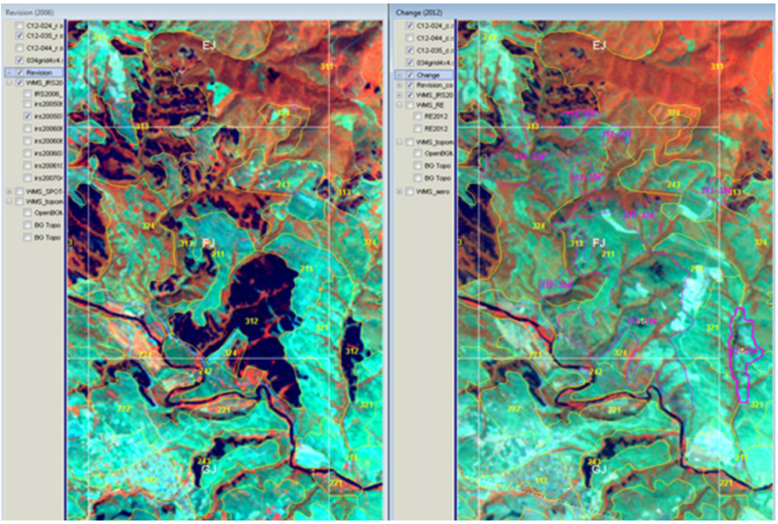

Fig. 5. Felled coniferous forests – 9 polygons „Change" with a total area of 591 ha.

erous forests" has a negative balance for all three parts of the 22-year period. This is explained by the market situation and the high demand for large construction timber as well as with the lack of coppice regeneration in these forests and the reduced area of planted conifers. When analyzing these changes, cutting of large massifs of coniferous forests makes impression. Even in polygons, specified as „mixed forests" only coniferous trees are cut down. The examples which may be given are numerous. Figure 5 shows such changes from the project „Corine Land Cover 2012". In the right window 9 polygons of changes „3.1.2. – 3.2.4." are delineated (north of Tserovo, District Pazardjik) with a total area of 591 ha.

In deciduous forests deforestation is contributed by illegal logging near settlements carried out in search of firewood, intentional fires, charcoal production etc. For the period 1990–2012, the forested areas in the forest territories of Bulgaria have decreased by 29,685.4 ha, which is 0.84 % of the total forested area in 1990. This is an alarming trend that after decades can redraw the map of the land cover types of Bulgaria.

It should be noted that a precise and fair comparison between those results and the official statistics of EFA, which is updated annually, is difficult to make. The reason is that the data for each Forest Range are updated every 10 years, but not simultaneously for all ranges. These data are of varying degrees of actuality for the different forest ranges.

Conclusions

The results of the computer assisted interpretation and the analysis of multitemporal satellite images within the EU project „CORINE Land Cover" allow an objective monitoring of the changes in the forest territories of Bulgaria. The analysis of these results leads to the following conclusions:

1. On the background of the increase of the total forested area in Europe, in Bulgaria for the 22-year period under consideration the process of deforestation predominates.

2. Although for the period 1990–2000 the balance is positive and afforestation dominates deforestation, under the

changed methodology of the project „CORINE Land Cover" and the identification of a large number of illegal cuttings for the period 2000–2012 the total balance is negative.

3. Out of the three forests classes only this of coniferous forests has a negative balance for all three parts of the 22-year period. This can be explained by the market situation and the high demand for large construction timber as well as with the lack of coppice regeneration in these forests and the reduced area of planted conifers.

4. In deciduous forests deforestation is contributed by:

- illegal logging near settlements carried out in search of firewood;
- intentional fires, since only the bark of deciduous trees is scorched but the quality of wood is not reduced;
- charcoal production, which leads to another harmful environmental effect – evolution of harmful emissions into the air etc.

5. In the afforestation process self-seeding has the largest share. This phenomenon (as it is in most of the European countries) in Bulgaria is most commonly seen over abandoned agricultural areas located close to forests.

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SEED QUALITIES AND SEED DORMANCY BREAKING OF SYCAMORE MAPLE (*ACER PSEUDOPLATANUS* L.) SEEDS

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Abstract

Mature seeds of Sycamore maple have a physiological dormancy. Different studies suggest fall sowing or cold stratification for a period varying from 3 weeks to 3 months. The aim of the study is to find optimal way for dormancy breaking of sycamore seeds in South-East Europe conditions. Variants of fall sowing, spring sowing of stored in dry substratum (untreated) seeds and sowing after 1, 2, and 3 months cold stratification were tested. The results showed that during a 2 and 3 months cold stratification the seeds germinate in substratum (relatively 51.5 % and 71.8) and they become useless for sowing. Only 7.0 % of seeds germinate during 1 month cold stratification period in the substrate. The germination after fall sowing is 68.8 %. After spring sowing of untreated seeds and 1 month cold stratificated seeds the germination is relatively 82.3 % and 82.75 %. The height of seedlings was smaller after fall sowing – average 9.76 cm. The seedlings produced after spring sowing of untreated seeds had average height 12.3 cm. The seedlings of seeds, which were cold stratified for 1, 2 or 3 months prior to spring sowing, exhibited the best growth in height (18.5–19.5 cm). The results bring to some conclusions. It is not recommended to store Sycamore maple seeds till spring in wet substrate because it has characteristics of cold stratification and lead to their early germination. The best way for seed dormancy breaking is 1 month cold stratification at 4 °C. The germination dynamics show that stratification have to begin 45 days before the date of last spring frost and the sowing have to be done 15 days before the determinate date. Second variant is spring sowing of seeds stored in dry substrate. In this case germination begins 25 days after the sowing and there is no danger of frost caused damages.

Key words: cold stratification, fall and spring sowing.

Introduction

In the recent years, there is a growing interest in broadleaves for afforestation in Bulgaria (EFA 2012). On the other hand, the conservation of biodiversity requires attention to the native species (Anonymous 2011). In this regard there is a reserve in usage of the species from genus *Acer*. It is considered that Sycamore (*Acer pseudoplatanus* L.) is one of the most valuable for the forestry (Pandeva 2004). It has relatively limited distribution in the country. It is found mostly in the mixed deciduous forests, separately, in small or bigger groups in all mountains, on fresh habitats in altitude up to 1400 m (Milev et al. 2004). It is spread over 2511 ha, which is 0.07 %

of the forest territory and 0.11 % of the deciduous forest territory (Dobrinov et al. 1982).

The information about Sycamore in the scientific literature in Bulgaria and other European countries is limited. Collection of reproductive materials and production of seedlings for afforestation in Bulgaria are not on the needed level from selection prospective (Pandeva 2004). The Sycamore seeds in Bulgaria are collected from the stands included in the National Register of Forest Seed Sources (National Register ... 2015), but the breeding work with it is limited to species level.

For the forest seed production specific characteristics of the Sycamore seeds are dynamic changes and high level of the moisture content which lead to perishability, as well as the physiological dormancy at the stage of ripping of fruits (Hong and Ellis 1990). In various researches, the authors recommend different periods of sowing and conditions of storage and stratification for successful germination of the Sycamore seeds.

It needs to be considered, that the optimal period of stratification depends on a lot of factors – the species, the origin, meteorological conditions in the ripping stage, physiological condition in the stage of collecting, period and conditions of storage (Milev et al. 2004).

Sycamore produces seeds every year, starting between 11th (Milev et al. 2004) and 25th year of age (Suszka et al. 1996, Joyce 1998) for the solitary growing individuals. In stands it starts around 25th (Milev et al. 2004) or around 30th year of age (Suszka et al. 1996, Joyce 1998). Harvests are regular, but abundant ones are in 1 or 2 years (Milev et al. 2004).

Collection of the seeds takes place in September (Krüssmann 1997), October or November. The yield of samaras is 80–90 % (Milev et al. 2004). The moisture content at this stage is 42–58 % (Joyce 1998, Milev et al. 2004, Iliev et al. 2015). Stejskalová et al. (2014) reported lower level of moisture content from 32.9 to 41.7 %. Suszka et al. (1996) differs the information for intact fruits (24–32 %), seeds (30–41 %) and pericarp (14–16 %). According to Milev et al. (2004) and Iliev et al. (2015) when the moisture level drops to 30 % the seeds die, Suszka et al. (1996) on the other hand reported lower minimum of 24 % moisture content.

The weight of a thousand seeds is approximately 85–95 g (Stilinović 1985) and varies from 70 to 221 g (Joyce 1998, Stejskalová et al. 2014), and in 1 kg there are between 6000 and 14,000 samaras (Milev et al. 2004).

Viability of the seeds varies from 50 % (Milev et al. 2004, Iliev et al. 2015) to 70–80 % and maintains for two years (Michev 1953, Stilinović 1985, Milev et al. 2004, Iliev et al. 2015). According to Suszka et al. (1996) the germination reaches 95 % in some cases.

There is unanimous opinion that fully ripened seeds fall in a physiological dormancy (Dirr and Heuser 1987, Krüssmann 1997, Joyce 1998, Milev et al. 2004, Hartmann 2010, Bertels 2012, Stejskalová et al. 2014, Iliev et al. 2015). Some authors assume presence of chemical compounds in the testa that inhibit germination (Thomas et al. 1973, Hong and Ellis 1990, Milev et al. 2004, Iliev et al. 2015).

In the literature recommendations to harvest seeds during the stage of physiological maturity is relatively rare (Bertels 2012). It is mostly recommended seeds to be sown in autumn (Michev 1953, Nestorovich et al. 1967, Dirr and Heuser 1987, Joyce 1998), as well as soaking the seeds one week before that (Hartmann 2010). Joyce (1998), however, takes note that very often the seeds germinate very early and the seedlings are damaged by the frosts.

Probably that is the reason most of the authors to recommend spring sowing of preliminary treated seeds.

For short storage from autumn to spring, there is no need for lowering the moisture content of the seeds, but for 2–3 years storage the moisture needs to be 24–32 % (for intact fruits) or 30–42 % (for seeds), and the temperature between -3 and -5 °C (Suszka et al. 1996). There are some opinions that Sycamore seeds can be stored up to 6–7 years, without losing their viability (Joyce 1998).

For the autumn ripping maples Hartmann (2010) gives a summarized recommendation for cold stratification at 4 °C for 60–120 days before spring sowing. For fruits with low moisture content (under 12 %) of the pericarp Bertels (2012) suggests even worm-cold stratification with one month warm stage at 20ºC or the alternative of 48 hours soaking at water at 40 ºC.

