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Condition and productivity of marginal oak and beech plantations in the southern part of the Right Bank Forest Steppe of Ukraine

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Abstract

The condition and productivity of marginal oak-beech plantations in the conditions of fresh hornbeam grove of the southern part of the Right Bank Forest Steppe of Ukraine, outside the natural habitat of European beech have been studied. It was found that with the observance of agricultural techniques and timely silvicultural care and felling of plantations at the age of 19–20 productive crops are formed with an average height of beech trees 9.2–10.4 m, diameter 11.8–12.7 cm, preservation of 352–386 pieces·ha^{-1}, which grow according to I^{b} class of quality. In the studied oak-beech crops of European beech trees lag behind in growth from common oak trees, but according to taxonomic indicators, hornbeam, sharp-leaved maple, birch and heart-leaved linden trees predominate. The prospects of growing of beech in non-range conditions have been established, as it can compete with aboriginal forest species.

Key words: average diameter, average heights, crop productivity, European beech, planting composition.

Introduction

As indicated by Krynytskyy et al. (2017), European beech is one of the main forest-forming species of the Ukrainian Roztochchia. In the forest fund of the region, beech is spread over an area of 12,810 ha (28.4 %). Productivity of beech stands reaches the I quality class, and some of them have I^{a}–I^{b} quality. In general, here beech stands are characterized by significant forest-typological potential and biotic resistance, achieve high productivity and are capable of self-reproduction.

According to Myklush and Myklush (2017), European beech forms forest stands mainly in barren forest types (over 85.6 % of the area of lowland beech forest stands), of which 48.8 % of areas are concentrated in the type of forest vegetation

conditions D_2 and 39.8 % – in D_3. Under conditions of fresh beech, both pure and mixed beech stands are formed, in which 2–4 species are mainly involved, where the participation of beech is three or more units. Under conditions D_2, pure and mixed beech forests are characterized by I^b–III quality classes.

According to Belous (1962), the condition of beech plantations indicates that in Vinnytsia and Khmelnytskyi regions beech grows successfully in plantation and is not inferior to oak in productivity. Plantation outside the eastern border of the natural range are characterized by good growth and development.

The southern limit of the natural range of forest or European beech (*Fagus sylvatica* L.) is located in the Mediterranean mountain belt to the limit of forest distribution (Cullotta et al. 2016). As indicated by Köcher et al. (2009) and Zang et al. (2014), the species *Fagus sylvatica* is more sensitive to drought than *Quercus petraea* (Matt.) Liebl. and *Q. robur* L., *Carpinus betulus* L., *Fraxinus excelsior* L., *Tilia cordata* Mill., *Picea abies* (L.) Karst.

Studies by Berry et al. (2002), Attorre et al. (2008) and Garzón et al. (2008), found that climate change, as indicated by Thomas et al. (2004) and Gessler et al. (2007), threaten the existence of European beech plantations on the southern borders of its range. According to the forecast of Kramer et al. (2010), both the southern and northern boundaries of the distribution of this species will shift to the north in the future.

Marginal peripheral populations of beech are threatened with extinction. These plantations are particularly vulnerable and require constant monitoring, conservation and protection. According to Popadynets (2002), the Podilsk variety of European beech is better adapted to the conditions of a drier continental climate, it is characterized by light-loving, xeromorphic leaf structure and a higher level of photosynthesis.

According to folklore, natural beech plantations used to grow in the Uman region in ancient times. Probably the name of the village Buky comes from them. However, due to intensive deforestation, unsatisfactory natural regeneration and without the creation of artificial plantations, beech forests in our region have gradually disappeared.

The urgency of the research is due to the need to study and make scientifically sound adjustments to the functioning of marginal populations of beech in climate warming and reducing the humidity of air and soil, reproduction of peripheral genetically stable resources of beech in the southern part of the Right Bank Forest Steppe of Ukraine.

The subject of research is the regularities of growth and development of forest beech trees of and aboriginal forest species in marginal artificial oak-beech plantations outside the natural range of beech depending on their species composition and mixing schemes.

The purpose of the study is to study condition and productivity of marginal oak and beech plantations in the conditions of fresh hornbeam wood, to establish the characteristics of the growth of beech, oak, ash and other native species with simultaneous growth in marginal plantations on the southeastern border area of European beech. To confirm or refute the hypothesis under these conditions, beech trees are not inferior in terms of biometric indicators to the trees of aboriginal species of forest species and successfully withstand competition with them, and artificial marginal oak-beech plantations are not inferior in terms of productivity to

native oak stands and are promising for creation in the southern part of the Right Bank Forest Steppe.

Object and Methods

Object of the study is marginal beech plantations in Synytsia forestry of Uman Forestry State Enterprise. Its geographic coordinates are 48°41′45″ N, 30°4′15″ E.

Uman Forestry State Enterprise is located in the Southern Forest-Steppe Region of the Dnipro Upland on the border with the Northern Steppe. According to long-term observations of Uman meteorological station, the average annual air temperature is 7.6 ºC, the absolute maximum is 36.8 ºC, and the absolute minimum is minus 32.9 ºC. The average rainfall is 508 mm per year, of which 50 % falls during the growing season. Relative humidity is 67 % with a climate coefficient of 0.6–0.7, which indicates an area of insufficient humidity. The growing season is 216 days. Gray forest loam soils predominate.

The research methodology involves the study of hardwood forests and is generally accepted for silvicultural and taxonomic research using general scientific methods of comparison, component analysis and synthesis of data to determine the status and productivity of European beech plantations. Forestry and assessment indicators of 18–19-year-old oak and beech crops were studied on temporary test plots of Synytske Forestry of the state enterprise 'Uman Forestry', which were established in accordance with normative and methodological materials 'Test areas are forested. Method of laying: SOU 02.02-37-476:2006' (Ministry of Agrarian … 2009). In sector 2 of block 93 (Object 1) and sector 1 of block 78 (Object 2), three trial areas were laid out. The researched plantings were created artificially. Rows of beech trees are planted with one-year-old saplings of the Podil variety of the European beech tree. The rows of oak trees are also planted with one-year-old saplings, trees of other types of trees of natural seed and shoot origin. The initial purpose of planting these plantations was to obtain a round forest. However, later, when it turned out that beech plantations are very rare in the research region, their research function was also added. The height of the trees was measured with an eclimemeter-altimeter EV1U1. Measurements of the tree DBH were performed by a measuring fork. Taxation indicators of plantations were determined according to assortment tables. The average diameter was calculated by the sum of the cross-sectional areas of the trunks, the average height of the trees – by the 'height curve'. The quality class was determined by the average age and average height of the forest element in accordance with the quality scale by Orlov (1931).

Statistical processing of research results was performed on a personal computer by the method of analysis of variance according to Dospekhov (1985).

Studies of the condition, composition and productivity of marginal artificial oak and beech plantations were conducted in Object 1 and Object 2 of Synytske Forestry in the conditions of fresh hornbeam grove. The height and diameter of trees of common oak, beech, ash and other species of aboriginal forest tree species were measured on the temporary test plots included in these species, and the stock of plantations was determined. The research was carried out during 2017–2019 in the third decade of April under favourable weather conditions and soil moisture, when the blooming of leaves on tree branches began and shoots began to grow.

Results

Forest crops in Object 1 were established in 2000 (Fig. 1). Type of forest vegetation conditions – fresh oak (D_2). The soil is gray forest and loamy. According to the FAO classification of soil types, they belong to the Podzol group. The category of forest area is a fresh felling. Soil preparation for forestry included lowering stumps on 1.8–2.0 m wide strips to provide proper conditions for tractor operation, removal of cutting residues and branches, autumn tillage and spring cultivation on these strips by KLB-1.7 cultivator.

The method of creating forest crops is mechanized planting. The scheme of planting – 6 × 0.7 m. The scheme of mixing wood species – 3 rows of common oak and 1 row of European beech. Black elderberry (*Sambucus nigra* L.), warty cowberry (*Euonymus verrucosus* Scop.), and hazel (*Corylus avellana* L.) were found in the undergrowth. Undergrowth up to 2.0 m is dense, placed on the square curtains. The living above-ground cover included: violet (*Viola sylvestris* Lam.), European ungulate (*Asarum europaeum* L.), medicinal bush (*Polygonatum officinale* All.), common primrose (*Aegopodium podagraria* L.). It is evenly distributed over the area, liquid. Survey data obtained in 2017 are shown in Table 1.

Fig. 1. A row of beech trees in Object 1.

Table 1. Forest and valuation characteristics of oak and beech crops (2017).

Object	Composition of forest stand	Forest	Average height, m	Average diameter, cm	Stock of timber, $m^3 \cdot ha^{-1}$
1	48 % oak	oak	8.4	11.6	39.1
	19 % ash	ash	8.2	12.1	17.9
	19 % beech	beech	7.1	11.2	13.2
	9 % maple	maple	5.1	6.6	4.8
	less than 5 % (elm,	elm	6.1	9.8	4.1
	aspen,	aspen	5.9	7.1	3.7
	hornbeam,	hornbeam	5.5	6.7	2.9
	little-leaf linden)	little-leaf linden	4.6	7.2	0.8
	LSD		0.23	0.46	0.39
2	48 % oak	oak	9.4	11.2	31.3
	19 % beech	beech	9.0	9.4	16.7
	19 % hornbeam	hornbeam	5.7	6.6	10.6
	9 % maple	maple	6.2	6.9	3.8
	less than 5 % (ash,	ash	6.1	8.4	3.6
	little-leaf linden)	little-leaf linden	6.1	7.2	2.1
	LSD		0.27	0.23	0.34

Note: LSD – least significant difference.

As it can be seen from Table 1, the average height of common oak trees in Object 1 is 8.4, common ash – 8.2 and beech – 7.1 m. According to analysis of variance,

this indicates a significant advantage and more intensive growth of trees of these two species in compared with beech. However, beech trees have a significantly higher average height than trees of related species: maple, birch, linden, aspen and hornbeam. In terms of average diameter, European beech plants significantly outnumber plants of these species, but have a smaller diameter than plants of common oak and common ash. Moreover, the advantage of ash trees over oak and beech trees is significant, and oak trees over beech trees is not significant.

Composition of plantation is 48 % of common oak, 19 % common ash, 19 % European beech, 9 % Norway maple and less than 5 % of others, such as elm, aspen, common hornbeam, and linden. Relative completeness 0.94 with a tent closure of 1.0. Average increase 5.1 $m^3 \cdot ha^{-1}$, stock 87 $m^3 \cdot ha^{-1}$, including beech – 13.2 $m^3 \cdot ha^{-1}$. The plantation is highly productive, it grows according to I^b class of quality, and currently requires of maintenance felling. Due to the late spring frosts in mid May 2000, some beech seedlings died (supplement made in the spring of 2001 with annual beech seedlings), but today there are 386 ha^{-1} (10.9 % of the plantation) (Fig. 2).

Preservation of trees of the main species – common oak (*Quercus robur*) is satisfactory and is 1034 pieces·ha^{-1} (30 % of plantation).

Sufficient natural seed and vegetative regeneration of ash (*Fraxinus excelsior*), maple (*Acer platanoides* L.), hornbeam (*Carpinus betulus*), linden (*Tilia cordata*), birch (*Ulmus minor* Mill.) and aspen (*Populus tremula* L.) grows in the plantation. On the forest outskirts and near the inter-quarter roads, there are trees of wild fruit species, among which white mulberry (*Morus alba* L.), cherry (*Cerasus avium* (L.) Moench) and plum (*Prunus cerasifera* Ehrh.) predominate. Young 2–3-year-old white mulberry seedlings from these plantations are used by researchers (Vitenko et al. 2019) as rootstocks for growing seedlings of ornamental forms.

The growth of oak, beech and other aboriginal trees can be traced on the graph of the ratio of average height and diameter of trees (Fig. 3).

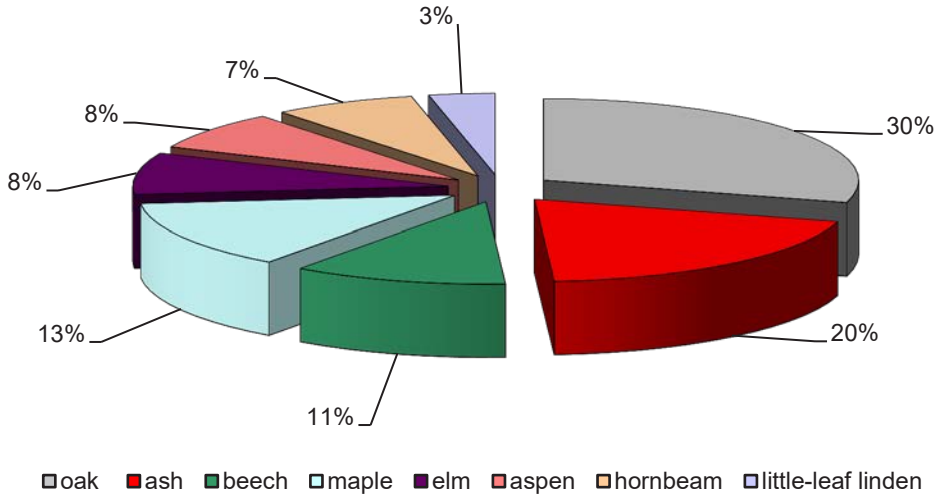

Fig. 2. The composition of plantation in Object 1.

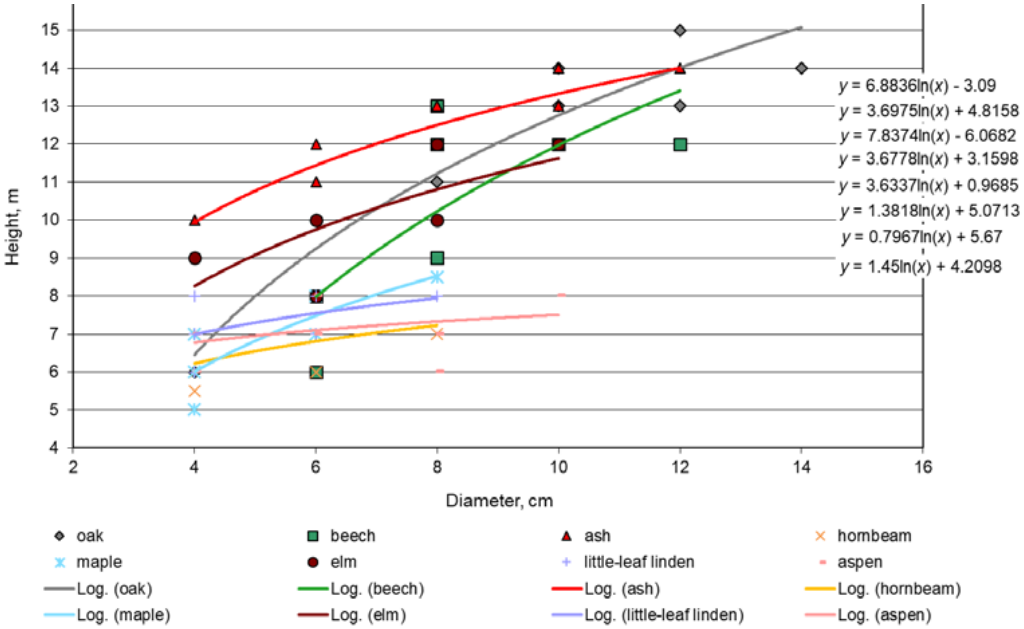

Fig. 3. The ratio of average height and diameter of trees in Object 1.

Analyzing the graph of the ratio of average height and diameter of trees, which is shown in Figure 3, it can be concluded that beech trees have a lower growth rate than oak and ash trees, but they are dominated by trees of other aboriginal forest species.

Oak and beech plantations in Object 2 were established in 1999 by planting annual seedlings of common oak and beech according to the mixing scheme – 1 row of common oak and 1 row of European beech. The type of forest vegetation conditions, soils, soil preparation technology on this forest area are the same as on the previous area. As of 2017, it was a highly productive plantation with a 48 % common oak, 19 % European beech, 19 % common hornbeam, 9 % Norway maple and less than 5 % (common ash, linden) with a total stock of 68 $m^3 \cdot ha^{-1}$ and an average increase of 3.8 $m^3 \cdot ha^{-1}$ (Fig. 4). Quality class – I^a, relative completeness – 0.77.

Fig. 4. A row of beech trees in Object 2.

Undergrowth is almost absent. As shown in Table 1, oak trees have an average height of 9.4 m, beech – 9.0 m, which is significantly (0.4 m) less than the average height of oak trees. The average diameter of beech trees is 9.4 cm, which according to the analysis of variance is significantly smaller (1.8 cm or 16.1 %) than the average diameter of oak trees, which is 11.2 cm. The average diameters of trees of other related species are significantly smaller than oak and beech trees and are 6.6–8.4 cm.

In the undergrowth warty elderberry, black elderberry, blood-red swine (*Swida sanguinea* L.) grow. It is dense, evenly distributed over an area up to 2.0 m high. In the living above-ground cover there are European ungulate, *Corydalis solida* L., medicinal tussock, *Stellaria holostea* L., *Betonica officinalis* L., *Anemone ranunculoides* L.

In Object 2 there are an average of 2546 pieces·ha^{-1} trees, including: oak – 703 and beech – 352 pieces·ha^{-1}. From natural regeneration, seed and undergrowth plants of hornbeam – 872, maple – 256, ash – 228 and linden – 135 pieces·ha^{-1} (Fig. 5) were found.

Analyzing the graph of the ratio of average height and diameter of trees (Fig. 6), we can conclude that beech trees have a lower growth rate than oak trees.

Thus, in Object 2 the average height and diameter of beech trees is smaller than that of oak trees, but larger than other species of aboriginal related species: hornbeam, maple, linden and ash. The decrease in the height of trees of related species was due to the maintenance fellings carried out in 2016.

During 2018–2019, similar to 2017, the growth processes of trees of the studied species were observed (Table 2). However, it should be noted that the average height and, especially, the diameter of the beech tree, began to approach oak and ash trees in Object 1 and the oak trees in Object 2. Thus, the first area of beech trees in 2018 on average reached a height of 8.2 m, while oak trees – 9.2 m, ash – 9.1 m. According to analysis of variance, the advantage in height of oak and ash trees over beech trees was significant, and

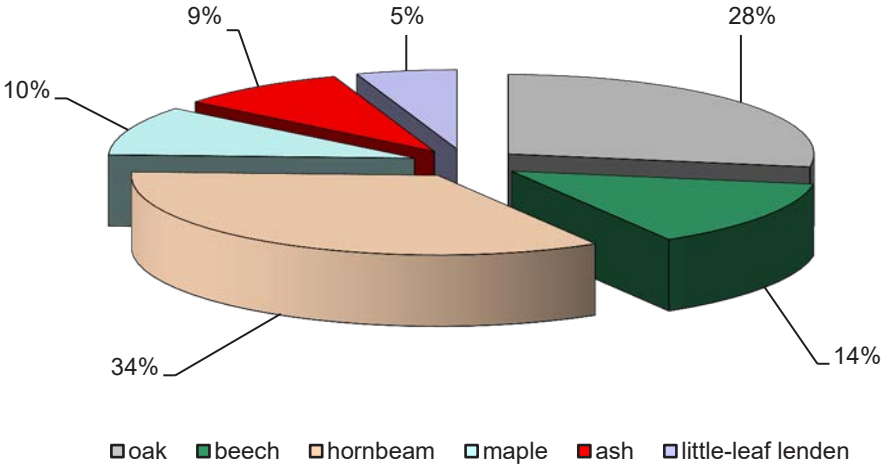

Fig. 5. The composition of plantation in Object 2.

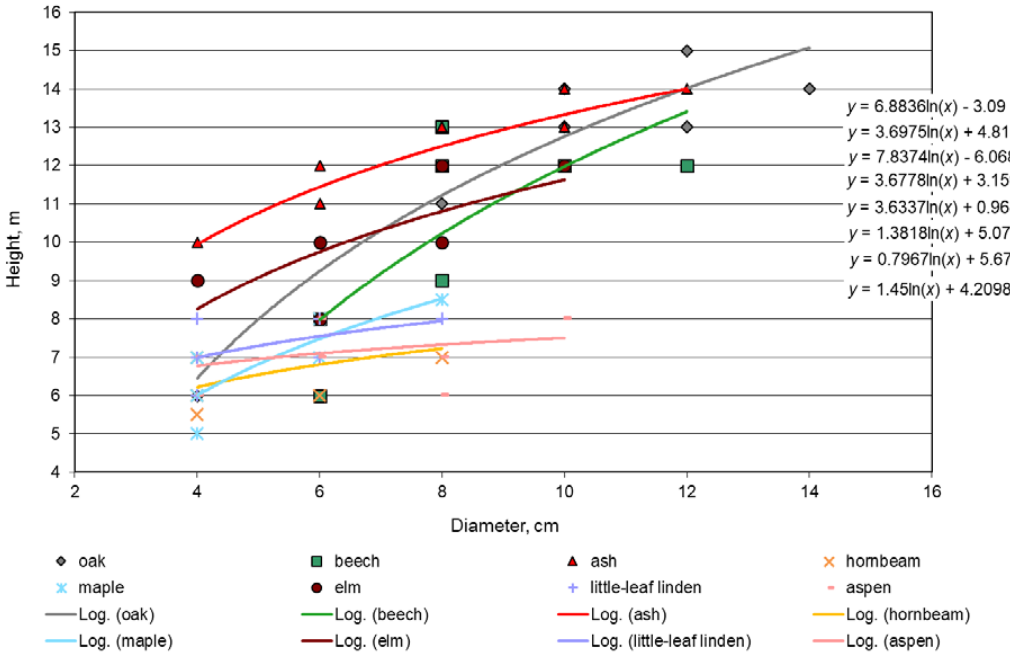

Fig. 6. The ratio of the average height and diameter of trees in Object 2.

Table 2. Average height and diameter of trees in oak and beech crops.

Object	Forest	Average height, m		Average diameter, cm	
		2018	2019	2018	2019
1	oak	9.2	10.1	12.2	12.9
	ash	9.1	9.9	12.5	13.0
	beech	8.2	9.2	11.8	12.7
	maple	6.4	7.6	7.0	7.8
	elm	6.6	7.5	10.1	10.6
	aspen	6.6	8.0	7.7	8.7
	hornbeam	6.5	7.7	7.5	8.1
	little-leaf linden	5.5	6.5	8.0	8.6
	LSD	0.24	0.25	0.19	0.21
2	oak	10.0	10.8	11.9	13.1
	beech	9.5	10.4	10.2	11.8
	hornbeam	6.4	7.4	7.1	8.5
	maple	6.7	7.7	7.4	8.7
	ash	6.6	7.9	8.8	10.2
	little-leaf linden	6.5	7.2	7.6	8.7
	LSD	0.09	0.21	0.14	0.23

oak trees over ash trees – insignificant, since it was less than LSD (0.24). In 2019, the average height of oak trees was 10.1 m, ash – 9.9 m and significantly exceeded the height of beech trees (9.2 m). The largest average diameter of 12.5 cm in 2018 was in ash trees and significantly exceeded 0.3 cm of oak and 0.7 cm of beech. In 2019, only ash trees significantly outperformed beech trees, and the predominance of oak trees over beech and ash trees over oak was insignificant, as it was less than LSD (0.21). According to the analysis of variance, oak, ash and beech trees had significantly higher trunk height and diameter com-

pared to trees of other forest species in this area.

As it can be seen from Table 2, on the second area of beech trees in 2018 on average reached a height of 9.5 m, which was significantly less than the height of oak trees 10.0 m, but significantly exceeded the average height of trees of other species. The average height of beech tree in 2019 reached 10.4 m and was significantly lower by 0.4 m than the height of oak trees. The results of analysis of variance show that the trees of these two species significantly outperformed the trees of other forest species in Object 2. On average, the largest indicators in 2018 were oak trees – 11.9 cm, which significantly outperformed beech trees (1.7 cm) and other studied species. In 2019, oak trees continued to significantly outperform beech trees (1.3 cm). In addition, the trees of these two species had a significant advantage over the trees of other forest species in this area.

In our opinion, it is impractical to compare the average stock due to the different number of trees of the studied species in the composition of plantations in these species.

Discussion

Vakulyuk and Samoplavskyi (2006) found that in extra-areal conditions beech can be marked by intensive growth, predominantly aboriginal species. In 104-year-old crops created in fresh oaks of Dzhurynske Forestry of Vinnytsia region, European beech trees had an average height of 30.2 m and an average diameter of 43.4 cm, oak trees – 29.9 m and 42.7 cm, respectively, ash trees – 32.3 m and 43.4 cm. Since the trees of all three species in fresh oak crops in mature age reached approximately the same size, we can conclude that the successful growth of beech in fresh oak outside its natural range.