The most common suggestion is cold stratification for 3 to 8 weeks (Krechetova et al. 1978, Krüssmann 1997, Joyce 1998, Milev et al. 2004, Stejskalová et al. 2014, Iliev et al. 2015) or 2–3 months (Michev 1953, Nestorovich et al. 1967, Thomas et al. 1973, Dirr and Heuser 1987).

The stratification can take place in moist sand (Krüssmann 1997) or 50:50 moist peat and sand (Suszka et al. 1996, Stejskalová et al. 2014). It has been reported for stratification without substrate in open plastic bags for seeds preliminary soaked for 24 hours (Suszka et al. 1996, Joyce 1998).

The optimal temperatures for stratification are from 0 to 5°C (Michev 1953, Krechetova et al. 1978, Thomas et al. 1973, Suszka et al. 1996, Joyce 1998, Hartman et al. 2010, Bertels 2012, Stejskalová et al. 2014) with optimum moisture levels for stratification 44–50 % for intact fruits or 50–58 % for seeds (Suszka et al. 1996).

It needs to be periodically checked for early germination during the stratification right after the 20^{th} day (Milev et al. 2004, Iliev et al. 2015). It is known that when 5–10 % of the seeds germinate and the sowing is not possible at the time, the seeds can be stored at 3°C up to 3 months without losing viability (Joyce 1998). The alternative is storage in snow up to 45 days (Krechetova et al. 1978).

For practical reasons the samaras are removed before sowing and the fruits become oval and free flowing (Milev et al. 2004, Bertels 2012).

A rate of 32.5 to 49.0 g/m^2 is recommended when sowing in furrow and 80–100 g/m^2 when sowing freely (Suszka et al. 1996). In Bulgaria there is practice for furrow sowing with rate of 4.4 g/m (Iliev et al. 2015).

The germination starts 2–4 weeks after sowing (Krüssmann 1997).

The production rate for furrow sowing is 100, and for free sowing – 300–500 seedlings per 1 m^2 (Suszka et al. 1996, Krüssmann 1997), or mean number of 25–30 one year old seedlings or 20 numbers of 2-years old seedlings per meter (Iliev et al. 2015).

The aim of the present study is to test the germination qualities and to determine the optimum method for dormancy breaking of the Sycamore seeds, in the South-East European region.

Material and Methods

Individual Sycamore tree in park 'Vrana' area near Sofia was used for a source of reproductive materials. The fruits were collected on 7 October 2013. The fruits were stored in conditions which keep their initial moisture level, in mixture with vermiculite in 1:3 ratio, at 4°C in polyethylene package. Not fully developed, damaged by insects and empty seeds were preliminary removed by hand from the mixture.

The sowing qualities of the seeds were tested according to requirements of ISTA (1996). The weight of 1000 seeds and the relative moisture were determined in the laboratory of 'Forest plantations' at the University of Forestry in Sofia.

The viability of the seeds was tested by tetrazolium method in Forest Seed Control Station – Sofia and by excised embryo test in the laboratory of 'Forest plantations' at the University of Forestry in Sofia, where the result was evaluated on the 10th day of incubation. Furthermore, germination of the untreated seeds was tested in germination device, where the evaluation was made on the 30th day (germination on Jacobsen apparatus).

The seeds, stored in vermiculite were moved prior to spring sowing to mixture of pearl-stone (1:3) for one, two and three months of cold stratification at 4°C and 80 % moisture.

The sowing was made on 1 March 2014 in 'Sequoia' ornamental nursery. The seeds were sown in open air, in plastic plates for seedlings production with parameters of the plug 5×5×18 cm. and volume of 240 ml. The substrate was microfibril sphagnum peat with parameters of 0.5 mm particles; 20 kg/m³ volume density; pH in water was 5.5–6.5; electrical conductivity 35–45 mS/m ±25 %; enriched with fertilizer NPK (14:10:18) 1.5 kg/m². The depth of the sowing was 2 cm.

To determine the type and deep of the seed dormancy the following variants ware tested:

1. autumn sowing – 13.10.2013;
2. spring sowing of dry stored (not stratified) seeds;
3. spring sowing after 1 month cold stratification;
4. spring sowing after 2 months cold stratification;
5. spring sowing after 3 months cold stratification.

All variants were placed in 4 replications of 100 seeds. The seeds were irrigated daily through automatic irrigation system.

Germination was evaluated daily to the 45th day, when it finished.

The mean germination and the energy of germination were evaluated up to the 15th day. For calculation of the average seed dormancy, the following formula was used:

$$S_d = \frac{a_1 \cdot n_1 + a_2 \cdot n_2 + ... + a_i \cdot n_i}{\sum n_i}, \text{ in days,}$$

where: a_i – day of evaluation; n_i – sum of germinated seeds at the a_i-day of evaluation.

As additional criteria for the efficiency of the different methods for dormancy breaking, the height of the seedlings was measured after the end of vegetation period.

All results were analyzed by analysis of variance (ANOVA) and post hoc LSD test using SPSS 10.0 (SPSS for windows 1999). Percentage values were transformed using arcsine square root ($\sqrt{p}$) (Compton 1994) to normalize error distribution prior variance analysis.

Results and Discussion

The preliminary evaluation of the sowing materials showed that 72.3 % of them are morphologically fully developed (Table 1). With not fully developed embryo or damaged embryo were 27.7 % of the seeds. There is statistically proved difference between the weight and the moisture level of the two categories (Table 1). The moisture content of the fruits with normally developed embryos is 51.5 %, and the weight of 1000 fruits is 246.41 g. The same parameters of fruits, with the not fully developed embryos, are 42.42 % moisture content and 79.95 g weight of 1000 fruits. With the removal of the wings the mean weight changes significantly to 223.54 g.

The results show higher weight of 1000 fruits compared to the data reported by Stilinović (1985), Joyce (1998), Milev et al. (2004), Stejskalová et al. (2014), which is due to the preliminary separation of the fruits with not fully developed embryos. This method gives a better opportunity for forming quality lots and equally spread germination and equal distribution of the seedlings in the nurseries.

The moisture content of the intact fruits is 51.50 %. This result is in agreement with Joyce (1998), Milev et al. (2004), Iliev et al. (2015) and is higher than the data reported by Suszka et al. (1996) and Stejskalová et al. (2014). The fruits with not fully developed embryos are with statistically different moister content, with mean value of 42.42 %.

The results for the viability (Table 2) from the tetrazole method and the excised embryo test are statistically identical – 90.0 % and 92.0 %, respectively, which refers to first quality in the Bulgarian National Standard (BNS) 208:1999.

The extremely low percentage of germinated seed tested in germination device (6.0 %) proofs the presence of physiologi-

Table 1. Physical parameters and quality of the fruits.

Parameter	Normally developed embryo	Not fully developed embryo	De-winged fruits
Morphological development, %	72.34 ±2.42a	27.66 ±2.42b	–
Weight of 1000 fruits (samaras), g	246.41 ±6.61a	79.95 ±3.74b	223.54 ±6.31c
Relative moisture content, %	51.50 ±0.64a	42.42 ±1.39b	–

Note: The means and standard error within a column followed by tvvhe same letter were not significantly different, estimated by One-Way ANOVA follow by a post hoc LSD test at $p \leq 0.05$.

Table 2. Viability of the seeds tested by different method.

Method	Viability, %
Tetrazole method	92.00 ±1.35a
Excised embryo test	90.00 ±3.46a
Germination	6.00 ±1.15b

Note: The means and standard error followed by the same letter were not significantly different, estimated by One-Way ANOVA follow by a post hoc LSD test at $p \leq 0.05$.

cal dormancy in the seeds and the need of breaking it based on the right time of sowing and the appropriate period of cold stratification.

It needs to be considered that the values of viability presented are for seed lots that have been separated preliminary from the not developed seeds. If we consider that the percentage of fully developed seeds was 72.34 % then the real percentage of viability is 65.1–66.6 %. Nevertheless those results confirm the high germination ability of the Sycamore seeds reported by Stilinović (1985), Suszka (1996), Milev et al. (2004).

The test on different periods of stratification showed that for one month of cold stratification 7.0 % of the seeds germinate in the medium. From that point of view it is reasonably recommended by Milev et al. (2004), checking for germination after the 20th day of stratification. This reaction is a landmark that the lot is ready, the dormancy is already broken and in good conditions after sowing, a high percentage of germination can be expected. Confirming that is the high percentage of germination during the 2 months stratification (51.5 %) and the 3 months stratification (71.7 %) (Table 3).

Those high percentages of germination in the second and third month are an indicator that the stratification has to last no more than one month, because the germinated seeds are not suitable for sowing. Identical results have been reported by Stejskalová et al. (2014). Bertels (2012) recommends the already germinated seeds during stratification to be frozen until coming of a good period for sowing, but this method can be used for limited number of species and Sycamore is not one of them.

The germination started earliest (1 March) in the seeds sown in autumn. Stratified seeds start to germinate on 11th day after sowing, while at the 25th day of the sowing start to germinate unstratified seeds sown in spring (control variant) (Table 4).

The statistically proved lowest germination was registered in the lot that was sown in autumn – 68.75 % (Table 4). It can be considered that exposure of natural conditions during winter leads to loss of some of the initial germination ability, probably due to frosts.

When the seeds were stratified one month or not stratified the germination percentages were statistically identical – 82.75 and 82.25 %, respectively. It means that the temperature was still low after the sowing on 1 March and provided the necessary conditions to break the non-deep seed dormancy.

From the seeds not germinated during stratification 24.25 % germinate from the 2 months stratified and 15.25 % germinate from the 3 months stratified. Together with the germination during stratification the percentage is very high – 75.75 % and 87.0 for the 2 and 3 months stratified respectively.

Considering the germination dynamic we find out that the process continues 41 days for the autumn sown seeds and a little bit longer (45 days) for the control lot sown in spring and the lot stratified for one month and sown in

Table 3. Germination in the medium during the stratification.

Period of stratification, months	**1**	**2**	**3**
Germination, %	7.00 ±1.35a	51.50 ±4.21b	71.75 ±2.84c

Note: The means and standard error followed by the same letter were not significantly different, estimated by One-Way ANOVA follow by a post hoc LSD test at $p \leq 0.05$.

Table 4. Germination of seeds depending on different methods of preparation and season of sowing.

Method	Germination, %	Beginning of germination, date	End of germination, date	Period, days	Eg, %	ASD, days
Autumn sowing	68.75 ± 3.75c	01.03	10.04	41	40.75	10.2
Spring sowing control	82.75 ± 2.32d	25.03	09.05	45	0.00	12.7
Cold stratification 1 month	82.25 ± 0.63d	11.03	24.04	44	1.00	13.1
Cold stratification 2 months	24.25 ±1.89b	11.03	10.04	31	0.25	10.5
Cold stratification 3 months	15.25 ± 0.75a	11.03	10.04	25	1.50	8.5

Notes: Eg – energy of germination; ASD – average seed dormancy. The means and standard error within a column followed by the same letter were not significantly different, estimated by One-Way ANOVA follow by a post hoc LSD test at $p \leq 0.05$.

spring (44 days). The longer stratification shortens the period of germination to 25–31 days.

The energy of germination was highest with the autumn sown seeds (40.75 %) and very low for spring sown seeds – from 0.0 % (spring control lot) to 1.5 % (spring lot after 3 months stratification).

The average seed dormancy of the autumn sown seeds was 10.2 days. For the spring control lot it was prolonged to 12.7 days, and for the spring lot stratified for 1 month it was 13.1 days.

The parameters of physiological qualities of the seeds recorded for the different methods of preparation are the main criteria for the efficiency of those methods. A secondary criterion is how those methods influence the growing of the seedlings (Table 5). The results show formation of three statistically different groups. With slowest growth are the seedlings from seeds sown in autumn (mean height 9.76 cm). The seeds stratified for 1, 2 and 3 months produce the highest seedlings with height from 18.46 ± 5.71 to 19.40 ± 5.49 cm (Table 5).

Statistical parameters that proof the significance (significance level p = 0.000) of the different methods for dormancy breaking on the seedlings growth during first vegetation period, are shown in Table 6.

Table 5. Influence of the different seed preparation methods on the seedlings height.

Method	Height of the seedlings, cm
Autumn sowing	9.76 ± 1.84a
Spring sowing control	12.27 ± 2.48b
Cold stratification 1 month	19.24 ± 4.68c
Cold stratification 2 months	19.40 ± 5.49c
Cold stratification 3 months	18.46 ± 5.71c

Note: The means and standard error followed by the same letter were not significantly different, estimated by One-Way ANOVA follow by a post hoc LSD test at $p \leq 0.05$.

In general our results show the opportunity which Suszka (1996) mentioned,

Table 6. Significance of the different preparation methods on the seedlings height.

Factor	*df*	*F*	Sig
Method of preparation	4	346.425	0.000

Fig. 1. Seedling with 3 cotyledons.

that the Sycamore seeds can be successfully sown in spring without stratification. This is possible because their high moisture content keeps their physiological activity during storage in dry conditions and the moderate physiological dormancy can be broken from the cold temperature influence, during this period of storage.

In addition we observed some anomalies in germination. Seedlings with 3 cotyledons appeared with small frequency 0.091 % (Fig. 1). Similar information exists for some other tree species as Black locust (Iliev and Chavdarov 1996) and Honey locust (Iliev et al. 2015).

Conclusion

The usual practice in Bulgaria of sowing the Sycamore seeds in autumn is not the most appropriate method of work with the species.

It is not recommended to store Sycamore seeds in high moisture conditions during winter, because it acts as a cold stratification and germination starts before the right period for spring sowing. Storage at dry conditions at temperature of 4 °C, for keeping the initial moisture content of the seeds, is appropriate.

The seeds are characterized with non-*deep* physiological dormancy. The best method to break it is cold stratification at 4 °C for one month, followed by spring sowing.

Stratification needs to start 45 days before the average date of the last spring frosts and sowing needs to take place 15 days before that date.

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EFFECTS OF GIBBERELLIC ACID AND COLD STRATIFICATION ON SEED GERMINATION OF TWO *SORBUS* SPECIES

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Abstract

The present study aims at investigating the effects of gibberellic acid (GA_3) application and cold stratification on the germination of *Sorbus domestica* L. and *Sorbus torminalis* (L.) Crantz seeds. In particular, seeds of both species were treated with 500, 1000 and 2000 ppm GA_3 for 30 hours and were, subsequently, cold stratified at 3–5 ºC for 0, 1, 2 and 3 months. In addition, seeds from each species were only cold stratified for 0, 1, 2 and 3 months (control). In both species, non-stratified and 1-month cold stratified seeds, despite having been treated with GA_3 solutions, exhibited very low germination percentages (0.8–4.2 % for *S. domestica* and 0.0–1.7 % for *S. torminalis*). However, in control seeds and seeds treated with GA_3 solutions an increase in the cold stratification period from 2 to 3 months increased germination percentages significantly in both species. In *S. domestica*, after a 2-month period of cold stratification, the germination percentage of the seeds treated with 2000 ppm GA_3 (30.8 %) was significantly higher than that of control seeds (18.3 %). In 3 months of cold stratification, there were no significant differences in the germination percentages between control seeds (87.5 %) and seeds treated with GA_3 solutions (89.2–91.7 %). In *S. torminalis*, the GA_3 application significantly improved the germination of 2 and 3-month cold stratified seeds. After a 2-month period of cold stratification, the germination percentage of seeds, which had been treated with 2000 ppm GA_3 (30.0 %), was higher than the germination percentages of the seeds which had been treated with 500 and 1000 ppm (15.8 and 14.2 % respectively). After a 3-month period of cold stratification there were no significant differences in the germination percentages between seeds treated with GA_3 solutions (88.3–91.7 %). The results demonstrated that the optimum germination percentages of *S. domestica* and *S. torminalis* were obtained after a 3-month cold stratification period. In both species, the application of GA_3 did not replace or shorten the required cold stratification period.

Key words: seed dormancy, service tree, *Sorbus domestica*, *Sorbus torminalis*, wild service tree.

Introduction

The genus *Sorbus* L. (Rosaceae) includes about 100 species of deciduous trees and shrubs which are widely distributed in the Northern Hemisphere (Krussmann 1984). Two of the most widespread European species of the genus *Sorbus* are *Sorbus* *domestica* L. (Service tree) and *Sorbus torminalis* (L.) Crantz (Wild service tree) (Tutin et al. 1968–1992). In Greece, where *S. domestica* is rather rare, scattered individuals were mainly found in the continental part of the country, whereas *S. torminalis* is mainly found in the northern part of the country, mostly in sub-mountain and mountain regions (Boratynski et al. 1992). Both of them grow in deciduous forests (Browicz 1982, Boratynski et al. 1992). In a forest ecosystem, the ecological significance of both species is great due to the forage (fruits) which they offer to wildlife species. Furthermore, their timber is valuable due to its excellent aesthetic and technical characteristics (Coello et al. 2013). As a result, the specific species have to be propagated in nurseries and, subsequently, introduced in reforestation programmes.

Sorbus species are usually propagated by seeds (Stein 2008). However, seeds of most *Sorbus* species, similarly to many other species of temperate deciduous forests, have only a dormant embryo (Dirr and Heuser 1987), which complicates germination. Therefore, overcoming seed dormancy is one of the major steps to ensure a rapid, uniform and high germination. Cold moist stratification is a technique, which is widely used for breaking embryo dormancy and enhancing the germination of seeds in numerous species (Macdonald 2006). According to Stein (2008), *Sorbus* seeds do not germinate readily and require a period of cold moist stratification. Despite the fact that the seeds of *Sorbus* species have a fully developed embryo (Martin 1946, cited in Baskin and Baskin 1998) warm followed by cold stratification is recommended for seeds of some *Sorbus* species (Dirr and Heuser 1987, Piotto et al. 2003). The length of the cold stratification period, which is necessary for dormancy removal, varies among the species of the genus. In general terms, the seeds of *Sorbus* species require at least a 2-month stratification period for good germination (Hartmann et al. 1997, Young and Young 1992). According to ISTA (1999), a 4-month stratification period at 3–5 ºC is required for the seeds of *Sorbus* species to release from dormancy. In *S. domestica* and *S. torminalis,* a 45-day period of cold stratification is sufficient to break dormancy in fresh seeds (Gultekin et al. 2007). Var et al. (2010), studying the seed germination of four *S. torminalis* provenances, found that the maximum germination percentage for all provenances was achieved after 4 months of stratification at 2 ºC. Apart from cold stratification, various chemical solutions are used to stimulate seed germination (Macdonald 2006). Gibberellic acid (GA) is one of the growth regulators that can be used to partially or fully replace the necessary period of cold moist stratification in a number of plant species (Baskin and Baskin 1998). In the relevant literature, however, the effects of GA_3 treatment on seed germination of *S. domestica* and *S. torminalis* have not been reported.

The objectives of the present study are to: i) examine the effectiveness of gibberellic acid and cold stratification on germination, ii) describe the effects of gibberellic acid and cold stratification treatment combinations on germination, and iii) propose effective treatments to maximize germination of *S. domestica* and *S. torminalis* seeds.

Materials and Methods

In autumn 2010, mature fruits of *S. domestica* and of *S. torminalis* were collected from trees growing in their natural habitat in Northern Greece (41º12´40´´N, 25º15´31´´E, 500 m elevation and 40º28´06´´N, 22º15´45´´E, 360 m elevation, respectively). In particular, fruits of *S. domestica* were collected in the middle of September and fruits of *S. torminalis* – in late October. After collection, the fruits of both species were pulped manually and the seeds were separated from the pulp using sieves and running water. It is worth noting that with flotation, apart from light debris, empty seeds were also removed. Subsequently, the cleaned seeds of both species were spread out on filter paper in laboratory conditions and left to dry. After drying, the seeds were stored in glass containers in refrigerator (3–5 ºC) until they were used in the experiments.