As established by Myklush and Myklush (2017), the intensity of growth in height of beech stands at different ages is not the same. By the age of 25–30, plain beech stands are mainly characterized by I, less often by II and III quality classes. After 30 years, the intensity of their growth increases and in the age range of 30–80 years, areas where beech is characterized by I^a and higher grades of quality prevail. Pure and mixed young and medieval stands are characterized by similar trends in height, so the average values of quality classes in them are very similar. Up to 40 years, the studied pure and mixed stands are characterized by a similar growth trend in height to normal stands and their average heights do not differ significantly. Not the same intensity of growth in height of beech stands in different age periods also affects their growth in diameter. It is established that the most intensive increase in diameter is observed up to 60 years, when the average increase in diameter of the studied stands reaches 0.52 cm.

According to Gordienko and Gordienko (2005), poor growth of European beech in the first years of life is a widespread phenomenon. The most intensive growth of beech trees in height in natural and artificial plantations of fresh oaks is manifested in the period from 10 to 45 years. In the plains of Ukraine beech shows high biological stability and intensive growth.

According to our research, at a young age (up to 20 years) in artificial plantations created on felling in the conditions of fresh hornbeam grove in the southern part of the Right Bank Forest Steppe of Ukraine, beech trees lag behind in growth from oak and ash, as they suffer from excessive lighting in the first years of life in the open,

but ahead of hornbeam, maple, birch, aspen and linden. Therefore, the results of our research confirm the data on the lower intensity of growth of beech trees under 25–30 years of age under the growth in pure and mixed plantations, previously obtained by Gordienko and Gordienko (2005), Myklush and Myklush (2017) in the conditions of fresh beech both within and outside the natural range of the European beech.

Unfortunately, no mature beech forest stands have been found in the studied area, but in Pashkevych V. V. Arboretum of Sofiyivka National Dendrological Park three mighty trees of ornamental garden forms of beech over 120-years-old grow, which are not inferior to aboriginal forest trees species growing in the same plantation (figs 7 and 8).

All this confirms the hypothesis that in the conditions of fresh hornbeam oak in the southern part of the Right Bank Forest Steppe of Ukraine beech trees are not inferior in average diameter and height to trees of aboriginal varieties of forest species.

Fig. 7. Three age-old trees of decorative garden forms of beech.

Fig. 8. Purple beech tree.

Conclusions

Studies have shown that European beech trees successfully grow and develop in artificial marginal plantations in the conditions of fresh hornbeam grove in the southern part of the Right Bank Forest Steppe of Ukraine and compete with oak and other native species.

It was found that with the observance of agricultural techniques and timely silvicultural care and felling of plantations at the age of 17–18, productive oak-beech crops are formed with an average height

of beech trees 7.1–9.0 m, diameter 9.4–11.2 cm, preserved 352–386 pieces·ha^{-1}, which grow according to I^{b} class of quality. At the age of 19–20, the average height of beech trees reaches 9.2–10.4 m, diameter 11.8–12.7 cm, the preservation (number) of trees in the plantation does not change, as maintenance felling is not carried out at this time, and natural waste has already ended.

Thus, according to taxonomic indicators in oak-beech plantations, European beech trees lag behind in growth from trees of oak and ash in Object 1 and common oak in Object 2, but are dominated by hornbeam, maple, birch, aspens and lindens. Given the continued sufficient preservation of trees, it can be predicted that the productivity of artificial marginal oak-beech plantations in the studied extra-areal conditions will not be inferior to native oak stands, and beech trees in height and diameter – trees of aboriginal forest species. Therefore, the cultivation of European beech in industrial plantations to obtain round assortments of wood has real prospects, but requires further study as the age of the plantations increases.

A positive solution to this problem will provide an increase in the number of European beech trees not only in arboreta, but also in artificial marginal plantations, which will contribute to the preservation of the beech gene pool and the expansion of biodiversity in the southern part of the Right Bank Forest Steppe of Ukraine.

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Bioinsecticidal activity of essential oil of *Mentha arvensis* L. grown in forest area on *Helicoverpa armigera* Hub. in tobacco and *Apis mellifera* L.

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Abstract

In laboratory conditions bioinsecticidal effect of *Mentha arvensis* L. essential oil (EO) on *Helicoverpa armigera* Hub. 3rd instar larva on tobacco and *Apis mellifera* L. was found. There were differences between the efficacy of the divers concentrations. Good bioinsecticidal effect of 48 % was detected at concentration of 1.25 % v/v, high effect of 92 % at concentration of 2.5 % v/v and 100 % efficacy at 5 % v/v. Phytotoxicity on tobacco plants was found only at 5 % v/v EO concentration. The concentrations of 2.5 % v/v and even the less than 1.25 % v/v could be used to control the pest *H. armigera*. Concentrations of 1.25 % v/v and 2.5 % v/v applied under the same conditions to *A. mellifera* showed 100 % toxicity to the bees as well.

Key words: cotton bollworm, honey bees, wild mint.

Introduction

The cotton bollworm *Helicoverpa armigera* Hub. is a polyphagous pest and one of the most dangerous worldwide. It attacks a huge variety of plant species (Gopalakrishnan et al. 2016). The use of pesticides had led to insect resistance (Sharpe 1999), some of them are restricted in Europe (Cressey 2017), and contain agents dangerous to human health (Yu et al. 2013). Essential oils (EOs) have a strong effect on insects (Boulamtat et al. 2021), and can be used (Radev 2022a). They show repellent and lethal effects (Ibrahim 2019). The plant extracts have physiological effects on insects (Rahimi et al. 2018), and their elaboration is a long process (Shcherbakova 2011). EO could be extracted from various plants such as *Mentha arvensis* L. distributed also in forestry areas.

The present work aims at study the bioinsecticidal activity of *M. arvensis* EO on *H. armigera* 3rd instar larvae and *Apis mellifera* L. under laboratory conditions.

Materials and Methods

EO from *M. arvensis* was produced from stems and flowers picked from forest area

in the Central Rhodopes mountain in blooming season, July 2021. The amount of 1.5 kg was hydro-distilled in a micro-distiller. The EO was stored in a glass container at room temperature.

The larvae collection of *H. armigera* was done by methodology of Papulwar et al. (2018) from tobacco plants at the Tobacco and tobacco products institute, Markovo. Larvae 3rd instar were determined according to DPI&F (2005) and placed in Petri dishes 90×20 mm with filter paper at the bottom. The test treatment was provided according to Boulamtat et al. (2021) methodology, and 0.01 % Tween 80 was used as emulsifier. The efficiency was calculated according to Abbott (1925).

The minimum concentration with highest efficacy was tested on 3 days old *A. mellifera* bees according to Radev (2022b), bees were treated with EO for 48 h under the same conditions as *H. armigera* larvae.

The results were statistically processed by using one-way Anova in Excel, at $p \leq 0.05$.

Results and Discussion

The results demonstrated bioinsecticidal effect of *M. arvensis* EO on *H. armigera* 3rd instar larvae. Good bioinsecticidal effect of 48 % was detected at concentration of 1.25 % v/v, high effect of 92 % at concentration of 2.5 % v/v and 100 % efficacy at 5 % v/v. There were differences between the efficacy of the divers concentrations ($F > F_{crit}$, $p = 0.00$) (Fig. 1). Effectiveness results for the concentrations of 1.25 % v/v and 2.5 % v/v were higher, while for 5 % v/v was the same compared to those of Boulamtat et al. (2021) for *M. spicata* EO. All the EO concentrations in laboratory study were applied over tobacco plants for phytotoxicity. Phytotoxicity (dried spots on tobacco plants) was reported only at 5 % v/v EO concentration.

The concentrations of 1.25 % v/v and 2.5 % v/v *M. arvensis* EO were tested for

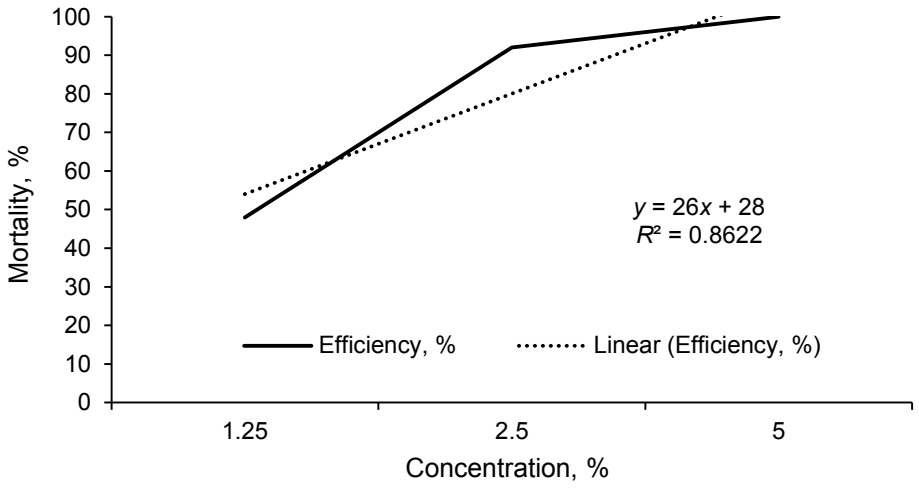

Fig. 1. Insecticidal efficacy of *M. arvensis* EO on *H. armigera* in varied concentrations applied.

toxicity on *A. mellifera*. Results indicated 100 % mortality (means ±SD 100 ±0.0). There is difference between the tested and control groups ($F > F_{crit}$, $p = 0.00$).

Conclusions

The results can be used as possible mean for the tobacco' pest control, and in bees' protection when performing treatments. More deep researches in this direction should be done.

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Test for contact toxicity of *Thymus pulegioides* L. essential oil on *Helicoverpa armigera* Hub. in tobacco and *Apis mellifera* L.

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Abstract

Thymus pulegioides L. essential oil (EO) in different concentrations was tested in laboratory conditions on *Helicoverpa armigera* Hub. 3rd instar larva on tobacco and *Apis mellifera* L. bees, and the results showed bioinsecticidal activity. Differences were found between the effectiveness of various concentrations as higher concentrations showed higher efficiency. Low toxicity of 36 % was found at concentration of 1.25 % essential oil, concentration of 2.5 % produced high toxicity of 76 % while concentration of 5 % produced 100 % toxicity. All the concentrations of *T. pulegioides* EO tested in the same conditions on *A. mellifera* showed 100 % toxicity.

Key words: cotton bollworm, honey bees, mountain thyme.

Introduction

The species *Helicoverpa armigera* Hub. is a major pest in agrocenoses (Santos et al. 2017). This species causing huge damage to many crops (Sharma 2007). When insecticides are applied incorrectly this could lead to pest resistance and oppression of beneficial entomofauna which makes the pest control difficult (Turchen et al. 2016). Some pesticides are limited for usage in Europe (Cressey 2017). Alternative for control, such as plants substances containing insecticide ingredients could be used (El-Wakeil 2013). Essential oils (EOs) have such impact (Boulamtat et al. 2021). According to Muturi et al. (2017) 10 % from the studied EOs are available as potential insecticides. The species *Thymus pulegioides* L. spread in forest areas, could be used for EO yield. Thyme EO is characterised by antimicrobial activity (Elfellah et al. 1984).

The aim of this study is to test contact toxicity of EO of *T. pulegioides* on *H. armigera* 3rd instar larva in tobacco and *Apis mellifera* L. in laboratory conditions.

Materials and Methods

In July 2021 stems and flowers from *T. pulegioides* were harvested from forest area in the Central Rhodopes mountain to

produce EO. The mass of 1.5 kg was distilled in a micro-distiller. The EO was kept in a glass package at room temperature.

The collection of *H. armigera* larvae was done by methodology of Papulwar et al. (2018) from tobacco plants at the fields of Tobacco and tobacco products institute, Markovo. Larvae of 3rd instar were determined according to DPI&F (2005) and placed in Petri dishes 90×20 mm with filter paper at the bottom. The test treatment was provided according to Boulamtat et al. (2021) methodology, 0.01 % Tween 80 was used as emulsifier. The efficiency was calculated according to Abbott (1925). Larva which not react on touching was counted as dead.

The same concentrations were tested on 3 days old *A. mellifera* bees according to Radev (2022), bees were treated with EO for 48 h under the same conditions as *H. armigera* larvae.

The results were statistically processed by using one-way Anova in Excel, at $p \leq 0.05$.

Results and Discussion

The data indicated toxicity effect of *T. pulegioides* EO on *H. armigera* 3rd instar larva. Low toxicity of 36 % was found at concentration of 1.25 % EO, quite high effect of 76 % at concentration of 2.5 % and 100 % toxicity at 5 % concentration. Differences between the effectiveness of the tested concentrations were found ($F > F_{crit}$, $p = 0.000$) (Fig. 1). The study results showed that 6 h after treatment mortality was reported only at 5 % concentration, after 24 h at the three tested concentrations. Bioinsecticidal effectiveness of *T. pulegioides* EO for all concentrations was much higher compared to those of Boulamtat et al. (2021) for *Thymus vulgaris* L. EO. The plant chemical substances show bioactivity against insects (Listiyani 2012).

All the concentrations of *T. pulegioides*

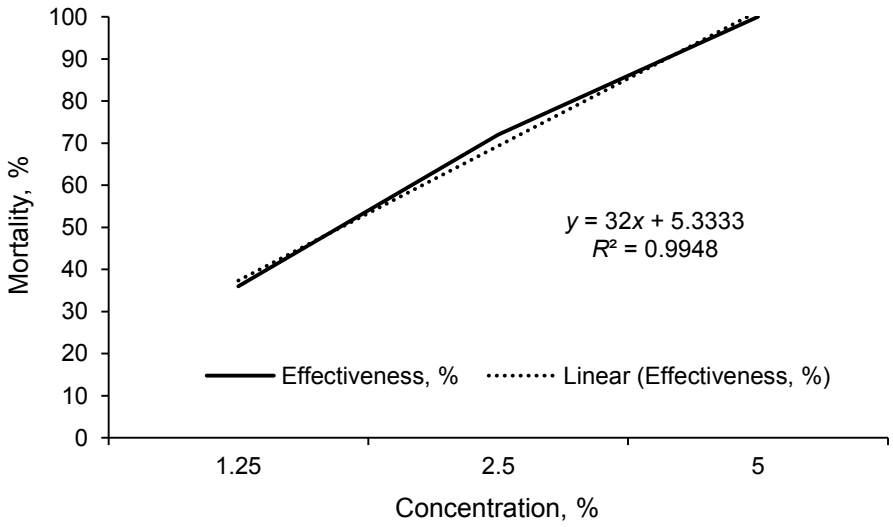

Fig. 1. Insecticidal efficacy of *T. pulegioides* EO on *H. armigera* in concentrations applied.

EO tested on *A. mellifera* showed 100 % toxicity (means ±SD 100 ±0.0). Differences between the treated and control groups were found to be highly significant ($F > F_{crit}$, $p = 0.000$).

Conclusions

These findings can be tested at field work with tobacco plants, but measures for bees' protection should be taken beforehand. Future field studies need to be done.

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Medicinal species of conservation importance from the Bulgarian flora

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Abstract

From antiquity to the present day, man has used plants for various purposes, most of them being used for the treatment of various diseases. However, some medicinal plants are of varying degrees of threat. The purpose of this study is to establish and analyse the conservation status of medicinal plants from the flora of Bulgaria. Of total 954 species of medicinal plants in the flora of Bulgaria, 290 are considered to be of conservation importance: Bulgarian and Balkan endemics; species listed in the Red Data Book of Bulgaria and their categories of endangerment; species in the Annexes of the Biodiversity Conservation Act in Bulgaria; species included in the Bern Convention; species in the Annexes to the Habitats Directive; species under the protection of the International Convention on Trade in Wildlife; medicinal plants in Bulgaria listed in the World Red List of Plants; species in the European Red List and European Red List of Medicinal plants of different categories; List of rare and endangered endemic plants in Europe; species, which are tertiary and glacial relicts. The collection of these species must take into account their condition and comply with the regulations governing their use and conservation.

Key words: Bern Convention, Biodiversity Act, protection category, Red Data book.

Introduction

From ancient times to the present day, man has used plants for various purposes, many of which are used to treat various diseases.

For the purposes of the present study, medicinal plants are those included in the Medicinal Plants Act (Anonymous 2000), as well as some species that are cited in the literature related to phytotherapy (Stoyanov 1972, 1973; Ivanov et al. 1977). This law regulates the management of activities for conservation and sustainable use of medicinal plants, including the collection and purchase of herbs obtained from them. It includes 733 species of plants. However, there are gaps in the list, such as *Rosa canina* L. In our manuscript we have attempted to fill these gaps.

Classification of medicinal plants in Bulgaria, depending on the regime of use or protection according to Medicinal Plants Act (Anonymous 2000), is as follows:

1. Protected species of medicinal plants, according to the Law of Biological Diversity (Appendix 3), prohibited for collection on the territory of the country for personal and commercial purposes – *Orchis laxiflora* Lam., *Diphasiastrum alpinum* (L.) Holub, *Taxus baccata* L., *Galanthus elwesii* Hook.f., *Rhodiola rosea* L., *Gentiana lutea* L., *G. punctata* L., *Thymus perinicus* (Velen.) Jalas*, Th. stojanovii* Degen.

2. Medicinal plants under protection and regulated-use regime, according to the Biodiversity Act (Appendix 4) – *Asparagus tenuifolius* Lam.

3. Medicinal plants under a special regime of protection and use, on the basis of the Medicinal Plants Act (Art. 10, para. 1, 2 and 3)

The special regime includes two groups of plants:

3.1. Species prohibited for collection from all over the country (restrictions and prohibitions do not apply to quantities of herbs collected for personal use) – *Asarum europaeum* L., *Arctostaphylos uva-ursi* (L.) Spreng., *Sideritis scardica* Griseb., *Orchis* spp. diversa.

3.2. Species, which are determined annually, eligible for collection for business and personal use – *Berberis vulgaris* L.

4. Species permitted for both personal and commercial use – these are plants that do not have conservation status compared to current regulations and are not subject to a restrictive collection regime with annual quotas.

Characteristic of wild medicinal plants resources in Bulgaria and recommendations for their long-term development can be found in publications of Hardalova et al. (1994), Gussev (2005) and Evstatieva et al. (2007). Biodiversity and problems of conservation of medicinal plants in protected areas are commented by Evstatieva and Hardalova (2000). The development of the status of medical resources in the country is considered by Vitkova and Tashev (2007). Tashev and Tsavkov (2008) point out that 180 species of the Bulgarian dendroflora have different medical applications. Tashev (2017) also presents the distribution of medicinal plants in Bulgaria by biological types, floristic elements and life forms. Dimitrova and Tashev (2019) identify 140 species of medicinal plants on the territory of Petrohan Training and Experimental Forest Range (PTEFR), indicating for them the usable part, content of biologically active substances and their action. Tashev and Dimitrova (2019) present ecological structure of medicinal plants in Bulgaria. The methods of herbal collection and storage of medicinal plants are discussed by Assenov et al. (1998). Many authors as Stoyanov (1972, 1973), Ivanov et al. (1977), Petkov (1982), Gateva et al. (2006), Ivanov (2007), Nikolov (2007), Ivanova (2009), Kaniskov (2011), Kunovski (2012) give a description of medicinal plants in Bulgaria, indicating their medicinal properties and what they can be used for. The cited plants vary between 777 (Ivanov et al. 1977) and 1020 (Kaniskov 2011). The difference is because some authors have included mosses, lichens and ornamental plants in their lists.

The above-mentioned publication, as well as Tauchen and Kokoska (2017), are referred in creating of present list of medicinal plants.

Medicinal plants have been also studied by many authors from abroad. Issues of conservation and sustainable use are discussed by Chen et al. (2016). The authors discuss *in situ* and *ex situ* conservation and cultivation practices, good agricultural practices and sustainable use solutions, as well as biotechnical approaches (e.g. tissue culture, micro-

propagation, synthetic seed technology, and molecular markerbased approaches, which should be applied to improve the yield of medicinal plants). Jamshidi-Kia et al. (2018) investigate and describe the process of using medicinal plants throughout history. Their review focuses on the important challenges in quality evaluation of medicinal plants. Other authors (Dihoru and Boruz 2014) make lists of naturally occurring medicinal plants, indicating the organs that are collected, the frequency of collection and whether they are included in the country's Red Book. For medicinal plants that are included in Red List of Romania, for example, it is recommended: to forbid their collection from spontaneous flora or limited collection, at 1–3 years; to cultivate and to multiplicate these plants in specialized centres in order to use their active substances; to designate protected areas; to preserve them *in vitro*. Redžić (2010) applies ethnobotanical method to gather information about wild medicinal plants used in traditional medicine in Bosnia and Herzegovina. He cited 96 medicinal species and their use for treatment of different illnesses. Ssenku et al. (2022) comment that conservation of medicinal plants is highly threatened by multiple factors, such as habitat loss, population pressure and the increasing dependence on herbal medicine as a source of income in some countries of Africa. The authors discuss different conservation strategies of medicinal plants as spiritually protected forests, gender restricted harvesting, avoiding destructive harvesting, preservation of propagules, safeguard from herbivores, homestead cultivation, night and naked restricted harvesting, and mouth restricted harvesting. Some organizations (Anonymous 1993), related to the conservation of natural resources, issue a handbook on the conservation of medicinal plants with guidelines for action, emphasizing the role of international organizations in identification of the medicinal plants, outline their distributions and assess their abundance; for utilization – wherever possible, to cultivate the medicinal plants as the source of supply; to ensure that any collecting from the wild is sustainable; to improve techniques for harvesting, storage and production; to conserve populations of medicinal plant species in natural habitats; to conserve populations of medicinal plant species *ex situ*; to build public support for the conservation of medicinal plants through communication and cooperation.

Despite all protective measures, some medicinal plants are of varying degrees of endangerment. Exploring their conservational significance is an important step towards protecting these resources of Bulgarian flora.

In relation with the above mentioned, the aim of the present study is to establish the conservational status of medicinal plants from the flora of Bulgaria and measures taken for their protection.

Material and Methods

The taxonomy of plant species is given according to: Yordanov (1963–1979), Velchev (1982, 1989), Kozhuharov (1995), Delipavlov and Cheshmedzhiev (2011), Kozhukharov and Anchev (2012), Stoyanov et al. (2021).

The conservation significance of medicinal plants of the Bulgarian flora at national level is presented according to the following documents: Red Data Book of Republic Bulgaria, Volume I – Plants and fungi (Peev 2015), the Balkan and Bulgarian endemics (Petrova and Vladimirov 2010, Assyov and Petrova 2012). The bi-

odiversity of relict vascular plants in Bulgaria is according to Zahariev (2016). The conservation significance at international level is according to Red List of Endangered Plants of IUCN (Walter and Gillett 1998), as well as the List of rare, endangered and endemic plants of Europe (Lucas 1983), European Red List of vascular plants (Bilz et al. 2011) and European Red List of medicinal plants (Allen et al. 2014).

The conservation measures taken are presented in accordance with Biodiversity Act (Anonymous 2002, amend. SG 98/2018), the CITES (1973), Bern Convention – the Convention on the conservation of European wildlife and natural habitats, 1979 (Council of Europe 2022) and Council Directive 92/43/EEC, 1992 (EUR-lex 2022).

Results and Discussion

Two hundred ninety (30.4 % of the medicinal plants in Bulgaria) of the 954 species of medicinal plants in the flora of Bulgaria are considered to be of conservation importance. They are presented in Table A1 (Annex A). They belong to 81 families and 176 genera. Many of these plants are included into protected areas and sites of community importance. For comparison, 748 species of medicinal plants have been described in Romania, of which 115 are in the country's Red Book, i.e. 15 %.

Twenty two percent of investigated species are listed in the Red Data Book of Republic of Bulgaria (2015) (Fig. 1): 36 % of them are critically endangered (CR): *Adiantum capillus-veneris* L., *Allium angulosum* L., *Myricaria germanica* (L.) Desv. and others; 59 % are endangered (EN): *Vaccinium arctostaphylos* L. (Fig. 2), *Castanea sativa* Mill., *Alkanna tinctoria* Tausch. and others; 5 % are vulnerable

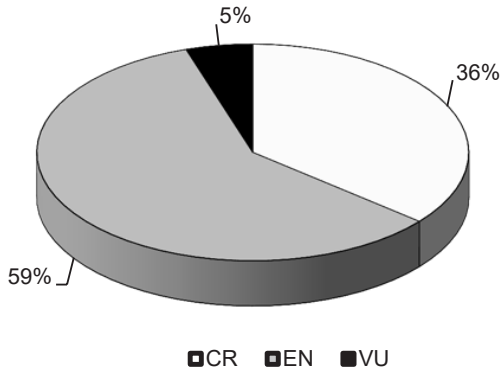

Fig. 1. Categories of endangerment (%) of medicinal species included in the Red Data Book of Republic of Bulgaria (2015).

Fig. 2. *Vaccinium arctostaphylos* (photo A. Tashev).

(VU): *Campanula lanata* Friv., *Echium russicum* Gmel. and *Opopanax chironium* W.D.J.Koch.