Seed treatment

Germination experiments were carried out in the following December in the laboratory of Silviculture, Department of Forestry and Natural Environment, Aristotle University of Thessaloniki. For each species, an experiment was carried out to determine the effects of gibberellic acid (GA_3), cold stratification (CS) and combination of GA_3 with CS on seed germination. Seeds of each species were soaked in solutions of GA_3 for 30 hours. The concentrations of GA_3 solutions were 500, 1000 and 2000 ppm. Subsequently, the treated seeds were placed in plastic containers and were mixed with moist sterilized river sand and left to stratify at 3–5 ºC for 0, 1, 2, and 3 months. For each species, three plastic containers, which corresponded to the concentrations of GA_3, were used and 12 treatments (combinations of GA_3 solutions with CS periods) were applied. In addition, seeds (control) from each species were soaked in distilled water for 30 hours and then were subjected to CS for 0, 1, 2, or 3 months. For each species there was a plastic container. During stratification, sand moisture was checked periodically and water was added, when necessary, to keep it moist.

Germination test

For each species, at the end of each CS 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). For each treatment, there were 4 replications of 30 seeds. The seeds were placed on sterilized river sand moistened with distilled water in 9-cm plastic Petri dishes. Prior to the arrangement of seeds in Petri dishes, the seeds were dusted with fungicide (Captan) to avoid fungi development. The Petri dishes were randomly arranged on the shelves of the growth chamber and were watered with distilled water, as necessary. The temperature in the growth chamber was set at 20 ºC for a 16-hour dark period and 25 ºC for an 8-hour light period. The germinated seeds were counted once a week for a period of 7 weeks. A seed with at least 2 mm long radicle was considered to be germinated (ISTA 1999). Finally, for each treatment of each species, the germination percentage (GP) was calculated as the average of the 4 replications.

Statistical analysis

For each species, a completely randomised experimental design was used. In both species, treatments, in which none of the seeds germinated or the germination percentage was lower than 5.0 %, were not included in the statistical analysis. The germination percentage data were arc-sine square root transformed before analysis (Snedecor and Cochran 1980). The transformed data were checked for normality and homogeneity of variances and then analysed by one-way ANOVA. Comparisons of the means were made using the Duncan test (Klockars and Sax 1986). All statistical analyses were carried out using SPSS 20.0 (SPSS, Inc., USA).

Table 1. Effects of GA_3 and CS on GP of *S. domestica* and *S. torminalis* seeds.

GA_3, ppm	CS, months	*S. domestica* GP, % ±S.D.	*S. torminalis* GP, % ±S.D.
Control	0	2.5 ±1.67	0.0
	1	1.7 ±1.92	0.0
	2	18.3 c ±4.30	7.5 e ±3.19
	3	87.5 a ±3.19	79.2 b ±5.69
500	0	0.8 ±1.67	0.8 ±1.67
	1	2.5 ±3.19	0.8 ±1.67
	2	20.8 bc ±5.00	15.8 d ±5.00
	3	89.2 a ±5.00	90.8 a ±5.00
1000	0	0.8 ±1.67	0.0
	1	4.2 ±1.67	1.7 ±1.92
	2	28.3 bc ±5.78	14.2 d ±4.19
	3	90.0 a ±6.09	88.3 a ±4.30
2000	0	2.5 ±1.67	0.8 ±1.67
	1	3.3 ±2.72	0.0
	2	30.8 b ±5.69	30.0 c ±6.09
	3	91.7 a ±6.38	91.7 a ±4.30

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

Results

There were significant differences in GPs ($\alpha = 0.05$) among the treatments applied in *S. domestica* and *S. torminalis* seeds [$F_{(7,24)} = 77.69$, $p = 0.00$ for the *S. domestica* and $F_{(7,24)} = 149.26$, $p = 0.00$ for the *S. torminalis*].

In both species, regardless of GA_3 treatment, the seeds, which were not stratified or stratified at 3–5 ºC for 1 month exhibited very low GPs (0.8–4.2 % for *S. domestica* and 0.0–1.7 % for *S. torminalis*) (Table 1). In control seeds, as well as in the seeds treated with GA_3 solutions, an increase in CS period from 2 to 3 months demonstrated a significant increase in GPs in both species.

In *S. domestica*, after a 2-month period of CS, the germination percentage of seeds treated with 2000 ppm GA_3 (30.8 %) was higher ($p < 0.05$) than that of control seeds (18.3 %). However, after a 3-month period of CS there were no significant differences in GPs between control seeds (87.5 %) and seeds treated with GA_3 solutions (89.2–91.7 %). Furthermore, in seeds stratified for 2 as well as 3 months, no significant differences in GPs among the treatments with the GA_3 solutions were observed.

In *S. torminalis*, GA_3 application significantly improved the germination of 2 and 3-month cold stratified seeds. After a 2-month period of CS, the germination percentage of seeds which had been treated with 2000 ppm GA_3 (30.0 %) was higher than the GPs of the seeds, which had been treated with 500 and 1000 ppm (15.8 and 14.2 %, respectively). However, after a 3-month period of CS there were no significant differences in GPs between seeds treated with GA_3 solutions (88.3–91.7 %).

Discussion

Non-stratified and 1-month stratified seeds of *S. domestica* and *S. torminalis*, regardless of GA_3 treatment, exhibited very low germination or failed to germinate, which confirms that the seeds of both species are dormant. As mentioned in the introduction, *Sorbus* seeds are characterized by having a fully developed but dormant embryo (Martin 1946, cited in Baskin and Baskin 1998, Dirr and Heuser 1987) and require a period of cold moist stratification to germinate (Stein 2008). According to Hartmann et al. (1997), hormonal changes occur during the CS of seeds with dormant embryo. A number of studies have shown that the ABA (a hormone, which is responsible for maintenance of dormancy) levels in embryo or seed decrease during the warm or cold stratification (Pinfield et al. 1987, Chien et al. 1998, Chen et al. 2007) and the gibberellin (GAs) (hormones that promote seed germination) levels in embryo increase during CS (Powell 1987, Chen et al. 2007). Furthermore, Oster et al. (1987) reported that germination inhibitors are present both in the seeds and fruits of *S. aucuparia*. The seeds of both species, receiving only a 2-month period of CS exhibited low GPs (18.3 and 7.5 %, Table 1), which demonstrates that a 2-month period of CS was insufficient to release dormancy of seeds. Possibly, a 2-month period of CS is not sufficient to drastically decrease the ABA levels and increase the level of GAs in seeds of both species. In contrast, Gultekin et al. (2007) state that a 45-day period of cold stratification is sufficient to break dormancy in fresh seeds of *S. domestica* and *S. torminalis*. In their experiment, *S. domestica* and *S. torminalis* seeds stratified at 6 ±1 ºC for 45 days exhibited GPs equal to 92.25 and 91.75 %, respectively. In the present study, the seeds of both species, which were only stratified at 3–5 ºC for 3 months, exhibited high GPs (87.5 and 79.2 %, respectively). According to Orsanic et al. (2009), stratification for 105 days at 3 ºC is sufficient to break the dormancy of *S. torminalis* seeds. However, Var et al. (2010), studying the seed germination of four *S. torminalis* provenances, found that the maximum GP for all provenances was achieved after a 4-month stratification period at 2 ºC. In addition, Taylor and Gerrie (1987) reported that CS of *S. glabrescensis* seeds at 1 ºC for 12 weeks resulted in the highest germination and they also demonstrated that the germination of *S. glabrescensis* seeds was optimum at temperatures of 10 and 15 ºC; in addition, they argued that high temperature (25 ºC) inhibited germination and induced secondary dormancy. However, ISTA (1999), for seed germination of *Sorbus* species, recommends a 4-month stratification period at 3–5 ºC and then alternating temperature regimes of 20/30 ºC. Furthermore, at about the end of the 3-month CS period germinated seeds appeared in both species and therefore, a longer than a 3-month period of CS of seeds could not be applied in the present study.

The results of the present study demonstrate that the application of GA_3 in the seeds of both species does not replace or shorten the required CS period. According to Pipinis et al. (2012a), the application of GA_3 or kinetin in *Pyrus pyraster* (L) Burgsd. and *Malus dasyphylla* Borh. seeds does not replace or shorten the required CS period. However, exogenous GA_3 application has been reported to be effective in breaking dormancy and substituting for the CS requirement in the seeds of many species (Karam and Al-Salem 2001, Pipinis et al. 2011, Pipinis et al. 2012b, Pipinis et al. 2014). In addition, the application of GA_3 improved seed germination of the two studied *Sorbus* species. In particular, GA_3 application in *S. domestica* significantly improved the germination of 2-month only cold stratified seeds, whereas in *S. torminalis* significantly improved the germination of 2 and 3-month cold stratified seeds (Table 1).

Conclusions

The results of the present study demonstrate that the *S. domestica* and *S. torminalis* seeds exhibit physiological dormancy. A 3-month period of stratification at 3–5 ºC is essential for breaking dormancy in seeds of both species. It is also worth emphasizing that the application of GA_3 in seeds of both species does not replace or shorten the required CS period. However, GA_3 application prior to a 3-month period of CS maximizes germination in *S. torminalis* seeds.

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PHENOLOGICAL OBSERVATIONS OF *FAGUS SYLVATICA* L. DURING 2012 IN THE BALKAN RANGE

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Abstract

In the frame of implementation of International Cooperative Program 'Forests' – second level – (ICP Forests), since 2011 phenologycal observations of beech vegetation in the station of 'Vitinya' were conducted. The targets of this study, along with the data from the many years of observations (2001), related to the assessment of defoliation, phytocenotic changes, leaf analysis, meteorological parameters, air pollution, soil, etc., were to identify the key factors and processes in the functioning of it. The results of observations of the phenologycal development of common beech (*Fagus sylvatica* L.) in 2012 are: duration of vegetation, start and end of leafing, timing of autumn discolouration of leaves, beginning and end of leaf fall. Assessment of the results was combined with the average minimum and maximum air temperature in 2011 and 2012 and average monthly relative air humidity in both years.

Key words: intensive monitoring, phenology, phenologycal stages.

Introduction

In the intensive monitoring program Assessment and Monitoring of Air Pollution Effects on Forests (ICP-Forest Manual 2000), forest phenology is defined as 'systematic observation and recording of the development of tree species, biotic and abiotic events and phenomena'.

Studies of processes during the development and duration of major life events (phenology) are important for assessing of the condition of the trees. They also have a significant impact on other components of the ecosystem (Menzel 2002, Badeck et al. 2004, Vitasse and Basler 2012). Shifting in the beginning and ending phases of development can be directly linked to climate change, due to natural climate changes or anthropogenic interference.