Two percent of the medicinal plants in Bulgaria are listed in the 1997 IUCN Red List of Threatened Plants (1998): *Alchemilla achtarowii* Pawl., *A. asteroantha* Rothm., *A. jumrukczalica* Pawl., *Campanula lanata* and *Rheum rhaponticum* L. In the latest version of the list on the Internet, four of the species have been dropped.

There are 41 % of species in the European Red List (Bilz et al. 2011) with different categories: 109 species – Least concern (LC) (Fig. 3): *Alisma plantago-aquatica* L., *Bidens tripartita* L., *Hydrocharis*

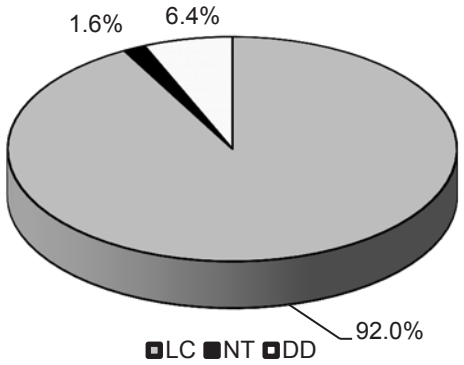

Fig. 3. Categories of endangerment (%) of medicinal species included in the European Red List of vascular plants (Bilz et al. 2011).

morsus-ranae L., *Asparagus officinalis* L., *Caltha palustris* L. and others; 2 plants – near threatened (NT): *Galanthus nivalis* L. and *Orchis spitzelii* Sauter ex W. Koch; 8 species – data deficient (DD): *Allium albidum* Fischer ex Bieb., *A. atroviolaceum* Boiss., *Campanula lanata*, *Galanthus elwesii* (Fig. 4), *Malus sylvestris* Mill., *Prunus cerasifera* Ehrh., *P. fruticosa* Pall., *Vaccinium arctostaphylos*.

Fig. 4. *Galanthus elwesii* (photo A. Tashev).

There are 19 % of species in the European Red List of Medicinal Plants (ERLMP 2014), for example: *Rhodiola rosea* (Fig. 5), *Ruscus aculeatus* L. etc.

Fig. 5. *Rhodiola rosea* (photo A. Tashev).

The List of rare and endangered endemic plants in Europe (1983) includes 2 % of the species: *Astragalus dasyanthus* Pall., *Ligularia glauca* (L.) O. Hoffm., *Rheum rhaponticum*, *Vaccinium arctostaphylos*, *Sempervivum erythraeum* Velen. and *S. leucanthum* Panc.

Six percent of plants are endemic – five of these species are Bulgarian endemics: *Alchemilla achtarowii*, *A. jumrukczalica*, *Colchicum diampolis* Delip. & Cheshm., *Rheum rhaponticum* and *Sempervivum leucanthum.* Eleven are Balkan endemics: *Sideritis scardica*, *Sambucus deborensis* Koš., *Alchemilla asteroantha*, *A. catachnoa* Rothm., *A. indivisa* (Buser) Rothm., *Campanula lanata*, *Opopanax chironium*, *Sempervivum erythraeum*, *Thymus stojanovii*, *T. perinicus* (Velen.) Jalas, *Verbascum pseudonobile* Stoj. & Stef.

Fourty one percent are relicts. Eighty-six species are tertiary relicts: *Adiantum capillus-veneris*, *Aesculus hippocastanum* L., *Butomus umbellatus* L., *Calluna vulgaris* (L.) Hulland, *Daphne pontica* L., *Ephedra distachya* L. and others. Twenty-seven are quaternary glacial relicts: *Dasiphora fruticosa* Raf., *Drosera rotundifolia* L., *Equisetum fluviatile* L., *Galanthus nivalis*, *Gymnadenia conopsea* (L.) R.Br.,

Menyanthes trifoliata L., *Rheum rhaponticum*, *Rhodiola rosea, Salix pentandra* L. and others. Four species are quaternary interglacial relicts: *Adonis vernalis* L., *Asparagus tenuifolius*, *Iris pumila* L., *Amygdalus nana* L. One plant is quaternary postglacial relict – *Rubus saxatilis* L. (Annex B).

The Annex III of the Biodiversity Act (Anonymous 2007) in Bulgaria lists 23 % of species of medicinal plants in the country: *Limonium vulgare* Mill., *Acanthus spinosus* L., *Acorus calamus* L., *Adiantum capillus-veneris*, *Alchemilla mollis* (Buser) Rothm., *Alkanna tinctoria*, *Artemisia lerchiana* Web., *Chamaecytisus ratisbonensis* (Schaeff.) Rothm., and others.

There are 8 % of the species under the protection of the International Convention on Trade in Wildlife (1973): *Platanthera chlorantha* Reich., *Orchis simia* Lam., *Gymnadenia conopsea*, *Anacamptis pyramidalis* Rich., *Cyclamen hederifolium* Aiton, *Galanthus elwesii*, *G. nivalis* and others.

Two percent of species are covered by the Bern Convention, 1979 (Council of Europe 2022): *Armoracia macrocarpa* (Waldst. & Kit.) Kit. ex Baumg., *Campanula lanata*, *Cyclamen coum* Mill., *Haberlea rhodopensis* Friv., *Orchis provincialis* Balb. ex Lam. & DC., *Rheum rhaponticum* and *Vaccinium arctostaphylos*.

Two percent of species that occur in Bulgaria, are included in the Annexes of the Habitats Directive: *Echium russicum*, *Galanthus nivalis* L., *Gentiana lutea*, *Himantoglossum jankae* Somlyay, Kreutz & Óvári, *Lycopodium clavatum* L., *Ruscus aculeatus* L.

As examples of overexploitated species, well-known species, such as *Gentiana lutea, G. punctata, Leontopodium alpinum (Ten.)* Huet ex Hand.-Mazz*., Haberlea rhodopensis*, etc., can be mentioned as more recognizable by people and collected in greater quantities for medicinal and ornamental purposes. As measures to prevent overexploitation of resources of rare and endangered species, it can be recommended to strengthen the control at the time when the relevant species are collected.

Conclusions

The medicinal plants with conservational significance in the Bulgarian flora are 290. Of these, 5 species are Bulgarian endemics; 11 are Balkan endemics. 63 species are listed in the Red Data Book of Bulgaria, of which 23 are critically endangered (CR), 37 are endangered (EN), and 3 are vulnerable (VU). The Annexes of the Biodiversity Conservation Act in Bulgaria list 81 species of medicinal plants in the country. There are 7 species covered by the Bern Convention. Of the species in the Annexes to the Habitats Directive, 5 species occur in Bulgaria. There are 23 species under the protection of the International Convention on Trade in Wildlife (CITES 1973). Of the common medicinal plants in Bulgaria 5 are listed in the World Red List of Plants. There are 119 species in the European Red List of different categories. Of these, 110 are Least concern (LC), 2 are Near threatened (NT), and 8 are Data deficient (DD). The list of species included in European Red List of Medicinal plants comprises 54 species. The List of rare and endangered endemic plants in Europe includes 6 species. There are 4 species in the Rare category (R), and the species *Sempervivum erythraeum* Velen. and *Sempervivum leucanthum* Pancic. are listed as taxa whose populations have been conserved. There are 86 species, which are tertiary and 33, which are gla-

cial relicts.

With the highest conservational status among them are: *Campanula lanata*, *Rheum rhaponticum* included in 7 documents. They are followed by *Galanthus nivalis*, *Vaccinium arctostaphylos*, which are present in 6 lists. *Orchis provincialis* is a part of 6 documents. *Haberlea rhodopensis* and *Galanthus elwesii* are part of four documents. *Mentha aquatica* L., *Hydrocharis morsus-ranae* and others are included only in one document.

The results indicate that substantial part of the medicinal plant species of the Bulgarian flora are of high conservation value.

The regulations governing their exploitation must be strictly observed during the collection of these species, in which the protection of their habitats by the competent state authorities is provided.

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Annex A

Table A1. Conservational status of medicinal plants of Bulgarian flora.

Species	Conservation importance						Measures taken				No of documents including the taxon
	Red Data Book of Bulgaria (2015)	IUCN	ERLMP	ERLVP	LRTEP	Endemic	Biodiv. Act (2002)	CITES Appendix 2	Bern Conv. (1979)	Habitats Directive	
1	2	3	4	5	6	7	8	9	10	11	12
Acanthus spinosus L.							+				1
Acorus calamus L.							+				1
Adiantum capillus-veneris L.	CR		+				+				4
Aesculus hippocastanum L.			+				+				3
Alchemilla achtarowii Pawl.	EN	+				Bul	+				4
Alchemilla asteroantha Rothm.	CR	+				Bal	+				4
Alchemilla jumrukczalica Pawl.	CR	+				Bul	+				4
Alchemilla mollis (Buser) Rothm.	CR						+				2
Alchemilla catachnoa Rothm.	EN					Bal					2
Alchemilla fissa Günther & Schummel	EN										1
Alchemilla indivisa (Buser) Rothm.	EN					Bal					2
Alchemilla plicata Buser	EN										1
Alchemilla pyrenaica Dufour	EN										1
Alisma plantago-aquatica L.				LC							1
Alkanna tinctoria Tausch.	EN		+				+				3
Allium albidum Fischer ex Bieb.				DD							1
Allium amethystinum Tausch				LC							1
Allium ampeloprasum L.				LC							1
Allium angulosum L.	CR						+				2
Allium atroviolaceum Boiss.				DD							1
Allium carinatum L.				LC							1
Allium flavum L.				LC							1
Allium fuscum Waldst. & Kit.				LC							1
Allium guttatum Steven				LC							1
Allium melanantherum Pancic				LC							1
Allium schoenoprasum L.			+	LC							2
Allium scorodoprasum L.				LC							1
Allium ursinum L.			+	LC							2

1	2	3	4	5	6	7	8	9	10	11	12
Allium victorialis L.				LC							1
Anacamptis pyramidalis Rich.			+	LC			+	+			4
Anethum graveolens L.	EN										1
Angelica archangelica L.			+				+				2
Apium graveolens L.				LC							1
Aquilegia nigricans Baumg.			+				+				2
Aristolochia rotunda L.	EN						+				2
Armoracia macrocarpa (Waldst. & Kit.) Kit. ex Baumg.	EN		+						+		3
Artemisia lerchiana Web.							+				1
Asparagus officinalis L.			+	LC							2
Asparagus tenuifolius Lam.				LC							2
Astragalus dasyanthus Pall.					R		+				2
Barbarea vulgaris W. T. Aiton				LC							1
Bidens tripartita L.				LC							1
Brassica nigra L.				LC							1
Bupleurum longifolium L.	CR						+				2
Butomus umbellatus L.				LC							2
Calluna vulgaris (L.) Hull			+				+				3
Caltha palustris L.				LC							1
Campanula lanata Friv.	VU	+		DD		Bal	+		+		7
Cardamine amara L.				LC							1
Cardamine pratensis L.				LC							1
Castanea sativa Mill.	EN		+								3
Chamaecytisus ratisbonensis (Schaeff.) Rothm.							+				1
Cichorium intybus L.			+	LC							2
Cicuta virosa L.	CR		+	LC			+				4
Colchicum diampolis Delip. & Cheshm.	CR					Bul	+				3
Cyclamen coum Mill.				LC			+	+	+		5
Cyclamen hederifolium Aiton			+					+			2
Dasiphora fruticosa Raf.		+					+				3
Daphne pontica L.	EN						+				3
Diphasiastrum alpinum (L.) Holub							+				1
Diplotaxis tenuifolia (L.) DC.				LC							1
Dracunculus vulgaris Schott							+				1
Drosera rotundifolia L.			+				+				3
Echium russicum Gmel.	VU			LC						II/IV	3

1	2	3	4	5	6	7	8	9	10	11	12
Ephedra distachya L.							+				2
Equisetum arvense L.			+	LC							2
Equisetum fluviatile L.				LC							2
Equisetum palustre L.				LC							1
Eriophorum angustifolium Honck.				LC							1
Eryngium maritimum L.	EN		+				+				3
Euphorbia peplis L.							+				1
Fragaria vesca L.			+	LC							2
Galanthus elwesii Hook.f.	EN			DD			+	+			4
Galanthus nivalis L.	EN			NT			+	+		V	6
Gentiana lutea L.	EN		+	LC			+			V	6
Gentiana punctata L.	EN		+				+				3
Glycyrrhiza glabra L.			+				+				2
Gratiola officinalis L.			+	LC							2
Gymnadenia conopsea (L.) R.Br.			+	LC				+			4
Haberlea rhodopensis Friv.				LC			+		+		4
Himantoglossum jankae Somlyay, Kreutz & Óvári				LC			+	+		II	4
Hippophae rhamnoides L.	CR						+				2
Hordeum vulgare L.				LC							1
Hottonia palustris L.	EX			LC			+				4
Hydrocharis morsus-ranae L.				LC							1
Hypericum androsaemum L.	EN						+				3
Ilex aquifolium L.	EN		+				+				4
Ilex colchica Pojark.	EN						+				3
Iris pseudacorus L.			+	LC							2
Juniperus sabina L.	EN		+				+				3
Juniperus excelsa M.Bieb.							+				2
Lactuca serriola L.				LC							1
Lathyrus sylvestris L.				LC							1
Lathyrus tuberosus L.				LC							1
Lemna minor L.				LC							1
Leontopodium alpinum (Ten.) Huet ex Hand.-Mazz.	EN	+	+	LC			+				5
Lepidium campestre (L.) W.T.Aiton				LC							1
Lepidium graminifolium L.				LC							1
Lepidium latifolium L.				LC							1
Lepidium perfoliatum L.				LC							1

1	2	3	4	5	6	7	8	9	10	11	12
Lepidium ruderale L.				LC							1
Ligularia glauca (L.) O. Hoffm.	CR				R		+				3
Limonium vulgare Mill.							+				1
Lolium temulentum L.				LC							1
Lotus corniculatus L.				LC							1
Lycopodium clavatum L.			+	LC							2
Lycopus europaeus L.				LC							1
Lysimachia nummularia L.				LC							1
Lythrum salicaria L.			+	LC							2
Lythrum virgatum L.				LC							1
Malus sylvestris Mill.			+	DD							2
Medicago disciformis DC.				LC							1
Melilotus alba Medik.				LC							1
Melilotus officinalis Pallas			+	LC							2
Mentha aquatica L.				LC							1
Mentha pulegium L.			+	LC							2
Mentha spicata L.				LC							1
Menyanthes trifoliata L.	EN		+	LC			+				5
Myricaria germanica (L.) Desv.	CR			LC			+				3
Najas marina L.				LC							1
Nuphar lutea Sm.	EN		+	LC			+				4
Nymphaea alba L.	EN		+	LC			+				4
Oenanthe aquatica L.			+	LC							3
Opopanax chironium W.D.J.Koch	VU					Bal					2
Orchis coriophora L.								+			1
Orchis laxiflora Lam.								+			1
Orchis mascula L.				LC				+			2
Orchis militaris L.	EN		+	LC			+	+			5
Orchis morio L.								+			1
Orchis pallens L.			+	LC				+			3
Orchis papilionacea L.							+	+			2
Orchis provincialis Balb. ex Lam. & DC.	CR		+	LC			+	+	+		6
Orchis punctulatum Stev.								+			1
Orchis purpurea Hudson			+	LC				+			3
Orchis simia Lam.			+	LC				+			3
Orchis spitzelii Sauter ex W. Koch	CR		+	NT			+	+			5
Orchis tridentata Scop.								+			1

1	2	3	4	5	6	7	8	9	10	11	12
Orchis ustulata L.								+			1
Osmunda regalis L.	CR		+				+				3
Pedicularis palustris L.	CR						+				2
Persicaria hydropiper (L.) Delabre				LC							1
Persicaria lapathifolia (L.) Delarbre				LC							1
Peucedanum ruthenicum M. Bieb	CR						+				2
Pimpinella major (L.) Huds.	EX										1
Platanthera bifolia Rich.				LC				+			2
Platanthera chlorantha Reich.			+	LC				+			3
Polygala amarella Crantz	EN						+				2
Polypodium cambriacum L.							+				1
Potentilla palustris (L.) Scop.							+				1
Prangos ferulacea (L.) Lindl.	EN						+				2
Prunus cerasifera Ehrh.				DD							1
Prunus fruticosa Pall.				DD							2
Prunus mahaleb L.				LC							1
Prunus padus L.				LC							1
Prunus spinosa L.			+	LC							2
Pyrola rotundifolia L.							+				1
Quercus coccifera L.	EN						+				3
Ranunculus flammula L.				LC							1
Ranunculus lingua L.				LC							1
Ranunculus repens L.				LC							1
Ranunculus sceleratus L.				LC							1
Raphanus raphanistrum L.				LC							1
Rhamnus alpinus L.	EN										1
Rheum rhaponticum L.	CR	+			R	Bul	+		+		7
Rhodiola rosea L.	CR		+				+				4
Rhododendron ponticum L.							+				2
Ribes nigrum L.	CR		+				+				3
Rorippa austriaca (Crantz) Besser				LC							1
Rorippa sylvestris(Crantz) Besser				LC							1
Rubus macrophyllus Weihe & Nees							+				1
Rumex hydrolapathum Huds.				LC							1
Ruscus aculeatus L.			+	LC						V	4
Ruta graveolens L.	EN		+				+				4
Salix pentandra L.	CR						+				3

1	2	3	4	5	6	7	8	9	10	11	12
Sambucus deborensis Koš.	CR					Bal	+				3
Samolus valerandi L.				LC							1
Sempervivum erythraeum Velen.					NT	Bal					2
Sempervivum leucanthum Panc.					NT	Bul					2
Senecio paludosus L.	EN						+				2
Sideritis scardica Griseb.	EN		+			Bal	+				4
Sideritis syriaca L.	CR						+				2
Sinapis alba L.				LC							1
Sparganium erectum L.				LC							1
Spiraea salicifolia L.							+				1
Spirodela polyrrhiza (L.) Schleid.				LC							1
Taxus baccata L.	EN						+				2
Tetragonolobus maritimus (L.) Roth							+				1
Teucrium scordium L.				LC							1
Thymus perinicus (Velen.) Jalas	EN					Bal	+				3
Thymus stojanovii Degen	CR					Bal					2
Trachomitum venetum (L.) Woodson	EN						+				2
Trifolium alpestre L.				LC							1
Trifolium arvense L.				LC							1
Trifolium pratense L.			+	LC							2
Trifolium repens L.				LC							1
Typha angustifolia L.				LC							1
Typha latifolia L.				LC							1
Urtica dioica L.			+	LC							2
Vaccinium arctostaphylos L.	EN			DD	R		+		+		6
Verbascum pseudonobile Stoj. & Stef.	EN					Bal	+				3
Veronica anagallis-aquatica L.				LC							1
Veronica beccabunga L.				LC							1
Vicia grandiflora Scop.				LC							1
Vicia sativa L.				LC							1
Vitis vinifera L.			+	LC							2

Note: CR – critically endangered; EN – endangered; VU – vulnerable; IUCN – International union for conservation of nature; ERLVP – European Red List of vascular plants; NT – near threatened; LC – least concern; DD – data deficient; LRTEP – List of rare, threatened and endemic plants in Europe; R – rare; Bal – Balkan endemic; Bul – Bulgarian endemic; CITES – Convention on international trade in endangered species of wild fauna and flora; Bern Conv. – the Convention on the conservation of European wildlife and natural habitats; Habitats Directive – Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora.

Annex B

List of relict medicinal vascular plants in Bulgaria

Tertiary relicts: *Adiantum capillus-veneris* L., *Juniperus communis* L., *J. oxycedrus* L., *J. sabina* L., *Ephedra distachya* L., *Abies alba* Mill., *Picea abies* (L.) Karst, *Pinus nigra* J.F. Arnold, *P. peuce* Griseb., *P. sylvestris* L., *Taxus baccata* L., *Butomus umbellatus* L., *Tamus communis* L., *Hydrocharis morsus-ranae* L., *Acer campestre* L., *A. platanoides* L., *A. pseudoplatanus* L., *A. tataricum* L., *Cotinus coggygria* Scop, *Rhus coriaria* L., *Oenanthe aquatica* (L.) Poir., *Sanicula europaea* L., *Ilex aquifolium* L., *I. colchica* Pojark., *Hedera helix* L., *Asarum europaeum* L., *Periploca graeca* L., *Berberis vulgaris* L., *Alnus glutinosa* (L.) Gaertn., *A. incana* (L.) Moench, *Betula pendula* Roth, *Campanula lanata* Friv., *Sambucus racemosa* L., *Carpinus betulus* L., *C. orientalis* Mill., *Corylus avellana* L., *C. colurna* L., *Arctostaphylos uva-ursi* (L.) Spreng., *Calluna vulgaris* (L.) Hull, *Rhododendron ponticum* L., *Vaccinium arctostaphylos* L., *V. myrtillus* L., *V. uliginosum* L., *Cercis siliquastrum* L., *Colutea arborescens* L., *Castanea sativa* Mill., *Cistus creticus* L., *Fagus orientalis* Lipsky, *Quercus cerris* L., *Q. coccifera* L., *Q. dalechampii* Ten., *Q. pubescens* Willd., *Gentiana lutea* L., *Haberlea rhodopensis* Friv., *Aesculus hippocastanum* L., *Hypericum androsaemum* L., *Juglans regia* L., *Lycopus europaeus* L., *Viscum album* L., *Fraxinus excelsior* L., *F. ornus* L., *Jasminum fruticans* L., *Phillyrea latifolia* L., *Syringa vulgaris* L., *Platanus orientalis* L., *Rumex acetosa* L., *Cyclamen coum* Mill., *Clematis vitalba* L., *Isopyrum thalictroides* L., *Ranunculus lingua* L., *Crataegus pentagyna* Waldst. & Kit., *Laurocerasus officinalis* Roem, *Mespilus germanica* L., *Potentilla palustris* (L.) Scop., *Prunus fruticosa* Pall., *Dictamnus albus* L., *Ruta graveolens* L., *Populus alba* L., *P. nigra* L., *P. tremula* L., *Salix alba* L., *S. caprea* L., *S. fragilis* L., *S. purpurea* L., *S. triandra* L., *Smilax excelsa* L., *Staphylea pinnata* L., *Daphne mezereum* L., *D. pontica* L., *Celtis australis* L., *Ulmus minor* Mill., *Vitis sylvestris* C.C.Gmel.

Quaternary glacial relicts: *Equisetum fluviatile* L., *Diphasiastrum alpinum* (L.) Holub, *Galanthus nivalis* L., *Eriophorum vaginatum* L., *Gymnadenia conopsea* (L.) R.Br., *Meum athamanticum* Jacq., *Antennaria dioica* (L.) Gaerth., *Rhodiola rosea* L., *Drosera rotundifolia* L., *Vaccinium vitis-idaea* L., *Gentiana asclepiadea* L., *G. verna* L., *Gentianella bulgarica* (Velen.) Holub, *Menyanthes trifoliate* L., *Parnassia palustris* L., *Bistorta major* S. Gray, *Oxyria digyna* (L.) Hill, *Rheum rhaponticum* L., *Pyrola chlorantha* Sw., *Dasiphora fruticosa* (L.) Rydb, *Dryas octopetala* L., *Geum montanum* L., *G. reptans* L., *Spiraea salicifolia* L., *Salix pentandra* L., *S. reticulata* L., *Ribes nigrum* L., *Myricaria germanica* (L.) Desv.

Quaternary interglacial relicts: *Adonis vernalis* L., *Asparagus tenuifolius* Lam., *Iris pumila* L., *Amygdalus nana* L.

Quaternary postglacial relict: *Rubus saxatilis* L.

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Rooting of sycamore (*Acer pseodoplatanus* L.) cuttings by etiolated shoots

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Abstract

The aim of the study is to investigate the possibility of shoots' etiolation and long-term auxin effect on rooting of stem cuttings from sycamore. 3-year-old seedlings and 40-year-old mature donor were used, phase-rejuvenated through cutting of the stem at the height of the root collar. Traditional and alternative rooting methods were studied as well. The alternative variant was an attempt for rooting of etiolated and de-etiolated shoots. In the beginning of May, etiolated shoots were obtained. The leaves from the first node were cut to base. The node was subject of auxin treatment with lanoline-based 8000 mg·L^{-1} IBA (Indole-3-butyric acid) paste. It was used through a single application after cutting up and long-term auxin treatment. The traditional methods for rooting did not give a positive result. Precondition for propagation by cuttings was i) donor plants darkening two months before the beginning of growing period to obtain etiolated shoots with one node; ii) treatment of the basal node in the beginning of May with 8000 mg·L^{-1} IBA paste and its protection with aluminium foil; iii) parallel uncovering of the donors for de-etiolation 4 weeks before planting. Rooting reached up to 72 %. Successful rooting was not achieved with cuttings from a mature donor. However, in 77.33 % of the etiolated cuttings with long-term treatment before separation from the donor plant callus was formed in the basis. Root caps were developed from the callus, lenticels and the basis of the cuttings. Their average number was 26.05 and reached up to 113. After planting of these cuttings root caps continued to grow and reached length up to 4 mm. Parallelly the above-ground part necrotised.