The main objective of the phenological observations in the plot of 'Vitinya' was to provide additional information on the status and development of trees observed during the vegetation period. The data obtained complement the collected information on the remaining components – plant communities, soils, air pollution, meteorological data, etc. to evaluate the effect of climate change on forest ecosystems (Pavlova et al. 2002–2011).

Materials and Methods

The phenologycal observations were made on *Fagus sylvatica* L. located in the western part of the Balkan Range, near 'Vitinya' Pass. The area is situated in the temperate forest vegetation area zone. The stand is dominated by Common beech, aged between 120 and 140 years, at an altitude of 950 m west, at 23°55´48" latitude and 42°55´39" longitude (Kolarov et al. 2002). The soil is Distric-Eutric Cambisols, CM (Kolarov et al. 2002, ICP-Forest Manual 2000).

The observed trees were 15, dominant, with good visibility of the entire crown, including its top, with an opportunity to conduct observations from the same side of the crown throughout. Surveys were conducted once a week during two periods of the active growing season (April–June and September–November). The observed phases were: bud burst and leafing, flowering, fruiting, fall colouring and autumn leaf fall (User Phenologycal Observations 1960, Slavov and Kazandjiev 2006, Dittmar and Elling 2006). Presence of abiotic or biotic damages was detected during the observations. The evaluation of phenological data was made by using information for humidity and air temperature for the preceding 2011 and the year of observations – 2012.

Results and Discussion

Leafing of the observed trees in 2012 started on April 21 and ended within the next 7 to 10 days. The duration of the process, compared with the previous year, was much shorter, as leafing ended with formation of the leaf stalks simultaneously in all individuals. Figure 1 shows the variety of the beginning of unfolding for the observed trees, and the timing of its end. On Figure 4 it is shown the duration of leafing of trees observed in the number of days. The duration of the process is 7 to 14 days (Fig. 2). The trees are divided in tree groups – 7, 10, and 14 days duration.

Flowering was observed in almost all the trees (except tree No 2) in May 2012. Surveys showed that the intensity of flowering is weak (excluding tree No 19).

The average minimum and maximum air temperature and average monthly rela-

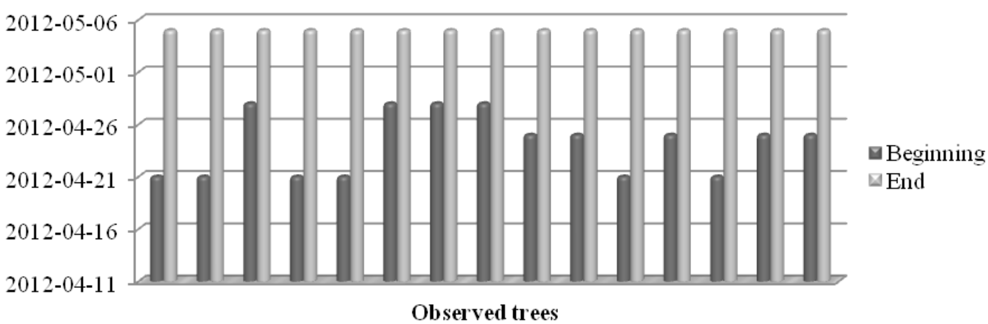

Fig. 1. Beginning and end of leafing, 2012.

tive humidity are shown in Figures 3 and 4. Minimum temperature in both years have a similar course. February 2012 is with the lowest average monthly minimum temperature. July is with the highest average monthly minimum temperature and it is higher than 2011.

The average monthly maximum temperature increase from March to December. For June, July and August excess is of 5 °C. Similar is the situation with the average monthly maximum temperature observed in October and November. In 2012 generally, relative air humidity was with lower values. Especially significant is the difference between the

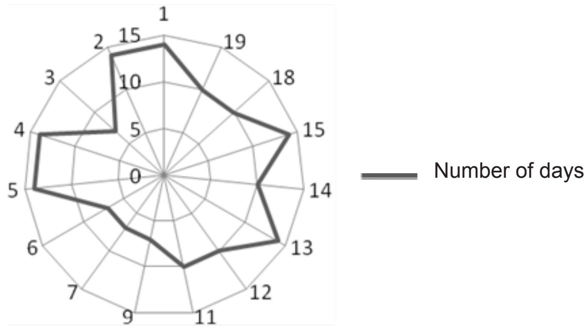

Fig. 2. Duration of leafing.

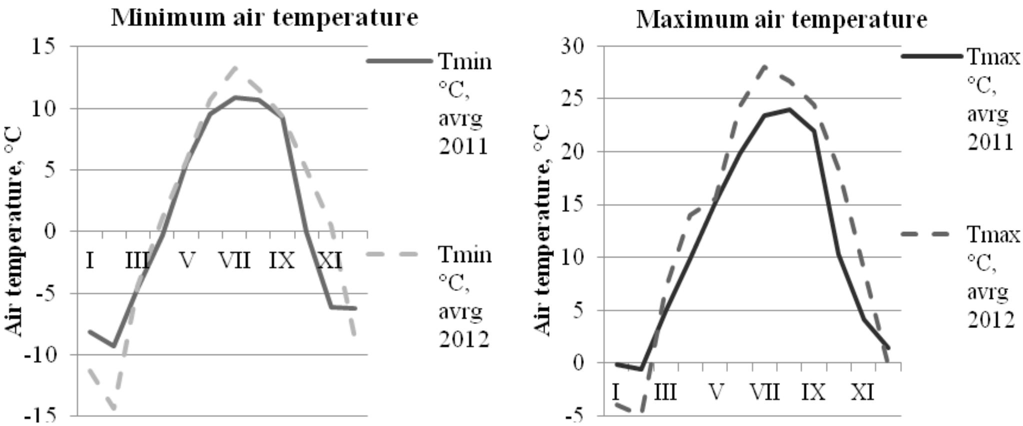

Fig. 3. Monthly average maximum and minimum air temperature in 2011 and 2012.

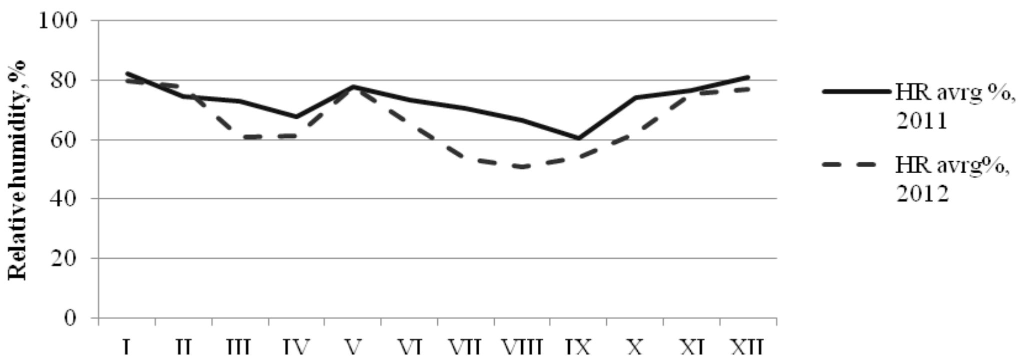

Fig. 4. Monthly average relative humidity in 2011 and 2012.

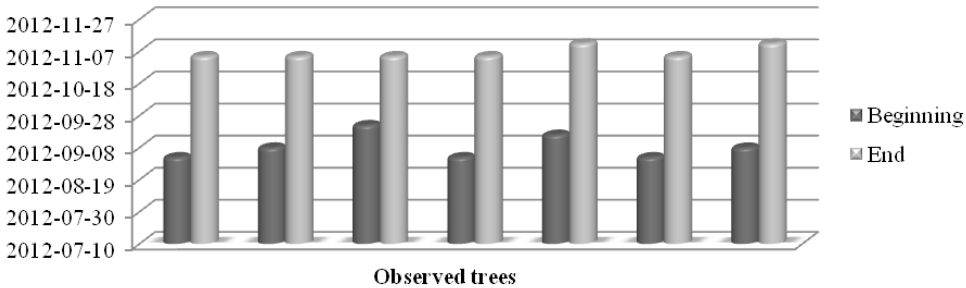

Fig. 5. Beginning and end of colouring in 2012.

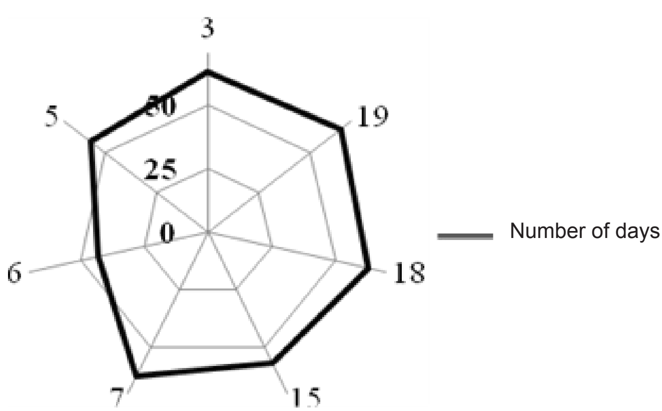

Fig. 6. Duration of yellowing in 2012.

two years in the period from May to November, when the accumulated air temperature and air relative humidity amounts directly related to the autumn colouring of the leaves.

During the reference year (2012) lack of fruiting was reported.

Autumn phenologycal phases in 2012 began very early. In the beginning of September instead of autumn colouring, was observed actively wilting of the leaves, which progressed to the middle of the month, and then the process was stable and relatively even (Fig. 7, 8, 9, 10, and 11). This is probably due to the low relative air

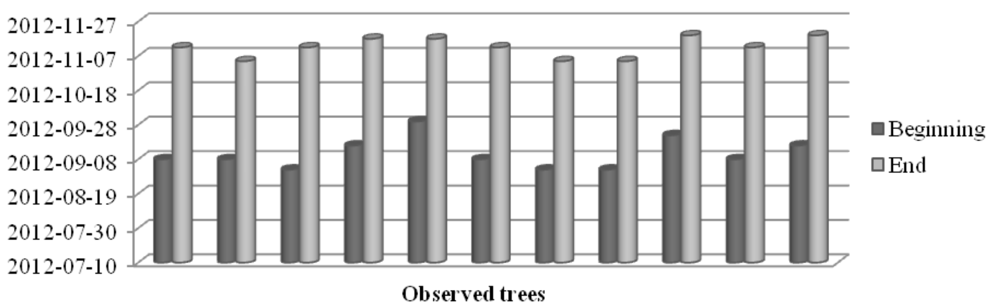

Fig. 7. Beginning and end of leaf fall in 2012.

humidity (Fig. 6) and high maximum air temperature (Fig. 5) recorded during the summer months. The combination of these weather indicators leads to difference in the occurrence of autumn phenologycal phases during 2012 compared to 2011. Yellowing of some of the trees continued 63 days in 2012. The longest phase in 2011 was 50 days. This is almost two weeks shorter than 2012.

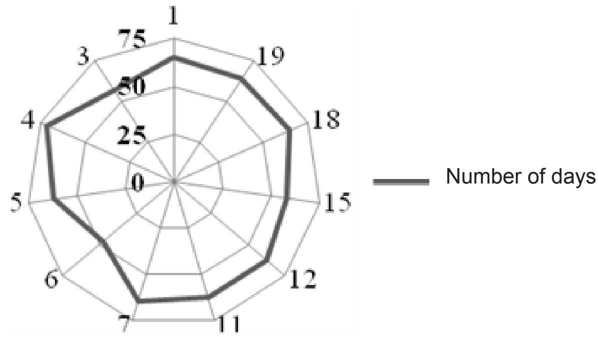

Fig. 8. Length of leaf fall in 2012.

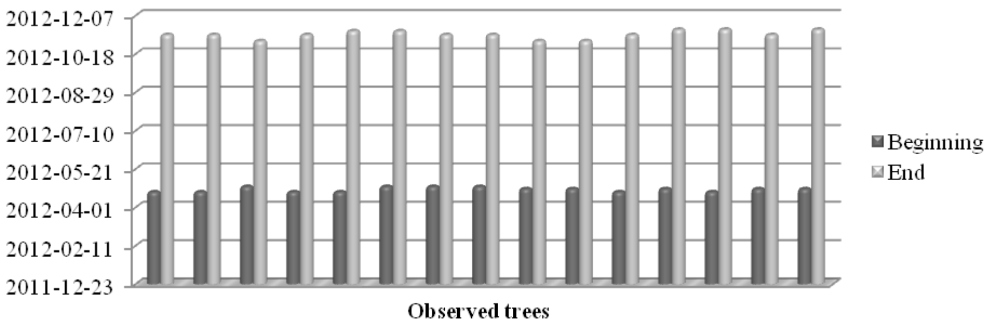

Fig. 9. Beginning and end of the growing season, 2012.

The autumn falling of leafs in 2012, and the autumnal colouring began very early (in the last days of August) due to the very high summer air temperature, low relative air humidity and drying of the leaves. After a rapid start, the process was gradually stabilized for about 65 days; all the trees passed through five levels of defoliation. Thus, during the reference year the ending of tree leafs falling was in the third decade of November unlike the previous year when it happened in the second decade of the month.

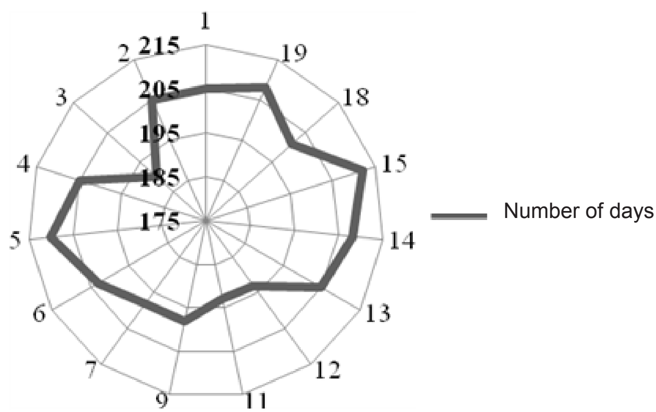

Fig. 10. Duration of the growing season, 2012.

In general, the vegetation period in 2012 started in the second decade of

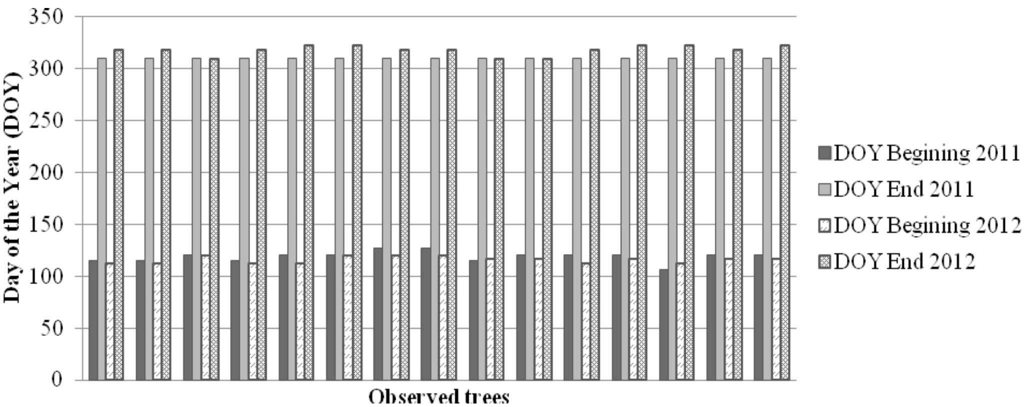

Fig. 11. Beginning and end of the growing season, 2011 compared with 2012.

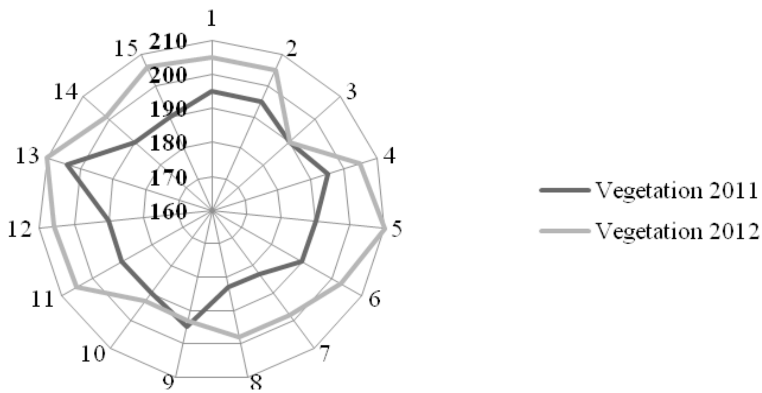

Fig. 12. Duration of the growing season, 2011 compared with 2012.

April with the bud burst, which – despite low minimum temperature in winter – almost coincides with those in the previous year. This is most likely due to the need to achieve a photoperiod of 13–15 h lighting of *Fagus sylvatica* L. (Čufar et al. 2012), suggesting little variation in leafing and spring phenologycal phases. In contrast, autumn phases were longer and, respectively, the growing season (Fig. 9 and 10) was extended in 2012. This is not the typical manner of an extension of the vegetation, as it generally is due to the extension of a flushing, but not in a delayed colouring and leafs falling.

Comparing 2011 and 2012 it is seen that the growing season began earlier and ended later in 2012 (Fig. 11). The duration of the process is longer in 2012 year and is 205 days, which is clearly seen in Figure 12 and in 2011 it is about 190 days.

Conclusions

In 2012 for *Fagus sylvatica* were observed the main phases of the development – buds burst, leafing, flowering, autumn colour change and leaf fall. The period of leafing in 2012 is in the range of 7–14 days, which is shorter than the previous 2011 year. There was an active flowering in all individuals. Extreme climate data (2012), air temperature and relative air humidity were leading to differences in autumn phenologycal phases. In early September, instead of changing the natural colour of the leaves, typical for this time of year, drying was observed. This period covered about 2 weeks and then colouring passed into relatively natural limits. In some trees, this stage (colouring) had duration of 63 days, which is almost two weeks longer than the previous year. Leaf falling followed the colouring lasting about a week more, and ended in the third decade of November. The vegetation period had a longer duration, due to the extension of the fall phenologycal stages (colouring and falling of leafs). This trend is not very common, because the most European tree species increase the duration of the vegetation period due to a greater degree of spring phases of development, not because of autumn once (Davi et al. 2011, Vitasse et al. 2011).

Acknowledgements

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STUDY ON SEED GERMINATION OF THIRTEEN DOUGLAS-FIR PROVENANCES

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Abstract

Rehabilitation of degraded forest ecosystems requires introduction of fast-growing productive and adaptive tree species, such as Douglas-fir. However, introduction of new species always carries an element of risk. This risk can be decreased by appropriate testing. Therefore, it is necessary to investigate the origin and success of species and to select the most suitable provenances. A very important stage in the process of provenance testing is the early control of seed material, which can improve and increase the growth of good-quality seedlings and provide better results in afforestation and establishment of new vegetation. Therefore, the Institute of Forestry in Belgrade set the germination test of Douglas-fir seed under different test conditions in order to identify and select the best seed sources for afforestation. Original Douglas-fir seed of different provenances in Canada was taken as seed material. The test included collection of seeds originating from thirteen different provenances in natural forests of Canada. The aim of the research was to determine the potentials and germination differences among the seeds of common provenances in different conditions – in laboratory and in nursery – and to select the best provenances. The relationship of seed germination and the geographic origin could be another important indicator for selection of Douglas-fir provenances. Regression and correlation analyses were used to determine the statistical dependence of the seed germination in different condition on the geographical origin of Douglas-fir seeds.

Key words: introduction, selection, seed transfer, seed quality, geographic origin,

Introduction

Introduction of adaptive allochthonous tree species has become increasingly important in forestry due to the modified growing conditions caused by the global climate change. Testing of new species with greater growth potentials and better chances to survive in the changed climate conditions will soon become a pressing need (Kjaer and Hansen 2013). The main objective of introducing exotic species into the existing and new forest ecosystems is to come up with the best solution that meets economic, ecological and aesthetic needs of the vegetation region of Serbia.

Introduction, as a model in forestry, entails a risk of selecting a bad provenance of the tree species of interest, which in turn causes long-term environmental effect on the vegetation structure of the region. Populations of species originating from different geographical locations (provenances) often show differential success due to the effect of genotype. Furthermore, provenances show significant differences in the characteristics of seedlings and the seed germination test reveals differences in their growth performance (Khurana and Singh 2001b).