Key words: auxin, juvenile, mature donors.

Introduction

Sycamore (*Acer pseodoplatanus* L.) is one of the most fast-growing deciduous species. Its fast growth rate and high wood price turns it into economically attractive species to be grown (Hein et al. 2008, Vachide 2009). It is characterised by wide range of tolerance towards geographical, altitudinal and edaphic conditions (Joyce et al. 1998). Sycamore can be used for erosion protection afforestation and urban landscaping (Pasta et al. 2016), and for establishment of forest protection belts. It is wind and soil salinity resistant (Joyce et al. 1998). Due to these arguments Spiecker et al. (2008) recommend Sycamore to be widely used in the

forestry in Europe.

Rooting sycamore cuttings is a difficult task (Dirr and Heuser 1987, Vachide 2009). According to Dirr and Heuser (1987), donor phasic status is of crucial importance, and cloning of mature donors using this method is practically impossible. The forming of adventives roots depends mostly on the genotype peculiarities (Rohr and Hanus 1987, Vachide 2009).

As optimal phenological status of the donor for preparation of cuttings it is recommended the shoots to be green (Jinks 1995). They have to be obtained straight after the growing begins in spring (Bertels 2012); or if they are half-lignified to be obtained in June and July (Spethmann 2007, Hartmann et al. 2010, Bertels 2012, Ewald and Naujoks 2015). The length of cuttings could be up to 20–35 cm (Hartmann et al. 2010, Ewald and Naujoks 2015).

Exogenic delivery of auxin is recommended as powder, water or alcoholic solution with concentration 2000–20,000 $mg \cdot L^{-1}$ (Hoogendoorn 1984, Spethmann 2007, Vachide 2009, Bertels 2012, Hartmann et al. 2010, Ewald and Naujoks 2015). The concentration of the auxins applied depends on the age of the donor specimen and its physiological status (Ermakov 1981, Dirr and Heuser 1987, Hartmann et al. 2010).

Besides the genotype, age and auxins, conditions during rooting are of great importance for the rhizogenesis. Peat (Hartmann et al. 2010), mixture of 2/3 peat and 1/3 perlite (Thompson et al. 2001) or sand (Ewald and Naujoks 2015) are usually used for substrate. It is recommended to keep high air humidity provided through a mist system (Ermakov 1981, Hoogendoorn 1984, Dirr and Heuser 1987, Jinks 1995, Thompson et al. 2001, Spethmann 2007, Hartmann et al. 2010, Ewald and Naujoks 2015). Thompson et al. (2001) determined switch-on regime system for 18 s every 30 min from 07:00 to 20:00 h every day. For good results, heating of the soil is also necessary (Hoogendoorn 1984, Bertels 2012).

Rooting is favoured to a large extent if donor plants are pruned and shaded before obtaining of the cuttings (Maynard et al. 1996). Etiolation of shoots or absence of light can induce or improve the percentage of rooting for species which under normal conditions are extremely difficult for vegetative propagation (Miske and Bassuk 1985; Maynard and Bassuk 1985, 1987, 1988, 1990; Podaras and Bassuk 1996; Sun and Bassuk 1999; Amissah and Bassuk 2007).

Adventive roots of green cuttings are set in the cambial area. Peripheral cells of the root germs form meristem cells later differentiated in vessel elements of the primary xylem. The formation of well-marked callus is not always observed and is not related to the formation of adventive roots. It can be definitely said that the callus formation results from the auxins effect (Vachide 2009).

In contrast to the generally accepted point of view, in rooting of cuttings, the callus formation in the potential root generation zone inhibits root formation. Large callus formations usually led to poor rooting (Vachide 2009). It reached up to 49–78 % (Jinks 1995, Thompson et al. 2001, Ewald and Naujoks 2015). Ewald and Naujoks (2015), however, pointed out that not all rooted cuttings are suitable for transplanting and that not all transplanted cuttings survive.

In an experiment of rooting of cuttings obtained from inoculated trees, they have been rejuvenated according to the stage. The percentage of rooting was 24 %. This low percentage was probably due to inoculated trees' fast ontogenetic ageing

(Thompson et al. 2001).

The low rooting rate of stem cuttings of some species has led to use of non-standard methods for improving the results. Such methods are shading and etiolation of donor plants. There are studies on lilac (*Bassuk* et al. *1985,* Maynard and Bassuk 1985, Miske and Bassuk 1985), *Acer griseum Franch, Acer saccharinum L., Betula papyrifera* Marsh., *Carpinus betulus L., Castanea mollissima* Blume*, Corylus Americana* Marshall*., Pinus strobus L., Quercus coccinea* M.*, Quercus palustris* M.*, Quercus robur L., Quercus rubra L.* (Maynard and Bassuk 1985), on common beach and hornbeam (*Bassuk* et al. *1985), on Quercus bicolor* Willd. and *Quercus robur* (Amissah and Bassuk 2007), *Cornus alba L. (Pacholczak and Szydło 2010), Acer platanoides L. (Tomov 2015), etc.*

The aim of the present study is to investigate the possible effect of etiolation of shoots and long-term auxin treatment on rooting of stem cuttings from sycamore.

Material and Method

For determination of stage influence of the donor on rooting capability of cuttings, the following donors were used:

- phase young specimen – 3-year-old seedlings produced through sowing-seeds in a container with capacity 3 L;
- mature donor at the age of 40, phase-rejuvenated through cutting of the stem at the height of root collar, from which the stump shoots have been used as cuttings.

The effect of donor phenological status and exogenic auxin on rooting was studied using both traditional and alternative impact approach. The traditional one included cutting cuttings with length 7–12 cm during two terms:

- green, half-lignificated cuttings – phenophase of mature (summer) wood formation (beginning of July);
- mature cuttings – phenophase of induced dormancy (end of March).

The auxin impact on the rooting process was studied through 4 experimental variants: control – non-treated cuttings and treated with talcum-based indole-3-butyric acid (IBA) powder in concentrations 1000, 3000 and 8000 $mg \cdot L^{-1}$.

The alternative variant was an attempt for rooting of etiolated shoots from two donor types. The containers and mature specimen were put in a darkened space (figs 1 and 2) before the start of growing

Fig. 1. Construction of crown darkening of phase mature donor.

Fig. 2. Construction of crown darkening of juvenile donors.

in the beginning of March for two months (until beginning of May). The space was established with a wooden construction around the donor covered with black mulching stuff Mypex.

In the beginning of May (1st May) etiolated shoots with at least two nodes were obtained. The mulching cloth was partially removed so the donor's shoots to be lightened but with indirect sunlight.

The leaves from the first node were cut to the base.

To test the auxin impact on the rooting of experimental variants 8000 mg·L^{-1} IBA lanoline-based paste was used. The first node with leaves removed was treated with it.

Part of the etiolated cuttings were cut and immediately planted without auxin treatment (var. 1, control 1).

Second part of etiolated cuttings were cut, treated with auxin paste and immediately planted (var. 2, single auxin impact).

Third part of the etiolated cuttings were treated with auxin paste without being separated from the shoots. The treated base was banded by 3 cm wide aluminum foil. The treatment was 4 weeks (until 28 May) (var. 3 etiolated cuttings treated with IBA paste in the base for 4 weeks) before cutting.

On 4th May the chlorophyll content in the leaves and shoots of the lightened donors was recovered. The cuttings obtained from them were considered as de-etiolated.

Part of de-etiolated cuttings were cut and immediately planted without auxin treatment (var. 4, control 2).

Second part of de-etiolated cuttings were cut, treated with auxin paste and planted immediately (var. 5, de-etiolated cuttings, single auxin impact.

Third part of de-etiolated cuttings were treated with auxin paste without being separated from the shoots. The treated base was banded by 3 cm wide aluminum foil. The treatment was 4 weeks (until 31st May) when they were cut and planted (var. 6, de-etiolated cuttings treated in the base with IBA for 4 weeks).

The etiolated cuttings with long-term auxin impact were cut and planted on 28th May, and the de-etiolated on 31st May.

The following experimental variants were developed for the juvenile seedlings and the mature donor as well (Table 1).

Table 1. Experimental variants developed for the juvenile seedlings and mature donor.

Variant	Cuttings' status
1	Control 1 – etiolated cuttings without auxin treatment
2	Etiolated cuttings, single treatment before planting
3	Etiolated cuttings, long-term treatment before separating of the donor
4	Control 2 – de-etiolated cuttings without auxin treatment
5	De-etiolated cuttings, single treatment before planting
6	De-etiolated cuttings, long-term treatment before separating of the donor

All cuttings were placed in a substrate of peat and perlite 1:1 (v/v). The peat parameters were: 0.5 mm particles; 20 kg·m^{-3} volume density; pH in water was 5.5–6.5; electrical conductivity 35–45 mS·m^{-1} ±25 %; enriched with fertilizer NPK (14:10:18) 1.5 kg·m^{-2}. The experiment was conducted in a glasshouse, at a temperature of 22–24 ºC and relative humidity of 85–95 %, maintained by an automatic mist irrigation system.

Each experimental variant was carried out in 3 repetitions, 25 cuttings each.

The result from the rooting was registered a month after the cuttings' planting.

To prove the reliability of the differences obtained, software products SPSS, ANOVA, Post Hoc test were used at confidential interval 95%. To determine the degree of influence of various factors, dispersion analysis components were used: Fisher criterion (*F* value) and significance level (*p*-value).

Results and Discussion

The traditional approach in auto-vegetative propagation did not lead to positive results both for the mature donor and juvenile specimens. Rooting was not achieved (0 %) during the two experimental phenological terms and for all variants of exogenic auxin delivery.

Alternative methods for rooting of cuttings through etiolation, however, provoked rhyzogenesis induction from juvenile donors (Table 2).

The highest percentage of rooting was obtained in setting of de-etiolated cuttings from juvenile donor specimens – 71.7 ±3.3 %, treated with IBA 8000 mg·L^{-1} for 4 weeks (Table 2, var. 6, Fig. 3). This result is in accordance with the obtained by a number of authors (Maynard and Bassuk 1985; Bassuk et al. 1986; Maynard and Bassuk 1988, 1990, 1991) – the etiolation in combination with auxin treatment and banding improved the rooting of cuttings. In their investigation Maynard and Bassuk (1996) underlined that the formation of lignificated sclerids inhibits the rooting but the etiolation delayed the sclerids development. In addition, the auxins deactivated under the light influence and its switching off by banding increased the auxins' level and improved the rooting (Sun and Bassuk 1999). But Pacholczak et al. (2005) pointed out that in rooting of *Cotinus coggygria* cuttings the shading was more effective than etiolation. The improved rooting of shaded cuttings was accompanied by decreasing of soluble sugars', proteins and abscisic acid con-

Table 2. Rooting of cuttings from a juvenile donor.

Variant	Rooting, %	Average number of roots	Average length of the root, cm
1 control 1, etiolated without treatment	60.0 ±0.0 c	5.6 ±0.5 abc	2.2 ±0.5 a
2 etiolated, single auxin treatment	35.0 ±0.0 a	7.0 ±0.8 c	3.2 ±0.3 ab
3 etiolated, long-term auxin treatment	46.7 ±4.4 b	4.5 ±0.4 a	2.2 ±0.2 a
4 control 2, de-etiolated without treatment	46.7 ±3.5 b	4.4 ±0.2 a	5.1 ±0.3 c
5 de-etiolated, single auxin treatment	44.0 ±6.1 ab	5.4 ±0.4 abc	4.6 ±3.9 c
6 de-etiolated, long-term auxin treatment	71.7 ±3.3 d	5.8 ±0.5 bc	3.2 ±0.2 b

Note: Means ± Standard error within a column followed by the same letter are not significantly different according Least Significant Difference (LSD) multiple range test at $p \leq 0.05$.

Fig. 3. Rooted cuttings from juvenile donor.

tent, and with increasing of chlorophyll, free amino acids, polyphenolic acids and free indole-3-acetic acid. In rooting of *Berberis thunbergii* 'Red Rocket' and 'Red Chief' cuttings Pacholczak et al. (2006a, b) explained the shading positive effect with less sclerenchyma development which can decrease the barriers for root primordia.

Etiolated cuttings without auxin influence rooted to 60.0 % (var. 1).

In the third group, with identical results from statistical point of view, were the variants 3, 4, 5: control; etiolated cuttings single treatment after cutting up; etiolated cuttings long-term treatment before cutting up. Rooting there reached up to 44.0–46.7 %.

The least rooting was found in etiolated cuttings single auxin treatment – 35.0 %.

Obtained results showed two basic facts.

First, it is seen that juvenile sycamore specimens have the opportunity for secondary differentiation of tissues and are capable to develop adventive root rudiments, to form adventive root system.

Second, etiolation as a tool for provoking physiological stress leads to increasing of tissues' morphogenesis activity. In support to this statement is the comparison between both controls in which rooting of de-etiolated cuttings (var. 4) reached 46.67 ±3.53 %, to differentiate from etiolated cuttings rooted 60.0 % (var. 1).

In the literature are given some general recommendations for donor plants shading before obtaining of the cuttings (Maynard et al. 1996). Etiolation of shoots or absence of light can induce or improve

the percentage of rooting for species, which under normal conditions are extremely difficult for vegetative propagation (Miske and Bassuk 1985; Maynard and Bassuk 1985, 1987, 1988, 1990, 1996; Podaras and Bassuk 1996; Sun and Bassuk 1999; Hawver and Bassuk 2000; Amissah and Bassuk 2007; Tomov 2015, 2017). Pacholczak and Szydło (2010) investigating the rooting of *Cornus alba* varieties cuttings found out that the intensity of shading maters and recommended 50 % shading. But in our experiment the complete limitation of light led to positive results. Shading of 4-year-old dogwood (*Cornus alba*) cultivars stock plants before harvest led to statistically significant increase of rooted cuttings by 6–8 % (Pacholczak et al. 2017).

However Hawver and Bassuk (2000) pointed out that the etiolation should be applied in combination with additional technics for rooting improvement. Such could be auxin treatment, root restriction or root pruning, red light application on donors, etc. According to the authors end-of-day far red light led to decreasing of cytokinins which inhibit the rooting and as a result it improved.

Maynard and Bassuk (1985) reported about positive effect of etiolation and treatment with 8000 ppm IBA powder through banding in the basis of the shoots on rooting of young (1–4-year-old) donors from *Acer griseum* (34 %), *Acer saccharum* (36 %), *Betula papyrifera* and *Castanea mollissima* (100 %), *Carpinus betulus* (94 %), *Pinus strobus* (83 %), *Quercus coccinea* (46 %). For *Quercus rubra*, however, well-expressed effect of etiolation has not been observed, and there were no experiments with *Pinus mugo*. Tomov (2015, 2017) rooted cuttings from 4-year-old *Acer platanoides* seedlings through etiolation, banding 0.8 % IBA powder (37.7 %), as well.

For *Acer platanoides*, the best reported result was 75 % rooting of etiolated cuttings (Bassuk et al. 1986). The results of this experiment were identical with the findings of Bassuk et al. (1986) and confirm the positive effect of complete shading of the donor plants or different parts of the shoots (taping) on the rooting ability of cuttings. Maynard and Bassuk (1990) pointed out successful rooting of cuttings from 5-year-old *Acer griseum* seedlings (54 %) as a result of etiolation. They also reported (1996) about 73 % rooting of cuttings from 5-year-old seedlings of *Carpinus betulus* '*fastigiata*' after etiolation and treatment for 5 s into 9.8 mM IBA in 50% aqueous ethanol.

Etiolation and banding could improve the rooting percentage of softwood cuttings from *Quercus robur*, *Q. macrocarpa* Mich and *Q. bicolor* (Amissah and Bassuk 2007). The authors reported that the influence on the rooting had the environmental conditions and more significant was the effect in field-grown cuttings in comparison with greenhouse-grown cuttings. Negative impact on rooting had cuttings' aging.

Amissah and Bassuk (2004) showed the importance of solvents when IBA was used for treatment of etiolated shoots without banding and outlined the advantages of ethanol and acetone, and rejected the positive influence of catechol (1,2-dihydroxybenzene). Our investigations showed that the lanoline as a base for the auxin paste did not lead to toxic effect. It could be pointed out as an advantage that the paste is more resistant to meteorological influences and does not dry out.

In their research Maynard and Bassuk (1992) pointed out that the rooting potential decreases if the time of their cutting

is delayed related to greening after etiolation. For *Carpinus betulus* during a period of 8 weeks positive effect was not observed. Our studies showed that for sycamore the rooting potential of cuttings was kept high if cutting was realised 4 weeks after greening. The same period was also suitable in rooting of etiolated cuttings of *Acer griseum* (Maynard and Bassuk 1990).

The analysis of root system development of juvenile cuttings showed that highest average number of roots in present in etiolated cuttings with single treatment with IBA after cutting up – 7.0 ±0.8 (var. 2). From statistical point of view, however, this variant did not differ from the de-etiolated cuttings, single treated with IBA after cutting up (var. 5), cuttings after etiolation, treated long-term with IBA before separation (var. 6), as well as non-treated etiolated cuttings (var. 1), where the average number of roots was from 5.4 ±0.4 to 5.8 ±0.5 (Table 2). Amissah and Bassuk (2004) reported significant increasing of the root average number in etiolated cutting of *Quercus bicolor* depending on the IBA solvent and found the positive effect of ethanol and acetone.

According to the length of roots (Table 2) it was determined that with biggest average length were de-etiolated cuttings – control 2, var. 4 (5.1 ±0.3 cm) and those single treated with IBA after cutting up - 4.6 ±3.9 cm (var. 5).

Differences of various indices obtained and discussed in various variants were with statistically proved significance (Table 3), significance level less than 0.05 %.

Successful rooting was not achieved with cuttings from a mature donor. However, interesting occurrence of morphogenesis was observed by the etiolated cuttings, treated long-term before separation (Table 4). In 73.3 % (from 68 to 84 %) of the cuttings, callus was formed in the basis as early as before their separation from the donor plant. Root caps were developed from the callus, lenticels and the basis of the cuttings. Their average number was 26.1 and reached up to 113. After planting of these cuttings root caps continued to grow and reached length up to 4 mm but parallelly above-ground part necrotised (Fig. 4). In some studies the negative impact of the callus on rhizogenesis was observed and it is recommended usage of band to fix up which inhibit callus formation eg velcro (Maynard and Bassuk 1987). Maynard and Bassuk (1985) successfully rooted by etiolation and treatment with 8000 ppm IBA powder through banding in the shoots' basis of mature donors from *Carpinus betulus* (72 %),

Table 3. Statistical significance of the criteria for assessment of juvenile donors rooting.

Criteria	Statistical indices	
	Fisher criterion (*F* value)	Significance level (*p*-value)
Rooting, %	12.7	0.003
Average number of roots	3.4	0.005
Average length of roots, cm	15.0	0.000

Table 4. Reaction of cuttings from mature donor.

Callused cuttings, %			Number of roots			Necrotised cuttings, %		
min	max	mean	min	max	mean	min	max	mean
68	84	73.3±4.8	10	113	26.1±1.9	16	32	22.7±4.8

Fig. 4. Formed callus (left) and root rudiments (right) of cuttings from mature donor.

Corylus americana (87 %) and *Quercus robur* (36 %). Amissah and Bassuk (2005) showed the positive effect on the rooting of *Quercus bicolor* and *Quercus macrocarpa* cuttings if shoots developed after preliminary pruning of stump root stocks to soil level. This approach was applied in our study as well aiming at stage rejuvenation of donor specimen.

Conclusions

The traditional methods for auto-vegetative propagation did not lead to a positive result in rooting of stem cuttings from sycamore.

Precondition for auto-vegetative propagation was: darkening for two months before the beginning of growing period in order to obtain etiolated shoots with one node; treatment of the basal node in the beginning of May with IBA paste with concentration 8000 mg·L^{-1} and protection with aluminium foil; parallel uncovering of the donors for de-etiolation for 4 weeks before planting. In these conditions rooting reached up to 72 %.

For rooting mature donors' cuttings optimisation in growing conditions should be applied, which would allow further development of the root system.

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Orchids of Buzuluk pine forest in South-east of European Russia: diversity and conservation issues

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Abstract

The data on orchid diversity of Buzuluk pine forest located in the South-east of European Russia on the border of steppe and forest-steppe zones have been summarised and updated. The occurrence of 12 Orchidaceae species can be reliably confirmed for this territory. This collection of species could be regarded as unique for such climatic and geographical conditions. Such a variety of orchids within the same Anatural site cannot be found anywhere else in Orenburg region, and in Samara region there are only few of similar locations. About half of the orchid species indicated for Orenburg and Samara regions are found in Buzuluk pine forest. *Hemipilia cucullata* and *Hammarbya paludosa* on the territory of Orenburg region and *Corallorhiza trifida* on the territory of Samara region are known only from Buzuluk pine forest. All the orchids found there are included in IUCN Red List. Four species are listed in Red Data Book of Russian Federation. Most of the species (except *Epipactis helleborine*) are in the regional Red Data Books, making up more than half of all protected species for each region. Orchids occur in localities near residential areas, in places visited by tourists, in the utility zone of the national park (where logging is carried out or may be carried out), in locations with unstable water regime, which may threaten their survival on this territory. All the orchids found in this woodland have at least one locality on the territory of Buzuluk Pine Forest National Park. The problems of the orchid conservation can be attributed to the insufficient knowledge of their populations (number, density, etc.), and the intensive development of oil production, tourism and recreation over the last decade.

Key words: national park, oil production, Orchidaceae, rare species, Red Data Book.

Introduction

Orchids are the second largest flowering plant family and of major conservation concern globally due to their high ratio of threatened genera and high number of threatened species (Swarts and Dixon 2009, Efimov 2010, Chase et al. 2015, Wraith and Pickering 2018, Wagner et al. 2021). Orchids occur on all continents except Antarctica, but most species are confined to tropical and subtropical zones. The flora of temperate countries usually comprises about 50–100 representatives of this family, 139 species are found in Russia (Efimov 2022). In most cases, orchid extinction is caused by the loss of habitats as a result of direct human

impact, and less so by plant collecting or climate change. (Efimov 2010, Koopowitz and Hawkins 2012, Seaton et al. 2013, Dix and Dix 2015, Go et al. 2020). The features of orchid ecology and biology amplify the effect of negative external impact, which results in a particularly high percentage of endangered species in this family (Efimov 2010).

Orchids are widely represented in Red Data Books of Russian Federation and all regions (Averyanov 2000, Efimov 2020). Around 60 % of the species occurring in Russia are listed in it (Bardunov and Novikov 2008), which is considerably higher than the similar indicators for other large families (Efimov 2010).

The hotspots of orchid flora warrant significant attention from scientists, conservation agencies and local stakeholders. By analysing geographic attributes associated with orchid hotspots, including environmental factors related to their distribution can be identified and explored to understand ecological relationships, identify suitable habitats and organise conservation (Crain and Fernández 2020). For the South-east of European Russia, such a hotspot is Buzuluk pine forest, thus studies of orchid distribution and conservation on its territory is of high relevance.

Records of some orchid species occurrence in Buzuluk pine forest, based on preserved herbarium specimens, date back to the late XIX and early XX century. Floristic reports and inventory lists (Kin 2009) compiled, as well as data on orchid diversity (Ryabinina 1998, Kin and Kalmykova 2002, Stetsuk 2009, Kin 2015) contained incomplete, partially erroneous and unconfirmed information.

The main purpose was to study orchid diversity, distribution, and to assess the problems and prospects of their conservation. The objectives were to: (1) summarise data on orchid diversity in Buzuluk pine forest; (2) consider the ecobiological and geographical composition of orchid species as a factor of sustainability; (3) identify possible threats to orchids and their conservation problems.

Materials and Methods

Buzuluk pine forest is located on the south-eastern edge of the Russian Plain, on the territory of Samara and Orenburg regions (Fig. 1). The area is 1111.18 km^2. Currently, most of its territory (1067.8878 km^2) is part of Buzuluk Pine Forest National Park established in 2007. The lands not included in the national park belong to residential areas, oil and gas production facilities, public roads, etc. (Chibilev 2008).

The complexity of the relief and landscape structure of Buzuluk pine forest defines the diversity of habitats on its territory (Kin 2009). Surface and underground waters have a great impact on the formation of the vegetation diversity. The hydrographic network of the pine forest and its surroundings comprises Samara River, its tributaries and numerous oxbow lakes in its floodplain. The main waterway crossing the pine forest is its tributary – Borovka River. Its banks form cliffs of high floodplain, less often the first and second floodplain terraces are covered with ancient dunes (Chibilev 2008).