Human beings have shaped forest ecosystems in Central Europe since prehistorical times, thus reducing the extent of forests to gain space for agriculture, thereby changing tree communities by cultivating the most useful tree species (Carnus et al. 2005).

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is a tree species with considerable growth potential, which makes it one of the most productive tree species of Canada and USA. As a fast-growing and highly productive species, it has become one of the most commonly introduced species in Europe. Douglas-fir was first introduced to Europe from USA more than 150 years ago. It was then planted on a large scale and it has now become economically most important exotic tree species in European forests (Schmid et al. 2014).

Knowledge on germination and seedling establishment is of pivotal importance for understanding such community processes as plant recruitment and succession and for the management of plant populations (Khurana and Singh 2001a). Douglas-fir, as an introduced species, has found an important place in the gene pool of forest tree species in Serbia (Šijačić-Nikolić and Milovanović 2007). Several sample plots of Douglas-fir trees of different provenances originating from USA and Canada were established with the purpose of testing characteristics of this species (Lavadinović and Koprivica 1999, 2000; Lavadinović et al. 1996; Radonja et al. 2003).

Seeds are one of the main sources of plants for restoration (Commander et al. 2010). Our aim was to get a deeper insight into the ecophysiology of seed germination of introduced species in different conditions. Understanding the seed germination and dormancy is important for many areas of plant science and technology, such as agriculture, restoration ecology and forestry (Baskin and Baskin 1998).

Germination under nursery or field conditions is often limited by unpredictable environmental factors. Epigeal germination is by far the most common in woody plants. All gymnosperms, and the major families of angiosperms have epigeal germination (Schmidt 2000). Knowledge on the ecology of germination and seedling growth is vital, not only for understanding the community processes of plant recruitment and succession, but also for developing strategies for the conservation of biodiversity and restoration of forests. In essence, seed is a miniature tree because it is responsible for its regeneration and ultimately for its reproductive success (Khurana and Singh 2001a). The objective of the present study was to determine the compatibility of seed germination of different Douglas-fir provenances under controlled laboratory and outdoor nursery conditions.

Material and Methods

In order to determine the characteristics of germination and differences between Douglas-fir provenances, the Institute of Forestry in Belgrade set a test of seed germination under different conditions. The seed from thirteen Douglas-fir provenances was used for the purpose of experimental analyses.

The seed was obtained through The National Tree Seed Centre of`Canadian Forest Service` in British Columbia, from the native range of Douglas-fir distribution in Canada. Spatial distribution of the provenances is shown in Figure 1, and their geographical characteristics are given in Table 1.

The germination of Douglas-fir seeds in laboratory were tested on the germination table (“Copenhagen table” or “Jakobson table”) where the water temperature was controlled by electronic digital temperature controller (Kamra 1968), temperature program of 25 °C for 10 hours (during day) and 20 °C for 14 hours (during night). The number of seeds that germinated during the test was recorded every two to four days. A seed was considered to have germinated when the emerged radicle was at least twice the length of the seed (Doody and O’Reilly 2005).

The basic seed pretreatment for nursery sowing included three-month seed stratification in a refrigerator at 5 °C. The seeds were rolled into a moist paper towel, sealed inside a plastic bag and placed in the refrigerator. The filter paper was changed every seven days in order to control and prevent seed infection.

In spring, the stratified seeds were sowed into containers in the nursery of the Institute of Forestry. Seed germination capacity was tested by applying the standard method of container seed

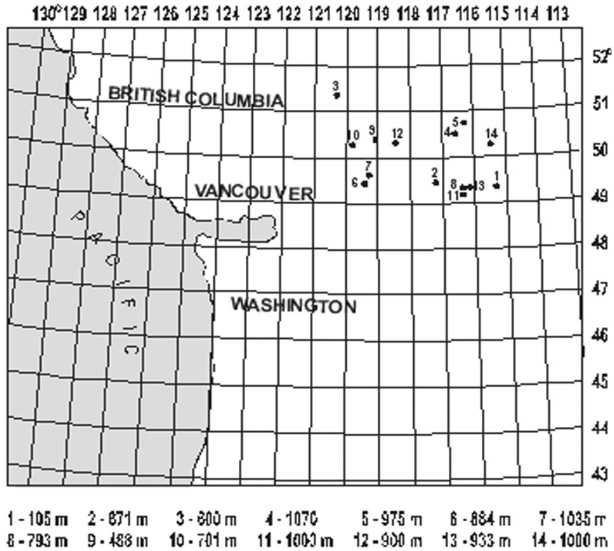

Fig. 1. Map of geographical distribution of the tested provenances.

Table 1. Geographic characteristics of the tested provenancesof Douglas-fir.

Provenance No	Provenance Code	Location	Geographic coordinates latitude	Geographic coordinates longitude	Altitude, m
1	03333	Cranbrook	49° 25'	115°20’	1050
2	00848	Inonoaklin	49° 50'	118°10’	671
3	30667	Mann Creek	51° 35'	120°10’	600
4	05227	Gavia Lake	50° 56'	116°35’	1070
5	05226	Nine Bay	50° 58'	116°32’	975
6	03356	Trout Cr	49° 40'	119°52’	884
7	03360	Michell CR	49° 54'	119°37’	1035
8	30460	Mara LK	50° 48'	119°00’	488
9	00278	Monte Crk	50° 37'	119°52’	701
10	03383	Sheep creek	49° 10'	117°15’	1000
11	30461	Cooke creek	50°38'	118°49’	900
12	03389	Benton creek	49° 12'	117°25’	933
13	05092	Sun Creek	50° 08'	115°52’	1000

sowing. We used 217 containers with 53 cells. Two seeds were sown in each cell, which made 21,412 seeds altogether. The containers were placed in the outdor under natural conditions of heat and light.

Several statistical methods, such as descriptive statistics, regression, correlation and testing, were used to process data of the germination rate of the seeds from different provenances. The methods of simple and multiple regression and correlation analysis were used to test the relationship of the seed germination rate with latitude, longitude and altitude of the seed source locations.

Results

Germination rates of Douglas-fir seeds from different provenances are presented in Table 2.

Table 2. Germination of Douglas-fir provenances under different conditions.

Provenance		Percentage of germinated seeds in the laboratory, %	Planted seeds in the nursery, number	Seedlings in the nursery, number	Seedlings in the nursery, %
No	Code				
1	03333	71	1 484	374	25.2
2	00848	96	1 802	808	44.8
3	30667	98	1 696	860	50.7
4	05227	95	2 014	632	31.4
5	05226	91	1 908	595	31.2
6	03356	70	1 696	568	33.5
7	03360	66	1 484	463	31.2
8	30460	98	1 590	731	46.0
9	00278	98	1 484	606	40.8
10	03383	86	1 590	586	36.8
11	30461	97	1 802	461	25.6
12	03389	81	1 484	354	23.8
13	05092	88	1 378	289	21.0
Average		**87**			**34.0**
Total			**21 412**	**7 327**	

The average germination rate of Douglas-fir seeds under laboratory conditions (laboratory climate chamber) amounted to 87 %, while it was 34 % under nursery conditions. Standard deviations were 11.71 % and 9.30 %, respectively, and coefficients of variation were 13.42 % and 27.34 % (Table 3). Provenance 7 (Michell CR – 03360) had the lowest germination rate under laboratory conditions 66 %, while provenances 3 (Mann Creek – 30667), 8 (Mara LK – 30460) and 9 (Monte Crk – 00278) had the highest germination rate of 98 %. The lowest rate of germination under nursery conditions (21 %) was achieved by provenance 13 (Sun Creek – 05092). Provenance 3 (Mann Creek – 30667) had the highest germination percentage (50.7 %) under the same conditions. There was a significant difference in the germination rate of the seeds from different Douglas-fir provenances.

Table 3 presents the main statistical parameters for the selected dependent (Y_1 and Y_2) and independent variables (X_1, X_2 and X_3).

Table 4 shows the coefficient matrix of the simple linear regression (r) between all variables.

The simple correlation coefficient of the germination of seeds in the laboratory with the latitude and altitude is statistically significant. However, the correlation coefficient between the seed germination in the nursery is statistically significant with the longitude and altitude, but not with the latitude.

Table 5 presents the coefficient matrix of the partial linear correlation (r) between all variables.

There is a statistically significant net correlation only between the seed germination under laboratory conditions and latitude at a confidence interval of 95 %. Other partial correlation coefficients are not statistically significant, suggesting that latitude, longitude and altitude have a common influence on seed germination. The statistical tests in laboratory and nursery are shown in Table 6.

(A) Laboratory Dependence of Douglas-fir seed germination in the laboratory (Y_1) on latitude (X_1)

The main parameters of parabolic regression are shown in Table 6, as the model number 1.

Model suggests that with increasing latitude provenance increases the parabolic their seed germination in the laboratory.

Dependence of Douglas-fir seed germination in the laboratory (Y_1) on longitude (X_2)

The main parameters of parabolic regression are shown in Table 6, as the model number 2.

The observed correlation is extremely weak. Only 7.68 % of the variance of seed germination under laboratory conditions can be explained by longitude. The correlation is not statistically significant (p = 0.67).

Table 3. Summary statistics.

Variables	Y_1	Y_2	X_1	X_2	X_3
Average	87.31	34.0	49.98	117.84	869.8
Standard deviation	11.71	9.30	0.70	1.65	190.7
Coefficient of variation	13.41	27.34	1.41	1.40	21.93
Minimum	66.00	21.00	49.10	115.20	488.00
Maximum	98.00	50.70	51.35	120.10	1070.00

Legend: Y_1 – percentage of germinated seeds in the laboratory; Y_2 – percentage of seedlings in the nursery; X_1 – latitude; X_2 – longitude; X_3 – altitude.

Table 4. Correlations.

Indicator	Y_1	Y_2	X_1	X_2	X_3
Y_1	-	**0.478**	**0.692**	**0.131**	**–0.577**
(p)		(0.098)	(0.0088)	(0.669)	(0.0391)
Y_2		-	**0.417**	**0.657**	**–0.821**
(p)			(0.156)	(0.015)	(0.0006)
X_1			-	**0.2764**	**–0.4392**
(p)				(0.3606)	(0.1332)
X_2				-	**–0.6504**
(p)					(0.0161)
X_3					-

Note: In all cases where the probability (p) is smaller than 0.05, the coefficient of correlation is statistically significant at a 95 % confidence interval, and if (p) is smaller than 0.01, the confidence interval is 99 %.

Table 5. Partial correlations.

Indicator	Y_1	Y_2	X_1	X_2	X_3
Y_1	-	**0.0812**	**0.6385**	**–0.4816**	**–0.4633**
(p)		(0.8234)	(0.0469)	(0.1587)	(0.1775)
Y_2		-	**0.0387**	**0.2898**	**–0.5346**
(p)			(0.9155)	(0.4166)	(0.1114)
X_1			-	**0.2759**	**0.1646**
(p)				(0.4403)	(0.6495)
X_2				-	**–0.4259**
(p)					(0.2197)
X_3					-

Dependence of Douglas-fir seed germination in the laboratory (Y_1) on altitude (X_3)

The main parameters of parabolic regression are shown in Table 6, as the model number 3.

We tested whether the seed germination rate under laboratory conditions shows a parabolic decrease with the increased provenance altitude, but this relationship was not statistically significant.

Dependence of Douglas-fir seed germination in the laboratory (Y_1) on latitude (X_1), longitude (X_2) and altitude (X_3)

Several regression models (additive and substitutive) were applied and additive models produced better results.

The additive model with fixed independent variables are shown in Table 6, as the model number 4. In model (4) some regression coefficients are not statistically significant. However, multiple regression, as a whole, was statistically significant. Latitude, longitude and altitude accounted for 89.02 % of the variance of the seed germination under laboratory conditions.

When using stepwise multiple regression, altitude emerged as the only significant variable. The results are shown in Table 6, as the model number 5.

Substitutive model (with the multiplication of independent variables) together with the method of stepwise multiple regressions are shown in Table 6, as the model number 6.

We can see that model (6), which includes the product of latitude with altitude and the product of longitude with altitude, produces better results than model (5), which includes only altitude.

(B) Nursery
Dependence of Douglas-fir seed germination in the nursery (Y_2) on latitude (X_1)

Dependence of Douglas-fir seed germination in the nursery on latitude was determined by the parameters in Table 6, model number 7.

The model point out that the nursery seed germination parabolically increases with the increased provenance latitude.

This dependence is, statistically speaking, expressed more clearly.

Dependence of Douglas-fir seed germination in the nursery (Y_2) on longitude (X_2)

Dependence of Douglas-fir provenance germination in the nursery on longitude was determined by the parameters in Table 6, model number 8.

The model shows that the nursery seed germination parabolically increases with the increased provenance longitude.

Dependence of Douglas-fir seed germination in the nursery (Y_2) on altitude (X_3)

Dependence of the provenance seed germination in the nursery on altitude was determined by the parameters in Table 6, model number 9.

The model point out that the nursery seed germination parabolically decreases with the increased provenance altitude.

Table 6. Statistical tests in laboratory and nursery.

(A) Laboratory			(B) Nursery		
Observed correlation	**Model No**	**Regression equation**	**Observed correlation**	**Model No**	**Regression equation**
Dependence of Douglas-fir seed germination in the laboratory (Y_1) on latitude (X_1)	(1)	$Y_1 = –6045.75 + 233.525 \cdot X_1 – 2.21678 \cdot X_1^2$ $R^2 = 48.64$ %, $S_t = 9.20$ %, $F = 4.74$ (0.0357)	Dependence of Douglas-fir seed germination in the nursery (Y_2) on latitude (X_1)	(7)	$Y_2 = 16790.1 – 674.828 \cdot X_1 + 6.7929 \cdot X_1^2$ $R^2 = 28.78$ %, $S_t = 8.59$ %, $F = 2.02$ (0.1833)
Dependence of Douglas-fir seed germination in the laboratory (Y_1) on longitude (X_2)	(2)	$Y_1 = –19123.4 + 325.669 \cdot X_2 – 1.37997 \cdot X_2^2$ $R^2 = 7.68$ %, $S_t = 12.33$ %, $F = 0.42$ (0.6707)	Dependence of Douglas-fir seed germination in the nursery (Y_2) on longitude (X_2)	(8)	$Y_2 = 1599.81 – 30.3381 \cdot X_2 + 0.144667 \cdot X_2^2$ $R^2 = 43.29$%, $S_t = 7.67$%, $F = 3.82$ (0.0586)
Dependence of Douglas-fir seed germination in the laboratory (Y_1) on altitude (X_3)	(3)	$Y_1 = 113.95 – 0.0242805 \cdot X_3 – 0.00000699164 \cdot X_3^2$ $R^2 = 33.29$ %, $S_t = 10.48$ %, $F = 2.49$ (0.1321)	Dependence of Douglas-fir seed germination in the nursery (Y_2) on altitude (X_3)	(9)	$Y_2 = 89.5408 – 0.0954227 \cdot X_3 + 0.0000347496 \cdot X_3^2$ $R^2 = 68.53$ %, $S_t = 5.71$%, $F = 10.89$ (0.0031)
Dependence of Douglas-fir seed germination in the laboratory (Y_1) on latitude (X_1), longitude (X_2) and altitude (X_3)	(4)	$Y_1 = –33209.4 – 37.8105 \cdot X_1 + 580.332\, X_2 – 0.0927229 \cdot X_3 + 0.499367 \cdot X_1^2 – 2.48145 \cdot X_2^2 + 0.0000809411 \cdot X_3^2$ $R^2 = 89.02$ %, $S_t = 5.49$ %, $F = 8.11$ (0.0112)	Dependence of Douglas-fir seed germination in the nursery (Y_2) on latitude (X_1), longitude (X_2) and altitude (X_3)	(10)	$Y_2 = 6146.55 – 484.514 \cdot X_1 + 101.413 \cdot X_2 – 0.125137 \cdot X_3 + 4.84896 \cdot X_1^2 – 0.424873 \cdot X_2^2 + 0.0000605416 \cdot X_3^2$ $R^2 = 78.19$ %, $S_t = 6.14$ %, $F = 3.59$ (0.0728)
	(5)	$Y_1 = –487.889 + 11.5091 \cdot X_1$ $R^2 = 47.89$ %, $St = 8.83$ %, $F = 10.11$ (0.0088)		(11)	$Y_2 = 68.8286 – 0.0400435 \cdot X_3$ $R^2 = 67.47$ %, $S_t = 5.54$ %, $F = 22.82$ (0.0006)
	(6)	$Y_1 = 124.403 + 0.00816858 \cdot X_1 \cdot X_3 – 0.00382914 \cdot X_2 \cdot X_3$ $R2 = 67.17$ %, $St = 7.35$ %, $F = 10.23$ (0.0038)		(12)	$Y_2 = 69.3253 – 0.000813673 \cdot X_1 \cdot X_3$ $R^2 = 66.84$ %, $S_t = 5.92$ %, $F = 22.17$ (0.0006)

Note: *p*-values are shown in the parentheses.

Statistically speaking, this dependence is so far the clearest one.

Dependence of Douglas-fir seed germination in the nursery (Y_2) on latitude (X_1), longitude (X_2) and altitude (X_3)

Several regression models (additive and substitutive) were applied. It is interesting to note that additive models produced better results.

The additive model with fixed independent are present in Table 6, model number 10.

Although 78.19 % of the variance of the provenance germination rate under nursery conditions was explained, some regression coefficients in model (10) were not statistically significant, which also applied to the regression as a whole. Therefore, we applied the method of (stepwise) multiple regression.

The results are present in Table 6, as model number 11.

Model (11) included only altitude, which accounted for 67.47 % of the observed correlation. The regression was statistically significant at a 99 % confidence.

Substitutive model (with the multiplication of independent variables) together with the method of stepwise multiple regressions are present in Table 6, as model number 12.

It is obvious that model (12), which included the product of latitude with altitude, didn't produce better results than model (11), which included only altitude.

Testing the difference in seed germination between Douglas-fir provenances under different conditions Comparison of medians

The difference between the medians of two samples (a provenance under laboratory and a provenance under nursery conditions) was tested Mann-Whitney W-test.

The result of the test is: $W = 169.0$, $P = 0.00002$

It was concluded that the difference between the seed germination medians in the laboratory and in the nursery was statistically significant at a 99 % confidence interval.

Comparison of proportions

The difference between the proportions of two samples (a provenance under laboratory and a provenance under nursery conditions) was tested by z-statistic test.

The result of the test is: $z = –40.0913$, $P = 0.0000$

It was concluded that the difference between the proportions of seed germination under laboratory and under nursery conditions was statistically significant at a 99 % confidence interval.

Both tests showed that the difference between the achieved seed germination rates under laboratory and under nursery conditions had a high statistical significance. Seed germination rate was much higher under laboratory conditions than in the nursery. The germination ratio was approximately 3:1.

Conclusions

The most important conclusion that can be drawn from this study is that the justification for performing the seed germination test of Douglas-fir provenances was proved. There are significant differences between the seed germination rates of Douglas-fir provenances under laboratory and nursery conditions. The compatibility of seed germination of thirteen common provenances under different conditions is

r = 0.478 (p = 0.098). The percentage of seed germination was higher in the laboratory and ranged from 66 to 98 %, while it was 21–51 % under nursery conditions. Provenances 3, 8, and 9 had the highest germination percentage.

The dependence of seed germination rate of Douglas-fir provenances on latitude, longitude and altitude was statistically determined under laboratory and under nursery conditions. We concluded that:

– The seed germination rate increases parabolically with the increased latitude of Douglas-fir provenances both in the laboratory and nursery. The relationship is stronger in the laboratory.

– The seed germination rate increases parabolically with the increased longitude of Douglas-fir provenances both under laboratory and under nursery conditions. The relationship is stronger under nursery conditions.

– The seed germination rate decreases parabolically with the increased altitude of Douglas-fir provenances both under laboratory conditions and in nursery. Dependence is stronger in the nursery. This dependence is statistically most pronounced.

Comparisons of medians and comparisons of proportions of two samples (thirteen provenances under laboratory and under nursery conditions) showed that the difference in the achieved seed germination rate of Douglas-fir provenances is statistically significant at a 99 % confidence interval. Considerably higher germination rate was achieved under laboratory conditions than in the nursery. The ratio was approximately 3:1.

Our future research studies will include the analysis of some additional aspects of Douglas-fir seed germination, all with the aim of getting a deeper insight into the problem of reliable selection of the provenances to be introduced into the forest sites of Serbia.

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