Over the past few decades, Buzuluk pine forest as a whole has been exposed to various types of human activities. Before the establishment of the national park (in 2007), large-scale logging, geological exploration and oil production (first started in the second half of the XX century and resumed in 2015) were carried out on its territory, wild fires were also quite common

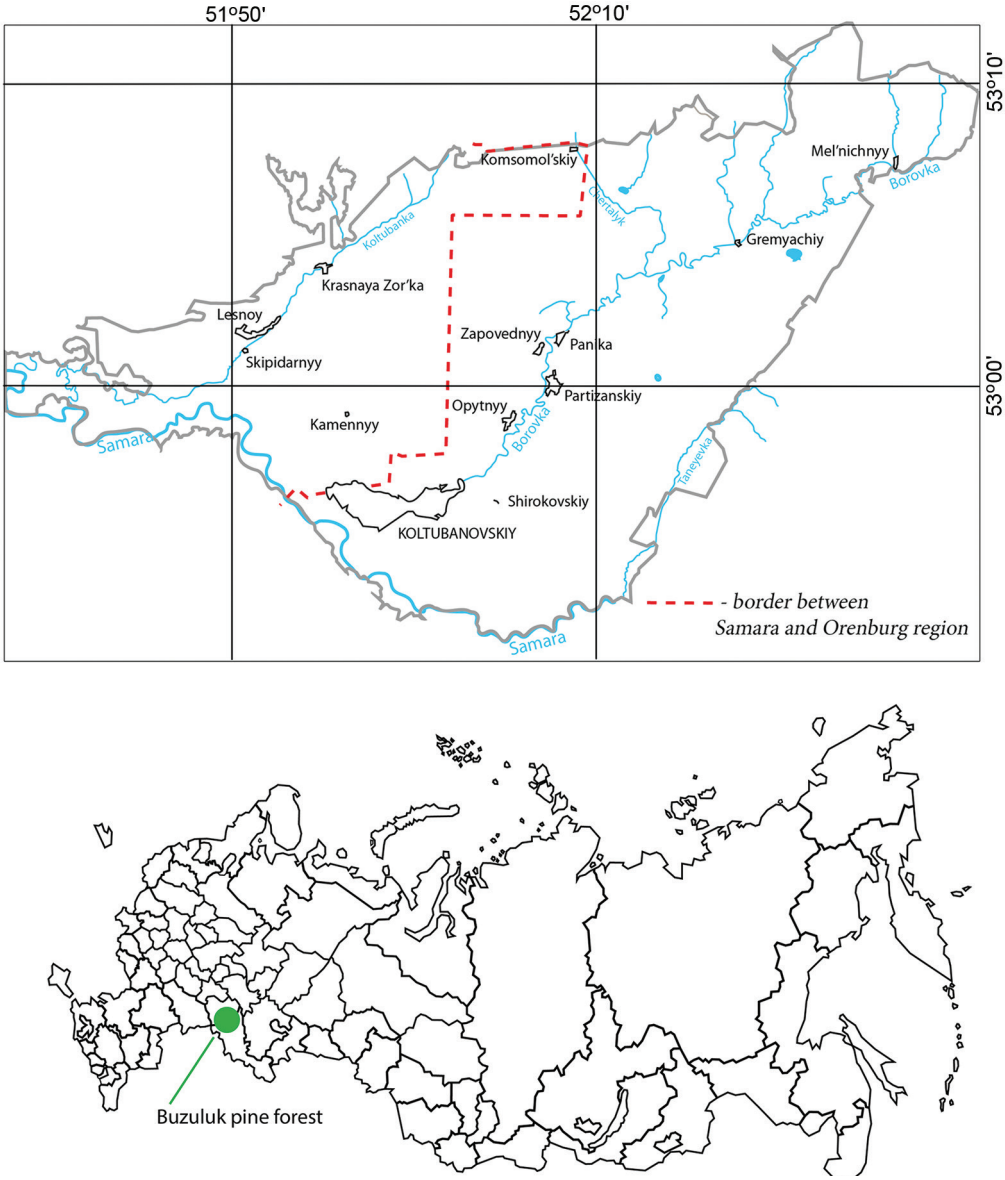

Fig. 1. Location of Buzuluk pine forest.

(Velmovsky and Chibilev 2019). Upon the establishment, the woodland included in its territory was divided into functional zones, and the impact of the above-mentioned factors has been reduced. However, there has been a significant increase in the impact of recreational and tourist activities, and urban development (often spreading beyond the borders of residential areas). The combined effects of natural and anthropogenic factors have resulted in the change (mainly decrease)

of groundwater level, river water content and, consequently, the moisture supply of orchid habitats in the forest.

The floristic survey of Buzuluk pine forest was conducted using the route method from 2000 to 2022. Sample collection and observations cover the entire territory of the woodland, including various types of habitats: meadows, floodplains, swamps, pine forests (both natural and plantings), broad-leaved woods, glades and forest fringes, dune slopes, residential areas and their surroundings, oil production sites (the old ones adjacent to suspended wells and new ones prepared for operation), transport routes between them and the surrounding area, sites of long-time burnt and logged forest. Herbarium specimens are stored in the collection of the Institute for Steppe Research of the Ural Branch of the Russian Academy of Sciences (ORIS). Archival materials were used (Anonymous 1927; Bulavkina-Onchukova 1935, 1938), as well as herbarium collections of: the Institute for Steppe Research of the Ural Branch of the Russian Academy of Sciences (ORIS), including the collection of the early XX century, contributed by Borovaya Forest Experimental Station, Komarov Botanical Institute of the Russian Academy of Sciences (LE), Lomonosov Moscow State University (MW), Lenin Moscow State Pedagogical University (MOSP), Kirov Saint Petersburg Forestry Academy. (KFTA), Penza State University (PKM), Nikitsky Botanical Garden (YALT).

The identification of potential threats to species in the surveyed territory was based on information from Red Data Books (Bardunov and Novikov 2008, Senator and Saksonov 2017, Belov 2019, IUCN 2020), as well as the analysis of the locality distribution in Buzuluk pine forest and threats presented. Wild fires, oil production, recreation, etc. are regarded as multifaceted threats that might have both a direct (habitat destruction) and indirect (habitat transformation) impact. The names of plant species are given according to The International Plant Names Index (Royal Botanic Gardens Kew 2022). IUCN Red List (IUCN 2020), Red Data Book of Russian Federation (Bardunov and Novikov 2008), Red Data Book of Orenburg Region (Belov 2019), Red Data Book of Samara region (Senator and Saksonov 2017) were used to characterise the species conservation status. Types of root systems and species geographical groups are presented according to Efimov (2022), species groups in relation to moisture content as well as ecocoenotic groups are given according to Kin (2009).

Results

Taxonomic diversity

For Buzuluk pine forest, different researchers (Yanishevsky 1898; Anonymous 1927; Sukachev 1931; Onchukova-Bulavkina 1935, 1938; Vasiliev 1998; Plaksina 1998, 2001; Matveev et al. 1999; Simonova 2001; Zadulskaya et al. 2002; Kin and Kalmykova 2002; Kin 2009; Ryabinina and Knyazev 2009; Stetsuk 2009; Korchikov et al. 2010; Saksonov and Senator 2012; Kin 2015; Kalmykova and Kin 2017; Belov 2019) indicated 22 species belonging to the Orchidaceae: *Cephalanthera rubra* (L.) Rich., *Corallorhiza trifida* Chatel., *Cypripedium calceolus* L., *C. guttatum* Sw., *C. macranthos* Sw., *C.* × *ventricosum* Sw., *Dactylorhiza majalis* ssp. *baltica* (Klinge) H.Sund., *Dactylorhiza cruenta* (O.F.Müll.) Soó, *D. incarnata* (L.) Soó, *Epipactis helleborine* (L.) Crantz., *Epipogiuma phyllum* Sw., *Goodyera repens* (L.) R.Br., *Gymnadenia conopsea* (L.) R.Br., *Hammarbya paludosa* (L.) Kuntze, *Her-*

minium monorchis (L.) R.Br., *Liparis loeselii* (L.) Rich., *Malaxis monophyllos* (L.) Sw., *Neottia ovata* (L.) Bluff & Fingerh., *N. nidus-avis* (L.) Rich., *Orchis militaris* L., *Platanthera bifolia* (L.) Rich., *Hemipilia cucullata* (L.) Y.Tang, H.Peng & T.Yukawa. More than half of these reports have been confirmed by herbarium materials and our current findings.

The data on the occurrence of seven species is doubtful: *Cypripedium guttatum*, *C. macranthos*, *C. × ventricosum*, *Epipogium aphyllum*, *Goodyera repens*, *Liparis loeselii*, *Malaxis monophyllos*. The occurrence of *Cypripedium macranthos*, *Epipogium aphyllum*, *Liparis loeselii* in Buzuluk pine forest is indicated in Red Data Book of Orenburg region (Vasiliev 1998), however, the analysis of the source material has revealed that there is no information confirming the occurrence of these species exactly in the forest. *Cypripedium guttatum*, *C. macranthos*, *C. × ventricosum*, *Epipogium aphyllum*, *Liparis loeselii*, *Goodyera repens* and *Malaxis monophyllos* are listed for the flora of Buzuluk pine forest (Stetsuk 2009) as questionable 'species indicated in the literature (without specifying sources of information), but not found in the course of field surveys'.

Two species, *Herminium monorchis* and *Dactylorhiza baltica* were misidentified and subsequently re-identified – the former as *Hammarbya paludosa*, and the latter as *D. incarnata*. We accept *Dactylorhiza cruenta* as *D. incarnata* (L.) ssp. *cruenta* (O.F.Müll.) P.D.Sell and consider it together with *Dactylorhiza incarnata*.

Thus, based on the results of our field survey and the analysis of literary, archival and herbarium materials, the occurrence of 12 Orchidaceae species can be reliably confirmed for Buzuluk pine forest: *Cephalanthera rubra*, *Corallorhiza trifida*, *Cypripedium calceolus*, *Dactylorhiza incarnata*, *Epipactis helleborine*, *Gymnadenia conopsea*, *Hammarbya paludosa*, *Neottia ovata*, *N. nidus-avis, Orchis militaris*, *Platanthera bifolia*, *Hemipilia cucullata.*

Ecobiological and geographical analysis

Most of the orchids growing in Buzuluk pine forest are polycarpics (11 species) and only one (*Neottia nidus-avis*) is a perennial monocarpic. In terms of the type of root system, rhizomatous species predominate (6 species: *Cephalanthera rubra, Corallorhiz atrifida, Cypripedium calceolus, Epipactis helleborine, Neottia nidus-avis*, *N. ovata*), tuberoids are typical of 5 species (*Dactylorhiza incarnata, Gymnadenia conopsea, Orchis militaris, Platanthera bifolia*, *Hemipilia cucullata*) one species has a pseudobulb (*Hammarbya paludosa*).

In relation to moisture content, among the orchids of Buzuluk pine forest, species of mesophytic fraction are prevalent – mesophytes (7 species: *Cephalanthera rubra*, *Cypripedium calceolus, Gymnadenia conopsea, Neottia ovata, N. nidus-avis, Platanthera bifolia, Hemipilia cucullata*) and hygromesophytes (3 species: *Dactylorhiza incarnata*, *Epipactis helleborine*, *Orchis militaris*). Only a species belongs to mesohygrophytes (*Corallorhiza trifida*) and hygrophytes (*Hammarbya paludosa*). In ecophytocoenotic terms, forest (*Cypripedium calceolus, Neottia ovata, N. nidus-avis, Hemipilia cucullata*) and forest-fringe (*Cephalanthera rubra, Epipactis helleborine, Gymnadenia conopsea, Platanthera bifolia*) species predominate, there is one in the forest-fringe–meadow (*Orchis militaris*), marsh-meadow (*Dactylorhiza incarnata*), marsh-forest (*Corallorhiza trifida*) and marsh (*Hammarbya paludosa*) groups (Fig. 2).

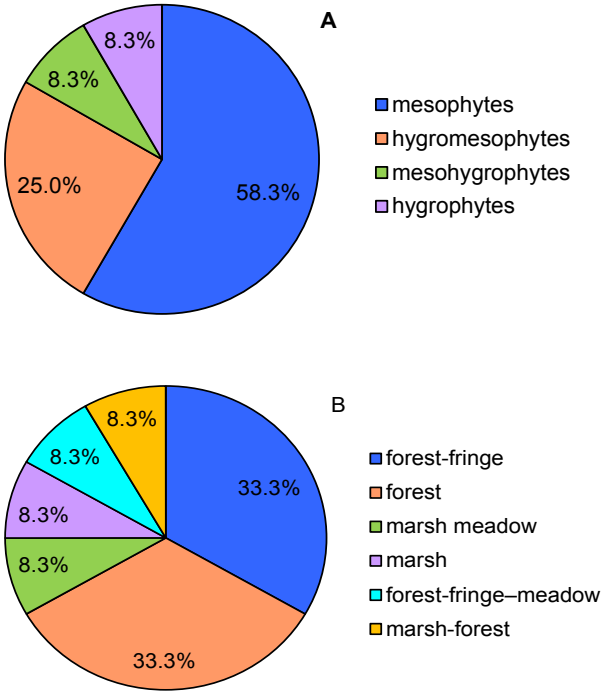

Fig. 2. Orchidaceae species spectrum of Buzuluk pine forest in relation to moisture content (A) and ecocoenotic spectrum (B).

Most of the geographical spectrum of Orchidaceae in Buzuluk pine forest is represented by Eurasian boreal-nemoral species (*Cephalanthera rubra, Cypripedium calceolus*, *Dactylorhiza incarnata, Epipactis helleborine, Gymnadenia conopsea, Neottia ovata, N. nidus-avis, Orchis militaris, Platanthera bifolia, Hemipilia cucullata*), 2 species belong to the mountain-boreal group (*Hammarbya paludosa*, *Corallorhiza trifida*).

Threats and conservation problems

Out of 12 species of orchids growing in Buzuluk pine forest, 5 were noted not only within the boundaries of the national park, but also in the territory allocated for oil production and its infrastructure: *Cypripedium calceolus, Hemipilia cucullata, Epipactis helleborine, Neottia nidus-avis, Platanthera bifolia*. Among them, *Cypripedium calceolus* and *Hemipilia cucullata* are of the greatest concern. They are less common in the pine forest and rarer in the regions than *Epipactis helleborine, Neottia nidus-avis, Platanthera bifolia*.

At the same time, all the orchids found within this woodland have localities on the territory of Buzuluk Pine Forest National Park (Table 1).

The localities of *Cephalanthera rubra* are found near residential areas where they are affected by grazing, recreation and tourism; on logging sites in the utility zone of the national park, on the site with an unstable water regime. Only the herbarium specimen (1885) indicates the occurrence of *Corallorhiza trifida* in Buzuluk pine forest. According to the existing unspecified indication of the species locality, it presumably occurs in the utility or recreational and tourist zones of the national park. The most significant limiting factors for *Cypripedium calceolus* in Buzuluk pine forest include: recreational impact, changes in coenotic conditions (the species occurs in areas with unstable water regime), collecting for bouquets, forest exploitation. The populations of *Dactylorhiza incarnata* and *Orchis militaris* are mainly impacted by changes in habitat conditions, mainly moisture, since

Table 1. Distribution of orchids by functional zones of the national park.

Species	Functional zone of Buzuluk Pine Forest National Park
Cephalanthera rubra	protected, utility
Corallorhiza trifida	utility, recreational and tourist
Cypripedium calceolus	reserved, protected, utility
Dactylorhiza incarnata	reserved, protected, utility
Epipactis helleborine	all zones
Gymnadenia conopsea	reserved, utility
Hammar byapaludosa	reserved
Hemipilia cucullata	reserved, recreational and tourist
Neottia nidus-avis	all zones
Neottia ovata	reserved, protected, utility
Orchis militaris	reserved, recreational and tourist
Platanthera bifolia	all zones

the species occurs in places with a sharp fluctuation in moisture content. In a locality, forest is occupying meadow. The species is noted on areas actively visited by people for recreation and tourism, in grazed areas near the village. For *Epipactis helleborine*, the major threats in Buzuluk pine forest are recreation and tourism, urbanisation, and oil production. *Gymnadenia conopsea* is threatened by the change in water regime, recreation and tourism. *Hammarbya paludosa* was found in Buzuluk pine forest in only one locality in the reserved zone of the national park, however, this habitat is characterised by an unstable water regime. *Neottia nidus-avis* is affected by forest exploitation, recreation and tourism. *Platanthera bifolia* was spotted in areas where a change in habitat conditions (moisture content, light regime) is likely to happen or already is taking place. This species is also exposed to recreational and tourist activities and logging. *Hemipilia cucullata* is very rare in Buzuluk pine forest, and currently oil production poses the most serious threat to this species, since one of its localities is on the transport route between industrial sites, and a road construction, if implemented, might change the light regime of the habitat. Another locality is in the recreational and tourist zone of the national park, and this may also threaten the species conservation.

Wild fires are one of the most serious potential threats to all the orchids of Buzuluk pine forest due to the high fire risk on the territory because of arid conditions. Thanks to the organisation of an effective warning and fire extinguishing system, today fires are rare in the national park. However, since the preparation for oil production is in progress and production processes are about to start in the future, fires become one of the most probable risks that cause concern for the safety of orchids.

Eleven of the 12 orchid species found in Buzuluk pine forest belong to LC (Least Concern) category of IUCN Red List (IUCN 2020), one species belongs to EN (Endangered) category (Table 2). The number of most species is estimated as decreasing. Four species are listed in the Red Data Book of the Russian Federation (Bardunov and Novikov 2008). All of them have the status of 3 – rare species. Ten of the 17 species in Red Data Book of Oren-

Table 2. Conservation status of orchid species.

Species name	IUCN Red List (2020)	Red Data Book of Russian Federation (2008)	Red Data Book of Samara region (2017)	Red Data Book of Orenburg region (2019)
Cephalanthera rubra	LC/decreasing	rare	rare	endangered
Corallorhiza trifida	LC/unknown	-	-	endangered
Cypripedium calceolus	LC/stable	rare	rare	endangered
Dactylorhiza incarnata	LC/decreasing	-	rare	rare
Epipactis helleborine	LC/decreasing	-	-	-
Gymnadenia conopsea	LC/decreasing	-	rare	vulnerable
Hammarbya paludosa	LC/declining	-	endangered	-
Neottia ovata	LC/unknown	-	endangered	vulnerable
Neottia nidus-avis	LC/decreasing	-	rare	rare
Orchis militaris	LC/decreasing	rare	rare	vulnerable
Platanthera bifolia	LC/decreasing	-	rare	rare
Hemipilia cucullata	EN/decreasing	rare	endangered	endangered

Note: LC – Least Concern, EN – Endangered.

burg region and ten of the 18 species of the Red Data Book of Samara region occur in the pine forest. In Red Data Book of Orenburg region, the conservation status of species is often stricter than in Red Data Book of Samara region. The view on the rarity of *Cephalanthera rubra* differs the most. The species is considered as endangered in Orenburg region and as rare in Samara region.

Discussion

Orchids are a family more common for tropical and subtropical latitudes, while the temperate zone is inhabited by a few species, mainly with extensive ranges. Orchid species are unevenly distributed over Russia. Arid and Arctic areas are characterised by a minimum number of orchid species, whereas afforested zones, especially humid subtropical regions, by a maximum number (Efimov 2020, 2022). Buzuluk pine forest is located on the border of steppe and forest-steppe zones, that is, in the area with moisture deficit. Orchids in these zones (including Samara and Orenburg regions), as a rule, are rare finds (Saksonov and Koneva 2006, Saksonov and Senator 2012, Efimov 2020). However, 1/3 of Orchidaceae species indicated for Volga region and about half of the orchids of regional floras occur within this forest alone. Such a number of orchid species in the surveyed territory is determined by local conditions. The natural conditions of Buzuluk pine forest and the history of its formation gave rise to a wide range of habitats on its territory that allowed the formation of an original flora. The diversity of habitats suitable for Orchidaceae species (the ratio of disturbed areas, pastures, hayfields and meadows, forests and wetlands) is known to have a great impact on the taxonomic diversity of this family (Kull et al. 2016). It may well be argued that such a variety of orchids within the same natural site is unique for Orenburg region, and in Samara region

there are only few of similar locations. *Hemipilia cucullata* and *Hammarbya paludosa* in Orenburg region and *Corallorhiza trifida* in Samara region are known only from Buzuluk pine forest. At the same time, almost all the orchids found in the surveyed area, except *Cephalanthera rubra*, are characterised by Efimov (2022) as 'widespread' in Russia.

Orchids are exposed to many factors that limit their distribution in the natural environment. These include both natural habitat changes and the loss of suitable biotopes as a result of human impact and direct destruction (for example, collecting for ornamental, medicinal purposes) (Soto Arenas et al. 2007, Vakhrameeva et al. 2008, Seaton et al. 2013, Le Roux et al. 2019), and recently climate change is also mentioned (Liu et al. 2010, 2015; Seaton et al. 2013; Efimov 2022). The features of orchid biology make them vulnerable to direct and indirect human impact (Soto Arenas et al. 2007). The main factors threatening the growth and spread of orchids in Buzuluk pine forest include oil production, recreation and tourism, natural and artificial transformation of habitats.

As in Russian flora of orchids in general, in Buzuluk pine forest Eurasian boreal-nemoral species predominate. These species are currently moving north and east as a result of climate change, namely warming and aridisation (Efimov 2022). Therefore, these species deserve close attention and regular monitoring since they can serve as a marker of the dynamics of abiotic (including climatic) changes.

The forest species of Orchidaceae more often belong to species with a stable and growing number of localities. Only a small part of forest orchids is confined to intact woodlands, whereas most species successfully exist in secondary small-leaved forests. Among the species with a decreasing number of localities, meadow-forest-fringe species are indicated (Efimov 2022). The predominance of forest species among the orchids of Buzuluk pine forest allows to consider this species collection as relatively stable. The meadow-forest-fringe group is represented in Buzuluk pine forest by *Orchis militaris* which, by the data of Efimov (2022), widely demonstrates an increase in the number of Russian localities.

It was proved by Kull et al. (2016) that tuberous orchids are significantly more vulnerable to the effects of limiting factors than rhizomatous taxa. Efimov (2022), using the example of orchids in Russia, showed that among species with a decreasing number of locations, tuberoid species predominate, while among species with a growing number of locations their proportion is minimal. On the contrary, the proportion of species with a pseudobulba is highest in the group of species with a growing number of locations. Among the orchids of Buzuluk pine forest, rhizomatous species, which are more resistant to external conditions, prevail; the threat of reduction in the number of locations (considering the type of root system) is higher in *Dactylorhiza incarnata, Gymnadenia conopsea, Orchis militaris, Platanthera bifolia, Hemipilia cucullata*.

The survival of orchids is possible only under minimal human impact or upon condition of precise management of orchid habitats in urbanised and/or populated areas out (Khapugin 2020). One of the tools to control and minimize human impact on rare species (including orchids) are Red Data Books. All the orchid species found in Buzuluk pine forest are listed in IUCN Red List (IUCN 2020), 4 in Red Data Book of Russian Federation (Bardunov and Novikov 2008), 10 in the regional Red Data Books. Thus, each species of

the studied group is protected by its status in Red Data Book at the global, federal or regional level. However, this approach to conservation of floral diversity is not efficient enough and should be combined with habitat protection.

Due to the natural transformation of some orchid biotopes, there are species that require a resumption of traditional farming methods to preserve their habitats (IUCN 1996). In Buzuluk pine forest, such species are *Orchis militaris* and *Dactylorhiza incarnata*. They have formed large, well-developed coenopopulations here that were preserved for a long time thanks to mowing. Currently, however, this habitat is being occupied by forest, which leads to a sharp reduction in the number of species. To prevent its decline, it is suggested to carry out a single mowing in the second half of summer (after fruit inception) to reduce competition from neighbouring species and to increase the illumination of the habitat.

Protected areas are considered the most effective effort to protect characteristic or threatened species, habitats and ecosystems and to counteract the biodiversity loss (Geldmann et al. 2013, Coetzee et al. 2014, Gray et al. 2016, Khapugin 2020). Considerable attention is paid to the study of the diversity, ecology and dynamics of orchid coenopopulations in such territories (Ishmurzina and Barlybaeva 2008, Lesina 2009, Nalimova 2010, Tsiftsis and Tsiripidis 2015, Kuznetsova et al. 2018, Baro et al. 2019, Gladkova and Sibirina 2019, Dikareva and Tarasova 2021). Almost all the orchids found in Buzuluk pine forest, except *Cephalanthera rubra* and *Corallorhiza trifida*, have at least one locality in the reserved zone of Buzuluk Pine Forest National Park, and they can be considered safe from direct destruction and the most common forms of human impact as long as the established here regime is strictly compiled with.

The problems of orchid conservation in Buzuluk pine forest can be attributed to the insufficient knowledge of their populations (abundance, density, etc.). The literature review showed that such data are available only for some localities of several species (*Platanthera bifolia, Hemipilia cucullata, Orchis militari, Dactylorhiza incarnata, Cypripedium calceolus*), mainly without dynamics description. Such data are found in the works of Stetsuk (2004, 2009); Stetsuk and Kin (2011).

Conclusions

Orchidaceae is represented in Buzuluk pine forest mainly by species that have the least threat of extinction on a global and national scale. These are mainly widespread species on the territory of the country. At the same time, a whole collection of orchid species is concentrated in one woodland, in the southern part of the range of orchids in Russia. This species collection can be regarded as unique for such climatic and geographical conditions. Taking into account current trends in the dynamics of species distribution in Russia, ecobiological and geographical spectra, it can be considered relatively resistant to possible external impacts and promising for conservation.

Currently, the inclusion of the species in the national and regional Red Data Books, as well as the status of a national park in most of the territory contributes to the preservation of this unique species collection.

On the basis of the available data on the distribution of orchids in the areas allocated for oil production, we recommended

the oil producing company to change the location of the facilities that might disturb orchid habitats. The recommendations were partially implemented. Owing to this, the localities of the rarest orchids in the area allocated for oil production have been preserved to the present. To minimize the impact of recreation and tourism, a more detailed survey of the recreational and tourist areas of the national park is recommended. At the same time, close attention should be paid to most-visited places. According to the results of the survey, it is necessary to optimize the functional zoning and limit the admission to the sites where orchids grow.

One of the important tasks is a more detailed study of orchid populations that will enable to optimize their protection. Observation of the dynamics of orchid populations in the south of their range can be used as a marker of global environmental changes.

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Development of community forests as locations for honey bee cultivation based on potential feed sources: case study of Mataram Village community forests, Indonesia

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Abstract

Honey bee cultivation is predominantly practised in community garden areas, although honey bee cultivation in community forest areas, which are typically monocultures, is still uncommon. In order to sustain honey production, it is crucial to assess the availability of food sources for honey bees in community forest regions. This research is aimed at discovering the potential sources of honey bee food in the Mataram Village community forest in order to promote the growth of honey bee cultivation. From July to October 2021, this research was conducted in the community forest of Mataram Village, Gading Rejo, Pringsewu, and Lampung Province, Indonesia. On the study site, vegetation data were collected, and the results were then matched with honey bee feed literature. The density, frequency, and diversity analyses of plant species are utilised. 17 species of plants were identified as food sources for honey bees, including 9 types of nectar-producing plants, 8 types of pollen-producing plants, and 4 types of nectar-pollen-producing plants. Coconut have the highest relative density (RD) and relative frequency (RF) values. H' value is 1.874, the importance of plant diversity as a source of food for honey bees falls within the moderate range. To promote the growth of beekeeping in community forests with low species variety, it is vital to increase the number of plant species, so that bees have a year-round food source.

Key words: beekeepers, biodiversity, insect visitor, private forests.

Introduction

Nowadays, the use of forest products is no longer limited to wood but has expanded to Non-Timber Forest Products (NTFPs). NTFPs were utilised for a variety of uses, including food, animal feed, traditional fiber medicine, agricultural facilities, household products, and construction materials (Talukdar et al. 2021)tHoney is one of the NTFPs that is currently expanding and in demand among forest farmers, particularly on community forest holdings. Because to the present COVID-19 epidemic, honey is one of the leading contenders for those seeking natural remedies that can maintain health and boost the immune system (Agus et al. 2021, Agussalim et al. 2021).

Moreover, honey in high demand internationally and utilised globally (Hinton et al. 2020). Diverse honey beekeepers took advantage of this possibility, and community forest farmers cultivate honey bees in forest regions. Apiculture seen to be more advantageous, particularly for community forest farmers. The cultivations was not based on land, thus it will not compete with other forest plants. Moreover, honey bees serves as pollinators for many plants (Rachersberger et al. 2019).

The Omah Tawon Mataram (OTM) farmer group is one of communal forest farmers groups that pursued for honey bee apiculture. Initially, the OTM group has just kept honey bees on their backyard. OTM relocated honey bee cultivation to community forest land due to restricted space and food supplies. The objective was moving the position of cultivation, in order to given broad access of higher quality and quantity of food sources, resulting in increased honey production. Honey bee cultivation necessitates that the quality and quantity of honey bee feed were met. Absence of feed sources in honey beekeeping might lead to a decline in honey production, which will affected in negative influence on beekeepers' income (Agussalim et al. 2017).

Bees require nectar or pollen from a variety of woodland or beehive-area plants in order to survive (Nicolson 2011, Hermita 2013). The proximity of honey bee nests to nectar sources has a positive effect on the growth of honey bee colonies. Colony populations will expand and procreate more rapidly and will not relocate in search of food. The availability of alternative food sources, such as pollen, will enhance the quality of life of bee colonies and extend their lifespan (Ferdyan et al. 2021). Thus, beekeepers and managers must position honey bee colonies near food sources. Also, the availability of feed sources must be considered at all times. Plants that provide bee food must be able to produce nectar or pollen in a single year, thus the location of honey bee cultivation must also take into account the types of vegetation present. This study seeks to identify possible honey bee feed sources in the community forest of Mataram Village, Gading Rejo District, Pringsewu Regency, Indonesia, in order to assist the development of honey bee farming.

Object and Methods

This investigation was conducted in the OTM group's honey bee cultivation region in Mataram Village, Gading Rejo District, Pringsewu Regency, Indonesia (Fig. 1). The farming location is adjacent to a protected forest and the Way Sekampung River. This study was conducted between July and November of 2021.

This study was utilized both primary and secondary data sources. At the location of the cultivation of plants that have the potential to be a source of honey bee food, primary research data were collected. Using a 20-by-20-meter square area, plant species data was collected. The obtained plant data was later recognised using community-specific identities and plant determination keys. The results of the plant inventory were then compared to the list of bee food source plants that had been identified through a previous literature review. The acquired data were evaluated by calculating the density (D), relative density (RD), frequency (F), and relative frequency (RF), as well as the Shannon-Wiener diversity index (H'), in order to determine which plants have the potential to serve as a food source for honey bees.

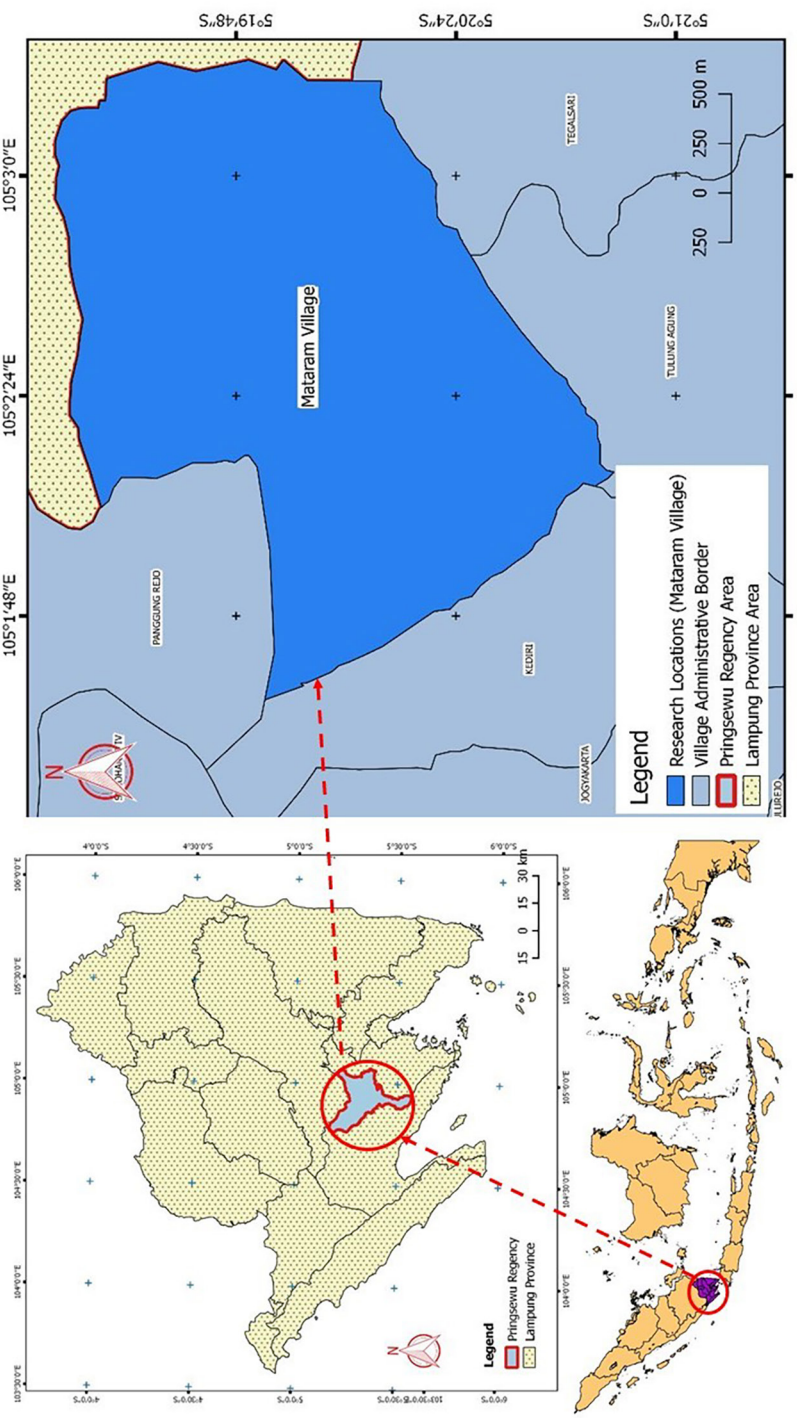

Fig. 1. Map showing the study area.

Results and Discussions

Types of pollen and nectar-producing plants

According to field identification results, 22 tree species were discovered, with 170 individual trees making up the forest. Unfortunately, not all plants are honey bee food sources. Honey bees have dietary preferences, namely on the sorts of flowering plants from which nectar or pollen can be extracted. Diverse flowering plants provide as a food supply for honey bees, however certain flowering plants release secondary metabolites that serve as a defence for the plant but are harmful to some flower visitors. This also applies to honey bees, hence honey bees have a preference for nectar and pollen sources (Villalona et al. 2020, Seshadri and Bernklau 2021).

The study result showed that from total of 22 tree species were discovered in OTM cultivation area, 17 species are honey bee food source. 17 species belonging to forest commodities, plantation commodities and fruit crop commodities. Forest commodities are bungur (*Lagerstroemia speciosa* (L.) Pers.), waru (*Hibiscus tiliaceus* L.), jati (*Tectona grandis* L. f.), pulai (*Alstonia scholaris* (L.) R.Br.), sengon (*Albizia chinensis* (Osbeck) Merr.), laban (*Vitex pubescens* Vahl.), petai (*Parkia speciosa* Hassk.), jengkol (*Archidendron pauciflorum* (Benth.) I.C.Nielsen), petai cina (*Leucaena leucocephala* (Lam.) de Wit), sonokeling (*Dalbergia latifolia* Roxb.) and suren (*Toona sureni* (Blume) Merr.). The plantation commodities are kelapa (*Cocos nucifera* L.), kakao (*Theobroma cacao* L.) and karet (*Hevea brasiliensis* Mull.Arg.). The fruit crop commodities are pisang (*Musa acuminata* Colla), durians (*Durio zibethinus* L.) and mangga (*Mangifera indica* L.). Bees can receive nourishment from a variety of flowering plants (forest, agricultural, plantation, horticultural, and wild species), which produce nectar as honey, pollen, and propolis ingredients. Honey bees gather pollen and nectar from flowers to sustain their colonies (Hermita 2013, Oguntuase et al. 2020).

The study revealed that honey bees consumed only a portion of the nectar and pollen from 17 tree species. Many species of these plants have been reported (Table 1) (Agussalim et al. 2017, Irwansyah 2018, De Lima et al. 2019, Priambudi et al. 2021) as a food source for honey bees. Nectar is the primary source of nutrition for honey bees, in addition to their own energy requirements, as well as an important portion of their larvae's diet. In the meantime, pollen is required to give the proteins, lipids, vitamins, and minerals necessary for larval development (Nicolson 2011, Portman et al. 2021). This feed was essential for the survival of honey bee colonies (Njurumana et al. 2021). This helped honey bee cultivation and productivity. Honeybees gather nectar and pollen from flowers. The flower's base, stamens, ovules, stylus, crown, and other organs contain nectar (Batoro et al. 2022). Meanwhile, pollen was a single cell containing the microgametophytes of seed plants, which produce the male gametes (sperm cells) (Stephen 2014).

Table 1 shown that some of the plants taken from nectar and pollen are kelapa, bungur, pisang, and manga. While waru, jati, pulai, sengon, petai, durian, karet, sonokeling, and suren are taken for nectar. Laban, jengkol, kakao and petai cina only taken for pollen.

Kelapa has the most potential source of pollen and a source of nectar (Jasmi et al. 2021). Kelapa inflorescence was monoecious, with male and female flowers in

Table 1. Tree species as a source of pollen and nectar.

No	Local name	Scientific name	Pollen	Nectar
1	Kelapa	*Cocos nucifera*	v	v
2	Bungur	*Lagerstroemia speciosa*	v	v
3	Waru	*Hibiscus tiliaceus*		v
4	Jati	*Tectona grandis*		v
5	Pulai	*ALSTONIA SCHOLARIS*		v
6	Pisang	*Musa acuminata*	v	v
7	Sengon	*Albizia chinensis*		v
8	Laban	*Vitex pubescens*	v	
9	Petai	*Parkia speciosa*		v
10	Kakao	*Theobroma cacao*	v	
11	Durian	*Durio zibethinus*		v
12	Jengkol	*Archidendron pauciflorum*	v	
13	Karet	*Hevea brasiliensis*		v
14	Petai cina	*Leucaena leucocephala*	v	
15	Sonokeling	*Dalbergia latifolia*		v
16	Mangga	*Mangifera indica*	v	v
17	Suren	*Toona sureni*		v

each spadix. Kelapa spadix is 1–2 m long, with 30 or more rachillae about 30–55 cm long, and has 200–300 male flowers from top to bottom (Regi and Rajkumar 2013). The high potential for male flowers in one tree produces a lot of pollen, which means that the feed source in the form of pollen was well available. Besides being the source of pollen, kelapa was also known as the source of nectar. Nectar containing 9–12 % sucrose was produced from the receptive flowers (Jay 1974). Nectar was produced from septal nectaries and hydathodes and it is secreted the stigmatic surface (Narayana 1937). The use of coconut as main food source for bees was carried out by beekeepers in Papak Hamlet, Genggelang Village, North Lombok Regency, West Nusa Tenggara Province, Indonesia (Agussalim et al. 2017).

Bungur has beautiful flowers that was able to attract insects to visit. Crescent flowers were purple and arranged in panicles with the length range of 10–15 cm. Usually, crepe flowers were located in the armpits of the leaves or the ends of the branches. Bungur can flower two times a year, from late November to December and from May to June (Rahmah et al. 2021). Crescent flowers have flower nectar and pollen, which honey bees use as a food source.

Pisang inflorescences at the end of the stem, protruding from the end of the stem, were pseudo, drooping, semi-curtained or even erect. The flowers of this plant will fall because the bracts are not deeply indented, twisted together or pinnately erect. Pisang have nectar and pollen, which were taken by honey bees for food sources. Another plant used as a source of nectar by honey bees was sengon. Sengon plants contain blooming flowers that produced seasonal nectar, which honey bees used as a source of nutrition (Agussalim et al. 2017). During the rejuvenation of fallen leaves during the dry season, this flowering process began in March and April.

Petai has the potential of a bee food, but only when it blooms. This was due to the Petai flowering season, which lasts from June to October. There were flower in some nodules, each nodule has *bracts* that form whorls and join to form a cup like structure called the cupula. Another plant used as a food source was durian. Climatic conditions influence the flowering season for durian plants. The flowers of this plant was arranged in a bowl-like arrangement on a stalk. Also, it has flowers of the

same sex that bloom in clusters right off the stem or branches at the base.

Another feed source in the field was kakao. Kakao become one of the food source for honey bees in Salut Village, Kayangan District, North Lombok Regency, West Nusa Tenggara Province, Indonesia (Erwan et al. 2022). Kakao plants have cauliflory-type flowers, namely the growth of flowers from the axillary tufts of leaves on stems and branches. Kakao flowers are hermaphrodite flowers, having two sexes (pistils and stamens) in one flower. This kakao plant is associated and symbiotic with insects that help pollinate (Nugroho et al. 2019).

Another food source plant was karet. Karet flowers consist of male and female flowers, which were found in sparse additional umbrella panicles. The base of the flower tent in bell-shaped. Flower tent length 4–8 mm. The female flowers were slightly larger than the males and contain three bear ovaries. The male flowers have ten stamens arranged into a pole. The anther was divided into two bouquets, one higher than the other. This karet flower was the source of nectar for honey bees.

As a source of pollen, Laban has an inflorescence arrangement at the end of the stem or in the leaf axils (terminal). The blooms were blue on the outside and slightly purplish and androgynous on the inside. The base of the petals are joined to form a tiny bowl, while the base of the corolla's irregular five lobes are joined. The crown was purplish-white, the stalk and anthers were within the crown's cavity, and the ovary was located above the flower's base (Alimah 2020). Pulai was another plant encountered as a source of feed. Pulai had been a type of flower that was reported as being bisexual. The flowers will form clusters on the leaf tips. Inside, the blossoms of this plant are greenish-white with rounded margins.

Mangga is a honeybee-pollinated fruit. Terminal branches produce thousands of panicle-shaped mangga blooms. Compound interest mango blooms have thousands of flower buds. Golden mango flowers with white crowns. Male mango blooms outnumber hermaphrodite ones. Insects helped pollinate mango blooms, which have male and hermaphrodite flowers. The honey bee, a helpful insect, takes mangga nectar and pollen.

Potential sources of honey bee feed

Mataram Village, Gading Rejo, Pringsewu, has a vast forest with many of resources. Agroforestry combines forestry, plantation, and crops to manage forests. Honey bee agriculture has been developed in Mataram Village, Gading Rejo, Pringsewu's communal forest over the previous year. Forest flora feed these bees. A total of 22 species from 170 trees in the forest were identified in the study area. However, not all of these trees are food source for honey bees. Ninety-three percent or 159 trees, representing 17 species, become food source for honey bees. Each species has different frequency and density values. RD and RF from 17 species are presented in Table 2.

Table 2 reveals that kelapa has the highest RD (45.28) and crepe, jati, island, sengon, laban, and sonokeling the lowest (0.63). Kelapa has a 17.65 RF value, while bungur, jati, island, pisang, sengon, petai, laban, kakao, durian, and sonokeling have 2.94. Kelapa plants have high RD and RF. Some plants have quite different RD and RF values than kelapa plants.

The field used for honey bee cultivation is directly opposite the protected forest area when viewed from the location of the investigation. This will indirectly alter the

Table 2. Potential sources of honey bee feed.

No	Local name	Scientific name	RD	FD
1	Kelapa	*Cocos nucifera*	45.28	17.65
2	Bungur	*Lagerstroemia speciosa*	0.63	2.94
3	Waru	*Hibiscus tiliaceus*	4.40	5.88
4	Jati	*Tectona grandis*	0.63	2.94
5	Pulai	*Alstonia scholaris*	0.63	2.94
6	Pisang	*Musa acuminata*	15.09	2.94
7	Sengon	*Albizia chinensis*	0.63	2.94
8	Laban	*Vitex pubescens*	0.63	2.94
9	Petai	*Parkia speciosa*	1.26	2.94
10	Kakao	*Theobroma cacao*	5.03	2.94
11	Durian	*Durio zibethinus*	3.14	11.76
12	Jengkol	*Archidendron pauciflorum*	1.89	8.82
13	Karet	*Hevea brasiliensis*	13.84	8.82
14	Petai cina	*Leucaena leucocephala*	1.26	5.88
15	Sonokeling	*Dalbergia latifolia*	0.63	2.94
16	Mangga	*Mangifera indica*	3.14	8.82
17	Suren	*Toona sureni*	1.89	5.88
		Total	100	100

sorts of vegetation on community-owned land. On community land, stands of woodland, agricultural, and plantation crops provide honey bees with sustenance. Kelapa predominates on community land since this plant is the primary crop farmed by the community. Its sap is used for palm wine and kelapa sugar production. This processed product is a source of income from community land and is sold by the community. The introduction of honey bees into this region is anticipated to generate additional money while preserving the land's biological qualities and being environmentally benign. The collection of Kelapa sap and the cultivation of honeybees can continue as usual. Kelapa sap consists of 85 percent water, 14 percent sugar, and trace amounts of aminoacids, organic acids, proteins, minerals, lipids, and colors (Iskandar and Darusalam 2020). This sugar content has been the principal source of food for honey-producing bees, therefore the prevalence of kelapa makes the potential source of food for honey bees more than adequate.

The presence or dominance of plant species with small RF and RD values was not uniformly distributed across all plots. Due to the agriculture location's proximity to a protected forest region, forestry plant seeds can transfer from the protected forest area to the cultivation area. Thus that certain forest plants are found in community-owned places; environmentally, this was a positive thing, as a variety of plants prevented monoculture stands and reduced the ecological value of property maintained by the community. In addition to woodland species, there were also fruit plants and plantation crops discovered. Although the value of RD and FP was not as high as that of kelapa, the existence of these plants benefited farmers by allowing them to cultivate crops other than kelapa sap, which is their primary output.

Diversity of food sources for honey bees

The diversity of flora kinds on the land of Mataram Village might be relatively significant. This was complemented by a diversity index result of moderate species diversity for the honey bee food supply plants. Table 3 displays the Shannon-Wiener (H') diversity index used to calculate the value of the plant diversity in Mataram Village as food sources for honey bees.

In Mataram Village, the value of plant diversity as a source of honey bee food was 1.874. This result shows that the community's land contains a medium diversity of plant species serving as a food source for honey bees. The variety value of honey bee food sources, which was previously classed as moderate, has the potential to expand. To improve the diversity of food supplies, it was still necessary to supplement additional plant species as honey bee food sources. The optimal carrying capacity of the region for the presence of honey bees is indicated by the abundance of viable food sources. With sufficient food sources, bees do not need to fly far or even travel to different regions to get nourishment.

A feed source determines the success of honey bee farmers, where the three most critical elements affecting bee populations are climate, food supplies and chemicals (Buchori et al. 2022). Bees thrive in environments where the diversity of plant species is high. This affects the diversity and quantity of available feed sources. Various food sources will increase the chances of honey bees finding suitable food sources (Parreño et al. 2022). So, the creation of diversity in honey bee cultivation locations dramatically affects the survival of honey bees.

Table 3. Diversity value of Shannon-Wiener (H') forage for honey bee plants.

No	Local name	Scientific name	$\sum pi \ln pi$
1	Kelapa	*Cocos nucifera*	0.359
2	Bungur	*Lagerstroemia speciosa*	0.032
3	Waru	*Hibiscus tiliaceus*	0.137
4	Jati	*Tectona grandis*	0.032
5	Pulai	*Alstonia scholaris*	0.032
6	Pisang	*Musa acuminata*	0.285
7	Sengon	*Albizia chinensis*	0.032
8	Laban	*Vitex pubescens*	0.032
9	Petai	*Parkia speciosa*	0.055
10	Kakao	*Theobroma cacao*	0.150
11	Durian	*Durio zibethinus*	0.109
12	Jengkol	*Archidendron pauciflorum*	0.075
13	Karet	*Hevea brasiliensis*	0.274
14	Petai cina	*Leucaena leucocephala*	0.055
15	Sonokeling	*Dalbergia latifolia*	0.032
16	Mangga	*Mangifera indica*	0.109
17	Suren	*Toona sureni*	0.075
		H'	1.874

Development of honey bee cultivation based on feed sources

Honey bee cultivation on forest land manifests the multifunctional use of the forest. Land management type, composition and density of forest vegetation affect honey yields (Sultanova et al. 2019). Based on the obtained data, it can be seen that the food source for honey bees in Mataram Village is not lacking. However, suppose you look again, where the composition of plant species is too dominated by kelapa and the value of diversity was classified as moderate. In that case, it still possible to enrich other food sources.

The Mataram Village community forest was initially focused on kelapa production, from which only the sap was taken. Then honey bees are introduced to be developed at this location. The initial objectives of forest management affect the management activities carried out and also affect the composition of the forest (Ojha and Dimov 2017). Based on this, the Mataram Village community forest stands were dominated by kelapa and when the bees were introduced, the main food source came from kelapa. As for other plants, not too much. This indicated by the val-

ue of RD and RF. Kelapa density has high and also spread throughout the plot, RD and RF values, which were quite different from other types indicate the dominance of this species compared to other types. The diversity index value also shows the level of diversity, which still classified as moderate.

Enrichment of honey bee source plants was considered very important, given the condition of the people's forest vegetation in Mataram Village. In dry and flower seasons, honey bees usually find it easy to get food. However, during the rainy season, bees will experience a lean season. It necessary to have various types of plants that can support food sources during the dry or rainy seasons, so that the bee feed is maintained throughout the year. The development and selection of plant species enrichment for honey bee feed must also pay attention to ecological and economic aspects comprehensively without reducing the primary function of the forest due to reciprocal use of the forest and society (Sukardi et al. 2022). This research could be used as a reference for species enrichment by looking at the species available in community forest areas. The selection of other types can be studied further by looking at the suitability of plant species, land conditions and the community's wishes.

Conclusions

This study concludes that honey bees food source in OTM consist in 17 plants species, including kelapa, bungur, waru, jati, pulai, pisang, sengon, laban, petai, kakao, durian, jengkol, karet, petai cina, sonkeling, mangga, and suren. Kelapa, bungur, pisang, laban, jengkol, kakao, mangga and petai cina, are source of pollen. While, kelapa, bungur, waru, jai, pulai, pisang, sengon, petai, durian, karet, sonokeling, mangga and suren are source of nectar. A Shannon-Wiener (H') index value is 1.874. The importance of plant diversity as a food source for honey bees falls into the moderate range. To encourage the growth of honey bee farming in Mataram Village, it remains necessary to diversify the types of plants from which honey bees were derived.

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Assessment of introduction success of *Viburnum* L. taxa under Right-Bank Forest-Steppe of Ukraine

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Abstract

On the territory of Ukraine, 24 species and 11 cultivars of the genus *Viburnum* were introduced at different times. We discovered 11 species and 5 cultivars in the Right-Bank Forest-Steppe of Ukraine. Therefore, it is important to assess the success of the introduction of *Viburnum* taxa in new growing conditions and it was analysed. The studied taxa belong to the groups that are quite promising and promising for introduction into culture. Plants of other groups were not detected. The studied species were well acclimatised in local conditions (acclimatisation number is from 83 to 95). The highest values were for *V. prunifolium* L. and *V. sargentii* Koehne. The degree of acclimatisation of all research objects was defined as good. The introduction capacity of the future viburnum introduction area for the Right-Bank Forest-Steppe of Ukraine is 0.85. The species were successfully acclimatised in the conditions of the Right-Bank Forest-Steppe of Ukraine; their seasonal rhythm of development corresponds to the climatic conditions of this region. The assortment of 22 species and 7 cultivars are proposed for further introduction work and the prospects for use in the region.

Key words: acclimatisation, introductory capacity, promising, viability.

Introduction

According to literature data, to the genus *Viburnum* L. belong up to 250 species (Egolf 1962, Hillebrand and Fairbrothers 1969). Two native species grow in Ukraine: *Viburnum opulus* L. and *Viburnum lantana* L. In total, 24 species and 11 cultivars from different geographical origins were introduced to the territory of Ukraine over the years (Kokhno and Kurdyuk 1994), which is only a tenth of the world's volume of the genus and represents almost all areas of natural habitats, except for Central and South America. According to the introduction test in the conditions of the Forest-Steppe of Ukraine (Kokhno and Kurdyuk 1994) 16 species and 5 cultivars of *Viburnum* successfully passed. We found there 11 species and 5 cultivars (Demchenko 2000, 2016). Analysis of the conditions of the Forest-Steppe of Ukraine and the experience of introduction of *Viburnum* species indicated that

this region is prospective for their cultivation. Special attention should be paid to cultivars and species of *Viburnum* such as *V. carlesii* Hemsl. ex Forb. & Hemsl. 'Aurora', *V. carlesii* 'Charis', *V. carlesii* 'Compactum', *V. carlesii* 'Diana', *V. rhytidophyllum* Hemsl. ex Forb. & Hemsl. 'Roseum', *V. opulus* L. 'Tatteri', *V. macrocephalum* Fortune 'Keteleerii' and so on (Egolf 1975, 1979, 1981a, b; Uleyskaya 1993).

The study of species in the conditions of introduction in different regions was devoted to the works of many researches (Petrova 1961; Rubtsov et al. 1974; Solodukhin 1985; Kokhno et al. 1986, 2005; Luchnyk 1988; Mamushkina 1988; Trofimenko et al. 2009; Chen et al. 2012; Acharya and Mukherjee 2014).

Research of species of genus *Viburnum* was carried out in many countries. In particular, useful properties and use of species in culture were considered in the works of Karatoprak and İlgün 2022, and Gopal et al. 2023. A work (Gonçalves Silva et al. 2009) was devoted to the study of species of genus *Viburnum* on the island of Madeira. The phylogenetic connections of species of genus *Viburnum* in Korea were highlighted in the work of Choi and Oh (2019).

It is impossible to speak about the prospects of using a particular introducer in crop without estimating the success of its introduction. Therefore, it is important to assess the success of the introduction of *Viburnum* taxa in new growing conditions.

According to the introduction area zoning of the territory of Ukraine (Kokhno and Kurdyuk 1994), Right-Bank Forest-Steppe belongs to the North-Eastern introduction area, the Right-Bank introduction sub-area, where the introduction and wide crop of all species of woody plants from the extreme northern regions of the Mediterranean, Caucasus, Northern regions – Eastern China, Korea, and Primorsky Krai, the northern part of Japan is possible; the northern and central parts of the Atlantic and Pacific regions of North America, that is, exactly those territories where the largest number of species of *Viburnum* naturally grows.

The success of plant introduction depends on the viability of plants in the new habitat, which is demonstrated by the peculiarities of plants passing through the cycles of seasonal development and ontogenesis.

The purpose of the study is a comparative analysis of introduction success and the viability and prospects of further introduction into the culture of species of the genus *Viburnum* in connection with the formation of their taxonomic composition in collections and rational use in landscaping and decorative horticulture in the Forest-Steppe of Ukraine.

Materials and Methods

The studies were carried out with the following taxa of the genus *Viburnum*: *V. opulus*, *V. opulus* 'Roseum', *V. opulus* 'Nanum', *V. lantana*, *V. lantana* 'Aureum', *V. lantana* 'Variegatum', *V. carlesii* Hemsl., *V. prunifolium* L., *V. rhytidophyllum* Hemsl., *V. buddleifolium* C.H. Wright, *V. burejaeticum* Rgl. et Herd., *V. lentago* L., *V. rufidulum* Raf., *V. sargentii* Koehne, *V. sargentii* 'Flavum', *V. veitchii* C.H. Wright. Plants grow in open ground in the collection plantations of the M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (Kyiv), O.V. Fomin Botanical Garden of T. Shevchenko National University of Kyiv, The National University of Life and Environmental Sciences of Ukraine (Fig. 1), arboretums 'Syrets'

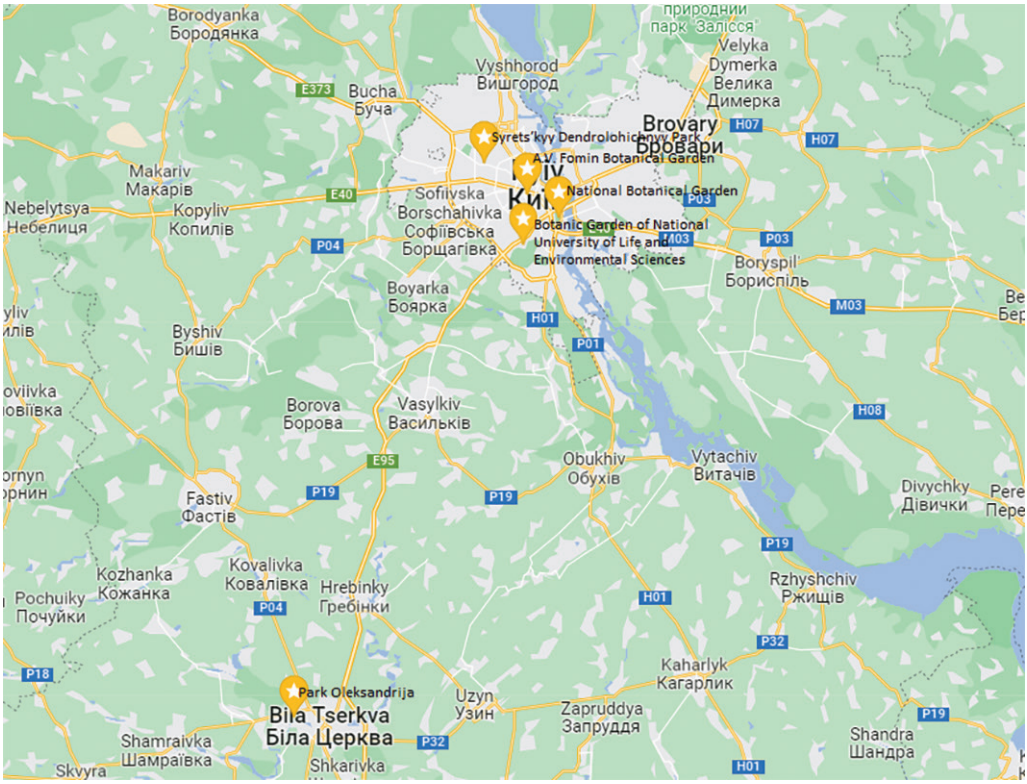

Fig. 1. Location of collection plantations of the genus *Viburnum*.

(Kyiv), 'Olexandriya' (Bila Tserkva), 'Sofiyivka' (Uman). Also, the city parks and gardens of Kyiv, Bila Tserkva, Uman, Vinnitsa, Cherkasy were examined. Stationary studies were conducted in 2009–2013. The age of the studied plants was 26–53 years. Up to 10 plants of each taxon were examined.

We conducted an evaluation of the introduction success in new growth conditions and an assessment of the viability and prospects of the introduction. The work was carried out using two methods based on regular observations of the seasonal development of plants and their ontogenesis.

The method of integral numerical evaluation of the viability and prospects of introduction of trees and shrubs based on visual observations of Lapin and Sidneva (1973) was used and evaluation of the introduction success was done according to Kokhno (1968). The introduction capacity of the area of introduction was determined by the method of Kokhno and Kurdyuk (1994).

According to the methodology of Lapin and Sidneva (1973), the perspective of the introduction was evaluated according to 7 main indicators determined by regular visual observations of the general and seasonal development of plants: the degree of annual maturation of shoots, winter hardiness, preservation of habitus, shoot-forming ability, regularity of shoot growth, ability to generative development,

available methods of propagation in crop. The method by which the assessment was carried out is given below. All 7 indicators were evaluated in points. The degree of annual maturation of shoots was assessed on a five-point scale: I – shoots ripen completely at 100 % of the length (20 points); II – mature at 75 % of the length (15 points); III – by 50 % of the length (10 points); IV – by 25 % of the length (5 points); V - shoots do not ripen at all (1 point). Winter hardiness was assessed on a seven-point scale: I – no damage (25 points); II – freezes no more than 50 % of the length of annual shoots (20 points); III – freezes 50–100 % of the length of annual shoots (15 points); IV – two-year-old and older parts of the plant frozen (10 points); V - the crown freezes up to the level of snow cover (5 points); VI – the entire above-ground part freezes over (3 points); VII – the plant freezes completely (1 point). Preservation of the habit was evaluated on a three-point scale: I – plants retain the life form characteristic in nature (10 points); II – they freeze partially, but restore the above-ground part to the previous height and volume (5 points); III – does not preserve the characteristic natural form of growth due to annual icing (1 point). Shoot-forming ability was assessed on a three-point scale: I – high shoot-forming ability: 6 or more shoots per two-year-old shoot (5 points); II – average shoot-forming ability: 3–5 shoots per two-year-old shoot (3 points); III – low shoot-forming ability: 2 shoots per one two-year-old shoot (1 point). The regularity of shoot growth was assessed by the presence or absence of annual growth of main shoots and branches, taking into account the age of the plants: annual increase – 5 points, not annual 2 points. The ability of plants to generative development was evaluated according to the scale: I – seeds ripen (25 points); II – the plants bloom, but the fruits do not ripen (20 points); III – bloom, but fruits not formed (15 points); IV – do not bloom (1 point). Available methods of reproduction of experimental plants in culture were evaluated on a five-point scale: I – self-seeding (10 points); II – artificial sowing (7 points); III – natural vegetative reproduction (5 points); IV – artificial vegetative propagation (3 points); V – repeated involvement of plants from the outside (1 point). The overall assessment of the introduction consists of the sum of points. Depending on the general assessment, the prospects of plant introduction are determined on a special scale. According to the methodology of Lapin and Sidneva (1973), six groups of introduction prospects were distinguished: I – quite promising (91–100 points); II – promising (76–90 points); III – less promising (61–75 points); IV – low-promising (41–60 points); V – unpromising (21–40 points); VI – totally unsuitable (5–20 points). Depending on the sum of the points, we determined a group of introduction prospects for each research object. Evaluation of the introduction success according to Kokhno (1968) was carried out by the method of acclimatisation number according to formula (1):

$$A = G \cdot w + Gd \cdot w + Wh \cdot w + D \cdot w, \quad (1)$$

where: A – acclimatisation number, G – growth indicator, Gd – generative development indicator, Wh – winter hardiness indicator, D – drought resistance indicator, w – coefficient of weight of the feature.

The acclimatisation number is the sum of indicators of growth, generative development, winter hardiness and drought resistance of plants; and was calculated by formula (1). Indicators of growth, generative development, winter hardiness and drought resistance were evaluated visually on five-point scales (Kokhno 1968).

The indicator obtained as a result of the visual assessment was multiplied by the indicator of the degree of importance of a specific feature – coefficient of weight (*w*). For winter resistance, its value is equal to 10, for generative development – 5, for drought resistance – 3, for growth – 2.

Depending on the value of the acclimatisation number, its degree of the research objects was determined. According to the methodology of Kokhno (1968), five degrees of acclimatisation were determined by the value of acclimatisation number (*A*): complete acclimatisation *A* equals 100, good *A* equals 80, satisfactory *A* equals 60, weak *A* equals 40, lack of acclimatisation *A* equals 20.

The introduction capacity was determined by formula (2) (Kokhno and Kurdyuk 1994):

$$Ic = \frac{n}{N'}, \qquad (2)$$

where: *Ic* is the introduction capacity, *n* is the number of species of the genus potentially appropriate for introduction in a certain area, *N* is the total number of species of the genus.

Results and Discussion

In consequence of our distribution of species of the *Viburnum* genus introduced to Ukraine by floristic regions (according to Takhtadjan 1978), it was found that the greatest number of introducers are species from the East Asian and Atlantic-North American floristic regions.

The distribution of these species by natural origin is presented in Figure 2.

Studies of the viability and prospects of introduction showed that the studied taxa belong to the groups of quite promising (I) (57.1 %) and promising (II) (42.9 %) for introduction into crop (Table 1). Plants of other groups were not found. We found that the plants of group I retain their natural growth form, have high shoot-forming ability, give annual growth of shoots, the vast majority of them bear fruit, reproduce by seeds and vegetatively. Life form and duration of life in nature and crop for these species were similar. Species of group II are characterised by regular shoot growth and good winter hardiness: no frost injuries, or occasionally no more than 50 % of

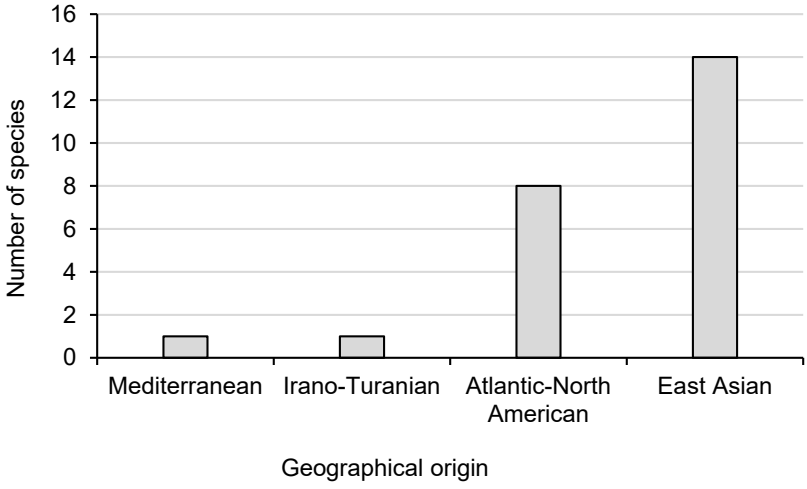

Fig. 2. Geographical origin of Viburnum species.

Table 1. Evaluation of viability of *Viburnum* spp. and the prospect of their introduction based on visual observations.

Species, cultivar	Viability indicators, points							General rating	
	Degree of shoot maturation	Winter hardiness	Preservation of habitat	Shoot-forming ability	Regularity of growth shoots	Ability to generative development	Methods of reproduction in culture	Sum of viability, points	Perspective group
V. burejaeticum	20	25	10	3	5	25	7	95	I
V. lentago	20	25	10	3	5	25	7	95	I
V. rhytidophyllum	20	20	10	3	5	25	7	90	II
V. carlesii	20	25	10	3	5	15	3	81	II
V. prunifolium	20	25	10	5	5	25	7	97	I
V. rufidulum	20	25	10	5	5	25	5	95	I
V. veitchii	20	20	10	3	5	25	7	90	II
V. buddleifolium	20	20	10	1	5	25	3	84	II
V. sargentii	20	25	10	5	5	25	7	97	I
V. sargentii ‘Flavum’	20	25	10	3	5	25	3	91	I
V. lantana ‘Aureum’	20	25	10	3	5	25	3	91	I
V. lantana ‘Variegatum’	20	25	10	3	5	25	3	91	I
V. opulus ‘Nanum’	20	25	10	3	5	15	3	81	II
V. opulus ‘Roseum’	20	25	10	3	5	15	3	81	II

annual growth freezes during severe winters (*V. buddleifolium*, *V. veitchii*). For species of group II, it is possible to propagate by seeds of local reproduction, except for non-fruiting *V. carlesii*, and cultivars that propagate vegetatively.

We estimated the degree of shoot maturation in all species by 20 points, as it was established that the shoots become woody along the entire length of the shoots. The winter hardiness of most taxa was estimated at 25 points (I no damage), the exceptions were the evergreen species *V. rhytidophyllum* and *V. buddleifolium*, as well as *V. veitchii*, in which no more than 50 % of the annual growth freezes (II 20 points). Preservation of the habit of all species was assessed at 10 points, as they preserve the life form characteristic of them in nature. The shoot-forming ability of most taxa was rated as medium (3 points), the exceptions being *V. prunifolium*, *V. rufidulum* and *V. sargentii*, which have a high shoot-forming ability (5 points). It should be noted *V. buddleifolium*, which has a low shoot-forming ability (1 point). The regularity of shoot growth was evaluated at 5 points in all research sites, as they all have annual shoot growth. The ability to generative develop-

ment was estimated at 25 points in most of the research objects, since the seeds of these species were maturing. Exceptions were *V. carlesii*, *V. opulus* 'Roseum', *V. opulus* 'Nanum' which do not bear fruit (15 points). The available methods of reproduction in culture are estimated at 7 points for most taxa, since they are propagated by artificial sowing of seeds. Exceptions were *V. rufidulum*, which naturally reproduces vegetatively (5 points), *V. buddleifolium*, *V. carlesii* and cultivars that are artificially propagated vegetatively (*V. sargentii* 'Flavum', *V. lantana* 'Aureum', *V. lantana* 'Variegatum', *V. opulus* 'Nanum', *V. opulus* 'Roseum'), (3 points).

Table 2 presents the assessment of the introduction success of research objects conducted using the method of acclimatisation number. Data show that the studied species were well acclimatised in local conditions (acclimatisation number from 83 to 95).

We estimated the growth rate of most of the research objects at 5 points; with the exception of *V. lentago*, *V. rufidulum* and *V. buddleifolium*, the growth of which was less intensive than in the natural range, but relatively good (4 points). We rated the generative development of most research objects at 4 points; exceptions were *V. carlesii* and cultivars *V. opulus* 'Roseum', *V. opulus* 'Nanum', which do not bear fruit (2 points). We assessed the winter hardiness of most of the research objects as 5 points, with the exception of *V. rhytidophyllum*, *V. buddleifolium* and *V. veitchii*, in which partial freezing of annu-

Table 2. Success assessment of the introduction of *Viburnum* spp.

Species, cultivar	Growth	Generative development	Winter hardiness	Drought resistance	Acclimatisation number (A)	Acclimatisation degree
V. burejaeticum	5	4	5	4	92	good
V. lentago	4	4	5	5	93	good
V. rhytidophyllum	5	4	4	5	85	good
V. carlesii	5	2	5	5	85	good
V. prunifolium	5	4	5	5	95	good
V. rufidulum	4	4	5	5	93	good
V. veitchii	5	4	4	5	85	good
V. buddleifolium	4	4	4	5	83	good
V. sargentii	5	4	5	5	95	good
V. sargentii 'Flavum'	5	4	5	5	95	good
V. lantana 'Aureum'	5	4	5	4	92	good
V. lantana 'Variegatum'	5	4	5	5	95	good
V. opulus 'Roseum'	5	2	5	5	85	good
V. opulus 'Nanum'	5	2	5	5	85	good

al shoots was observed (4 points). The drought resistance of most of the research objects was estimated at 5 points. The exceptions were *V. burejaeticum*, in which a temporary loss of leaf turgor during drought was observed (4 points) and *V. lantana* 'Aureum', in which leaf edge damage was observed (4 points). The degree of acclimatisation of all research objects was determined by us as good.

They grow intensively, are winter-hardy (only some of them have sometimes damaged annual shoots), bear fruit regularly, and form similar seeds, except for *V. rufidulum*, which bears fruit not annually, but reproduces well by root shoots, and *V. carlesii*, which flowers profusely but does not bear fruit in Kyiv conditions. According to literature data, flowering and fruiting were observed for this species in the conditions of Kyiv in previous years (Rubtsov et al. 1974; Malyugin 1985; Kokhno et al. 1986, 2005). We observed the formation of single fruits on young plants of this species, but they soon fell. The reasons for the lack of fruiting require further research. When comparing the degrees of acclimatisation of *Viburnum* species, we found that the highest values were found in *V. prunifolium* and *V. sargentii*, which turned out to be the most plastic under the conditions of introduction.

The Right-Bank Forest-Steppe of Ukraine belongs to the zone of relatively wide introduction of species of the genus *Viburnum*.

The introduction capacity as a quantitative assessment was determined according to formula (2) and is 0.85. The number of potentially suitable *Viburnum* species (*n*) is 213 species, the total number of species of the genus (*N*) is 250.

The experience of the successful introduction of viburnum indicates the possibility of significantly expanding the assortment of viburnum. According to literature data (Rubtsov et al. 1974, Kokhno et al. 1986, Mamushkina 1988), *Viburnum* species were presented in the collection plantations of botanical institutions of Ukraine, which were considered promising for further introduction into landscaping, however, over time, they did not become widespread. Currently, some of these species are represented in a limited number of botanical gardens and arboretums. Perhaps this is due to the lack of a sufficient number of queen cells for reproduction.

For further introduction work and prospects for use in the region, we offer an assortment of 22 species and 7 cultivars of *Viburnum*, common in the countries of Western Europe, from among species potentially suitable for introduction. First of all, these are species from North America: *V. cassinoides* L., *V. dentatum* L. *V. pubescens* (Ait.) Pursh., *V. xjuddii* Rehd., *V. xjackii* Rehd., *V. alnifolium* Marsh. *V. molle* Michx., V*. trilobum* Marsh. Species of interest from East Asian region are *V. fragrans* Bge., *V. mongolicum* (Pall.) Rehd., *V. utile* Hemsl., *V. macrocephalum* Fort., *V. phlebotricum* Sieb. et Zucc, *V. wrightii* Miq**.,** *V. sieboldii* Miq., *V. setigerum* Hance., *V. betulifolium* Batal., *V. hupehense* Rehd, *V. cotinifolium* D.Don., *V. dasyantum* Rehd., *V. dilatatum* Thunb., *V. orientale* Pall.

Some of the mentioned taxa have been tested in M.M. Gryshko National Botanical Garden, but there is no information about the causes of failure.

Conclusions

The results of the introduction of the researched species and cultivars of viburnum in the Right Bank Forest Steppe of Ukraine are summarised, the prospects

for the further introduction of representatives of this genus are determined.

According to the results of the studies of viability and prospects of introduction, we found that the studied viburnum taxa belong to the groups that are quite promising and promising for introduction into culture. As a result of the assessment of the introduction success by the method of acclimatisation number, it was determined that all the studied taxa were well acclimatised in the research region and are promising for further introduction.

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Optimization of the depth of the cages in the Dospat Dam by studying two environmental limiting factors in the rearing of rainbow trout (*Oncorhynchus mykiss,* Walbaum, 1792)

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Abstract

Aquaculture is one of the fastest growing livestock sectors for food production. In the future, sustainable and efficient development of this sector can be achieved only through a more rational use of resources. Optimization of the rearing process in rainbow trout (*Oncorhynchus mykiss*) cage farm, located in the Dospat Dam, by determining the optimal net cages depth, is an opportunity to reduce the costs for nets, save labour time for nets cleaning and reduce fish stress during the harvesting. The research of two environmental factors – water temperature (°C) and oxygen saturation (%) is important for the determination of the rainbow trout nets cage's depth. The results show that the longest period of the year with favourable conditions for the culture of the species in the dam is at a depth of 9 m. Here, water temperature and oxygen saturation are outside the optimal ranges for rainbow trout only in 11 weeks of the year, compared to 22 weeks at 1 m and 27 weeks at 20 m. Based on these results and taking into account the shape of the nets of the cages that are used in the aquafarm, the optimal depth of the rainbow trout nets in the Dospat Dam is 10 m.

Key words: aquaculture, water temperature, oxygen saturation, efficiency, costs.

Introduction

Rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) is the most widely introduced and cultured cold-water species in the world (Singh et al. 2016, Zhelyazkov and Stratev 2019). In Bulgaria, rainbow trout is the main object of cold-water aquaculture, too (Stoyanova and Staykov 2017, Uzunova 2020). In the past 5 years, its production varies between 4000 and 5000 t annually.

Trout farming is growing and to reduce costs and continue the industry forward, research and management improvement are needed (Pucher et al. 2015, Craveiro et al. 2019). In Bulgaria breeding of trout species is concentrated mainly in the mountainous and semi-mountainous regions, as the most commonly used intensive production systems are raceways and net cages. The last one is more eco-

nomically efficient (Sedgwick 1990, Stoyanova et al. 2016). The biggest rainbow trout cage aquafarm in Bulgaria is situated in the Dospat Dam.

A few economic analyses of farms that produce trout in cages shows that, the most important share of capital in the total assets are building-pool-cage and changing and maintaining the net cages (Karabulut et al. 2017, Myrli and Khawaja 2019). To reduce the costs and to ensure the economic efficiency of fish cages, it is important to determine the optimum submergence depth of them (Huang et al. 2008, Liu et al. 2019). It usually varies from 3 to 15 m (Salie et al. 2008, Yüngül et al. 2016, Dirican 2021). Transportation of fish from one facility to another causes stress to fish (Tacci et al. 2015). The deeper the cage is 20 m, the longer it takes fish to be hauled to the surface during the harvesting, resulting in fish drawing longer together into a confined space and causes it additional stress. It leads to negative effects (damage to fish health and meat quality) and economic losses (Stevenson 2007, Wu et al. 2021). On the other hand, the frequent changing of nets for on-shore cleaning is a capital- and labour-intensive process (Hodson et al. 1997). Costs and problems associated with mooring also increase with depth (Beveridge 2004). Determining the most appropriate depth for the cages in the Dospat Dam can lead to the optimization of the technological process and reduce the costs of the farm (Hadzhinikolova and Iliev 2011).

Rainbow trout occurrence in lakes and dams mostly depends on the dissolved oxygen and water temperature (Swales 2009). To define the depth of the net, it is necessary to establish its vertical distribution.

Water temperature (°C), oxygen saturation (%) and the amount of dissolved oxygen ($mg \cdot L^{-1}$) in the water are interrelated, and in general the saturation is a function of temperature. Using a stepwise linear regression model for randomly selected cages Stancheva (2021a) tested the effect of these factors on rainbow trout growth cultured in the Dospat Dam and established that the temperature and oxygen saturation has the greatest influence on it.

The optimal water temperature for rainbow trout is 12–18 °C (Purser and Forteath 2003). Growth rates are markedly reduced as the temperature is 4 °C (Hilton and Slinger 1981). The minimum temperature at which the growth of the species is reported is about 3 °C (Fornshell and Hinshaw 2009). At temperatures above 20 °C the digestive system does not use nutrients well and feed is only partially digested (Hinshaw 2000). High temperatures impaired the immunity, digestion, and growth of rainbow trout (Jiang et al. 2021).

For trout species, oxygen saturation is recommended to be over 70 % (Purser and Forteath 2003). There was no difference in growth or feed conversion ratio in the cultivation of rainbow trout in over-saturated water (180 %) with oxygen (Edsall and Smith 1990, Doulos and Kindschi 1990).

The present work aims to study the two most important environmental factors for rainbow trout rearing – water temperature and oxygen saturation in the different depth layers of the Dospat Dam and to determine the depth conditions that are favourable for culturing the species.

Materials and Methods

The study was conducted in the Dospat Dam (41°39’40” N and 24°09’05” E) which is situated in the Western Rhodopes

Mountains at 1200 m a.s.l. It was built on the Dospatska River in 1969 for the production of hydroelectrical power. Its flooded area is 22 m^2 and the catchment area is 432.30 km^2. Its length is 19 km and the average width is 1.3 m. The depth of the dam in some places exceeds 50 m. One of the largest farms for breeding rainbow trout in net cages in inland waters on the Balkan Peninsula is located in the dam. The aquafarm consists of over 150 cylinder shape with a conical base cages of 3 different diameters – 15 m, 22 m, and 30 m and its area is 3.5 ha. The cages are located in a 35 m area depth of the dam and the nets are at a depth between 15 and 20 m. Fish are grown from fingerlings (5 g) to market size (300–400 g). For the study period, the main chemical factors on which the welfare of rainbow trout depends (pH, ammonia, nitrates, sulphates, nitrogen, phosphorus, etc.) are within the permissible limits for the species. The water samples have been performed in licensed laboratories according to the respective standards.

To establish the water temperature regime and to determine the oxygen saturation of water in the Dospat Dam for the period 2017–2019, data from the aquafarm and personal reports were used (Table 1).

The measurements were made in the area of the cages 3 times a week in situ at each meter depth from 1 to 20 m. The values were reported with an electronic thermometer and combined oximeter FiveGo/F4. For the needs of aquafarm management, the results are present for weeks, and changing water temperature and oxygen saturation in depth was monitored at every 3 meters. The data for water temperature and oxygen saturation are averaged from 2017, 2018, and 2019, because no statistically significant difference ($\chi^2 = 2.229$, $p = 0.3279$) was found between their values in these years.

The obtained data were analyzed statistically using Microsoft Excel 2016 and Statistica 8.0 (Weiß 2007).

Results and Discussion

The water temperature in the Dospat Dam is outside the optimal range for rainbow trout for two periods of the year. During the winter (January–March), between the 2nd and 11th weeks, the average water temperature maintained below 4 °C (3.1 ±0.6°C, mean ±SD) in all depth layers from 1 to 20 m (Fig. 1). At these temperatures the digestion is delayed, due to which the consumption of feed with high protein content often causes severe liver damages to rainbow trout, which can lead to serious fish losses (Boyadzhiev 2011).

The second unfavourable period for species rearing is in the summer. The highest temperatures are between the 28th and 35th week of the year (Fig.1). Water temperature in these weeks in each meter depth up to 9 m exceeds 20 °C. The average temperature is 20.8 ±0.5 °C and the absolute water temperature maximum (22.9 °C) is recorded on 10.08.2019 at 1 m depth. Similar temperature values

Table 1. The number of measurements of water temperature and oxygen saturation in different depth layers of the Dospat Dam.

Depth, m	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Sample, n	224	224	224	165	162	162	162	162	162	162
Depth, m	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Sample, n	161	161	160	160	160	160	160	134	131	79

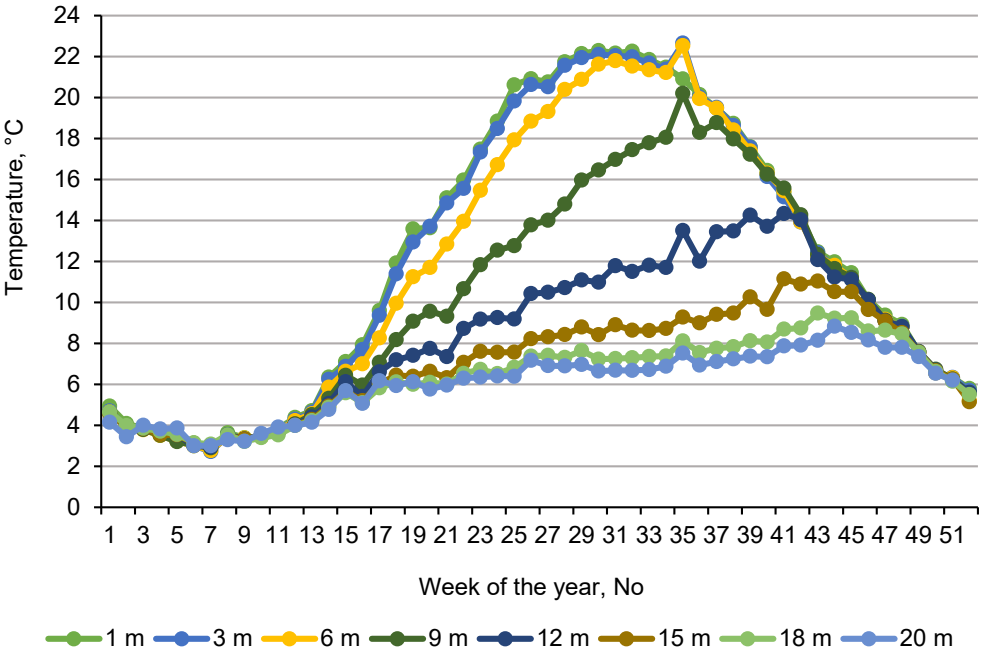

Fig. 1. Dynamics of water temperature in the Dospat Dam in different depth layers by weeks.

(22 °C and 23 °C) for the surface layer are reported respectively by Naumova and Zhivkov (1988) and Hadzhinikolova and Iliev (2011). High water temperatures in the Dospat Dam in July and August have a negative impact on the most important aquaculture efficiency parameters – fish growth, survival rate and feed conversion ratio (Stancheva 2021b).

The environment is a very important variable in aquaculture because it affects fish production and should be taken into account to make a reliable management decision (Casini et al. 2015). The analysis of the vertical dynamics of water temperature and oxygen saturation in different depth layers can be used as a tool to determine the most effective depth for rainbow trout cages in the Dospat Dam (Fig. 2a, b).

By going into depth water temperature decreases both in value and retention period (Fig. 2a). In the summer samples at a depth of 1 m, the average water temperature is 21.6 ±0.7 °C, which is above the temperature tolerance (21 °C) for rainbow trout farming, according to FAO (2023). These temperatures persist for 11 weeks (between the 25th and 36th week) at 1 m depth, and at 9 m the temperature is above 20 °C (20.2 °C) for only 1 week (35th week). Rainbow trout is highly susceptible to heat stress. It occurs when the water temperature exceeds 21 °C. The increase in temperatures may cause problems with the survival rate of fish and the occurrence of diseases due to a weakened immune system (Huang et al. 2018). For the entire study period, water temperatures above 20 °C were not measured in the depth layers between 12 m to 20 m.

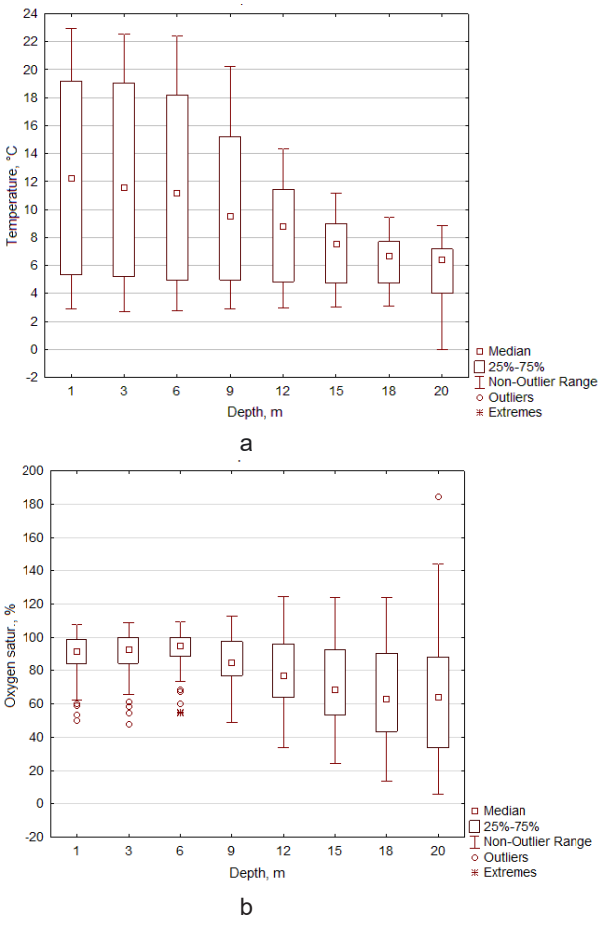

Fig. 2. Dynamics of the two environmental limiting factors in different depth layers in Dospat Dam: a) Dynamics of water temperature, b) Dynamics of oxygen saturation.

Rainbow trout growth does not differ significantly when farmed at 92 % and 79.4 % saturation, a difference occurs at 52.1 % when growth performance and survival rates are significantly lower (Welker et al. 2019). The water oxygen saturation in the dam at depths up to 9 m throughout the year is 90.3 ±12.9 %, which is above the required minimum (70 %) (Purser and Forteath 2003) for rainbow trout farming (Fig. 2b). In most unfavourable weeks in terms of water temperature (between the 25th and 35th week), the saturation remains high (96.8 ±7.06 %), which is in the optimal range for the species. Below the recommended 70 %, but within the allowable limits (40 %) for the species, saturation falls to 61.1 ±5.41 % in the period between the 48th and 51st week. This confirms the data of Hadzhinikolova and Iliev (2011) that at a depth of up to 10 m, there is no problem with oxygen levels for rainbow trout farming.

After a depth of 9 m, the average values of both factors decrease. The water temperature is below the optimal range (12–18 °C) for rainbow trout, resulting in the reduction of daily feed intake and respectively fish growth. It leads to a longer farm production cycle and higher costs (Stancheva 2021b). Oxygen saturation of water also decreases as the lowest reported levels at 12 m (33.7 %) are below the minimum required (40 %) and reach the minimum 5.7 % at a depth of 20 m, resulting in hypoxia and fish death (Williams et al. 2019). These fluctuations in the water temperature and oxygen saturation after 9 m depth are probably due to the thermocline existing at a depth of 8–15 m (Hadzhinikolova and Iliev 2011).

On this basis, the most effective depth for the site of cages is determined, as well as the most risky periods for rearing trout in terms of environmental factors. The longest period with favourable conditions for breeding rainbow trout in the Dospat Dam is at a depth of 9 m (Fig. 3). At this depth,

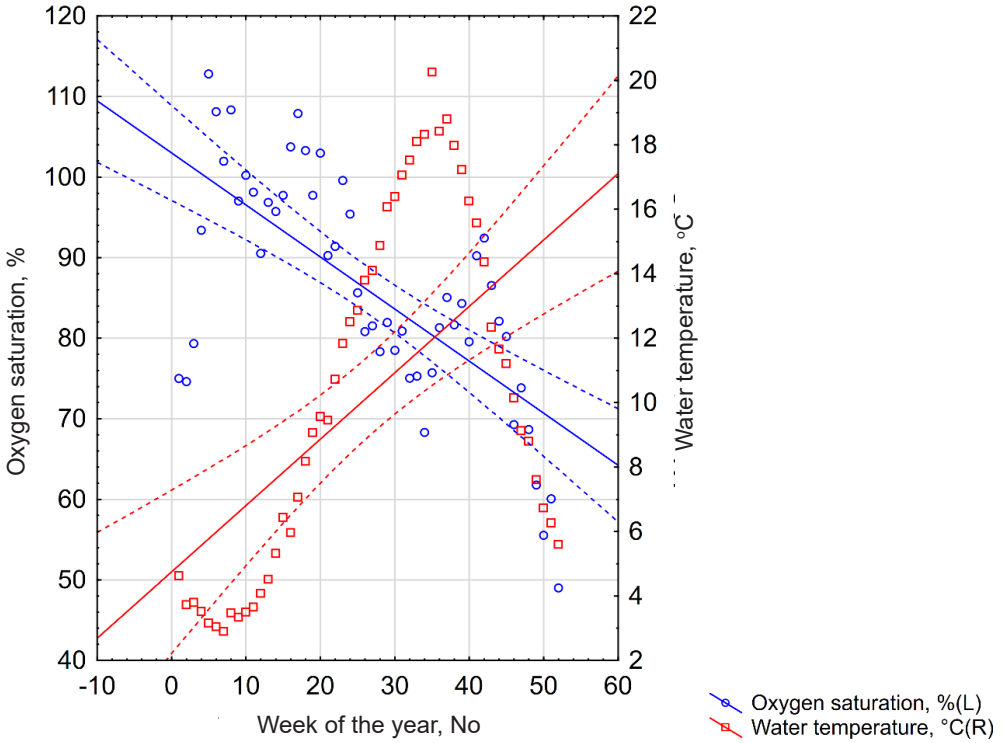

Fig. 3. Average values of water temperature and oxygen saturation at 9 m depth by weeks for the period 2017–2019.

the number of weeks in which the indicators do not meet the requirements of the trout is 11 weeks, for comparison, at 20 m they are 27 weeks, and at 1 m – 22 weeks. Water temperature and oxygen saturation are within the tolerance range for growing the species throughout the year at a depth of 9 m. Here, as in the other layers, temperatures are below 4 °C between the 2nd and 11th weeks, but the unfavourable summer period, with water temperatures above 20 °C, is only one week.

The results obtained apply to the entire water area of the dam, because according to Hadzhinikolova and Iliev (2011), there are no significant differences between the water indicators measured at different stations in the dam.

The technical efficiency of the aquafarm is related to the production technology (Farrel 1957, Brümmer et al. 2002). Optimizing the depth of the net cages will reduce the cost associated with this capital- and labour-intensive process. The cost of nets varies depending on the width of the mesh and the material that they are made of. For example, the cost of a 22 m diameter cage net used in the Dospat Dam, is about € 12 kg^{-1}, and the weight of such a net is approximately 250 kg, i.e. € 3000. For a cage with a diameter of 15 m, the price of the net is about € 2300. Reducing the depth of the cages, in addition to the price of the nets, also reduces the volume of nets that need to be cleaned. This is labour-intensive maintenance activity and

it is time- and water-consuming, and the efficiency of living labour is expressed primarily by saving time (Piralkov 2003).

According to FAO (2015) the depth of cages should be no more than 40 up to 50 % of the cage diameter, i.e. 7.5 m depth for cages with a diameter of 15 m, 11 m for cages with a diameter of 22 m and 15 m for cages with diameter 30 m. The depth of the cages may depend on the net shapes, too. Cage net shapes may be cylindrical or conical. A cylindrical shape maximizes the volume of the cage. In our case, the nets have cylinder shape with a conical base (Oliveras 2003). To use the maximum volume of the base net in the optimum environmental conditions, we recommend the depth of the base net to be up to 9 m plus 1 m depth of the net cone base height. In the specific conditions of the Dospat Dam and the nets‘ shape that is used, the optimal depth of rainbow trout nets is 10 m. The nets should be not more than 12 m, because after that the conditions for rearing the species are unfavourable and risky. It also confirms Beveridge's thesis (Beveridge 1996) that a cage depth of 3–10 m would be acceptable for most species.

Conclusion

To develop sustainable aquaculture in the future, producers need to look for opportunities to optimize production, which will reduce costs and resource use. This can be achieved by adapting technological processes to the specific needs and conditions – environmental, economic, and technological. The research of the two main environmental factors for rainbow trout rearing and optimization of the cages' depth give an opportunity to improve the production technology and reduce the costs and labour intensity in the future farming of the species in the Dospat Dam.

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Arbuscular mycorrhizal fungi status of *Nepenthes* spp. around Lore Lindu National Park, Central Sulawesi, Indonesia

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Abstract

Conservation of local and endemic biological resources of Lore Lindu National Park, Central Sulawesi Indonesia is of utmost importance as it is one of the most important ecosystem protected areas. Currently, the conservation area is threatened by forest encroachment, illegal logging and illegal mining which pose threat to the sustainability of the biological resources in it. Furthermore, there is still a lack of studies on endemic flora in this area, particularly research on the status of arbuscular mycorrhizal fungi (AMF) in Sulawesi's endemic flora in conservation areas. The present study aims to determine the species and colonisation levels of AMF on *Nepenthes* spp. plants that grow inside and outside the Lore Lindu National Park area. The results revealed that there were 3 species of *Nepenthes* in the study area, viz. *N. mirabilis*, *N. maxima* and *N. tentaculata* and as many as 4 species of AMF that have symbiosis with *Nepenthes* spp. namely, *Glomus* sp. 1, *Glomus* sp. 2, *Glomus* sp. 3 and *Gigaspora* sp. The degree of AMF colonisation on the roots of *Nepenthes* spp. varied from 2.4 % to 16.2 %. While the spore density varied between 4 and 60 per 15 g of soil. These results shows that *Nepenthes* spp. enter into symbiosis with AMF. Acquisition of more information is required in developing conservation plans and management strategies of *Nepenthes* species in the future.

Key words: colonisation, conservation, *Gigaspora*, *Glomus*, symbiosis.

Introduction

Nepenthes spp. ('pitcher' plants) are with the ability to prey on insects, where they use special traps called 'pouches' to attract, capture and digest insects as the main source of nutrition (Lee et al. 2016). Insect predation is a way for *Nepenthes* to overcome the lack of nutrients from the soil. This genus contains species that can grow as a liana or grow terrestrially (Mansur 2008). This plant has a unique way of trapping insects. Besides being able to trap insects, *Nepenthes* spp. are also able to trap frogs or birds that enter its pouch.

According to Sanusi et al. (2017), *Nepenthes* has long been used in tradi-

tional medicine in India and countries in Southeast Asia. Mansur (2006) reported that liquid in the pouch can be used as an eye, cough and burnt skin remedy. The stems are used to tie bird cages and fences (Dariana 2009) and root is used traditionally in the treatment of dysentery and colic (Schwallier et al. 2015). Some of the Miyanmin people at Yapsiei Station, West Sepik Province, Papua New Guinea, give the liquid from the plant pouches to infants and children as a treatment for cognitive disabilities and developmental delays (Hagwood 2019). In Malaysia, *Nepenthes* spp. which has been blended is used as an astringent drug (Aung et al. 2002).

It is estimated that there are 100 species of *Nepenthes* and 64 of them are found in Indonesia (Hariyadi 2013). It is known to be very well adapted to growing in nutrient-poor soils which have very low essential nutrients such as nitrogen, phosphorus and potassium as well as high soil acidity and the poor soil is a limiting factor for plant growth (Rembold et al. 2010, Bauer et al. 2012, Cheek and Jebb 2016). This plant grows in various ecological habitats, ranging from an altitude of 0–3500 m a.s.l., from fresh water swamps on the coast to high mountains. *Nepenthes* tends to grow in nutrient-poor, low pH and nitrogen-poor places (Wistuba and Fleischmann 2007) with an abundant supply of water and light (Givnish et al. 1984).

Research on the symbiotic association between carnivorous plants and arbuscular mycorrhizal fungi (AMF) in the world has been carried out, but similar studies have not been conducted in Indonesia. According to Macdougal (1899), Juniper et al. (1989) and Brundrett (2009) it is generally considered that carnivorous plants are non-mycorrhizal because there is no clear benefit for their association with fungal symbionts, for example through facilitating the acquisition of nutrients. However, Fuchs and Haselwandter (2004) and Chambers et al. (2008) reported sporadic colonisation by facultative arbuscular mycorrhizae on the roots of carnivorous plants. This is confirmed by the results of recent research reporting that *Nepenthes* plants can form symbiosis with AMF (Ducousso et al. 2008, Santiago and Darnowski 2012). Furthermore, Harikumar (2013) reported that two species of carnivorous plants, namely *Drosera burmanii* Vahl. and *Drosera indica* L., were also colonised by AMF.

Colonisation of *Nepenthes* spp. by AMF in Indonesia has never been reported, especially on the island of Sulawesi. Therefore, this study aims to determine the level of colonisation and species of AMF that are symbiotic with *Nepenthes* spp. located inside and outside the Lore Lindu National Park area, Central Sulawesi, Indonesia.

Material and Methods

Study sites

Observation and sampling of roots and rhizosphere soil of *Nepenthes* spp. was carried out in three locations, namely: Padang Padaeha (Inside the Lore Lindu National Park Area) with coordinates 01°19.986’ S and 120°19.581’ E, pine stands (Outside Lore Lindu National Park Area) with coordinates 01°35.156’ S and 120°25.822’ E and Padang Wanga (buffer zone of Lore Lindu National Park) with coordinates 01°29.784’ S and 120°19.273’ E. The maps and conditions of each research location are presented in figures 1 and 2.

a) b) c)

Fig. 1. The physical condition of each study location: a) Padang Padaeha, b) Padang Wanga, c) Pine stands.

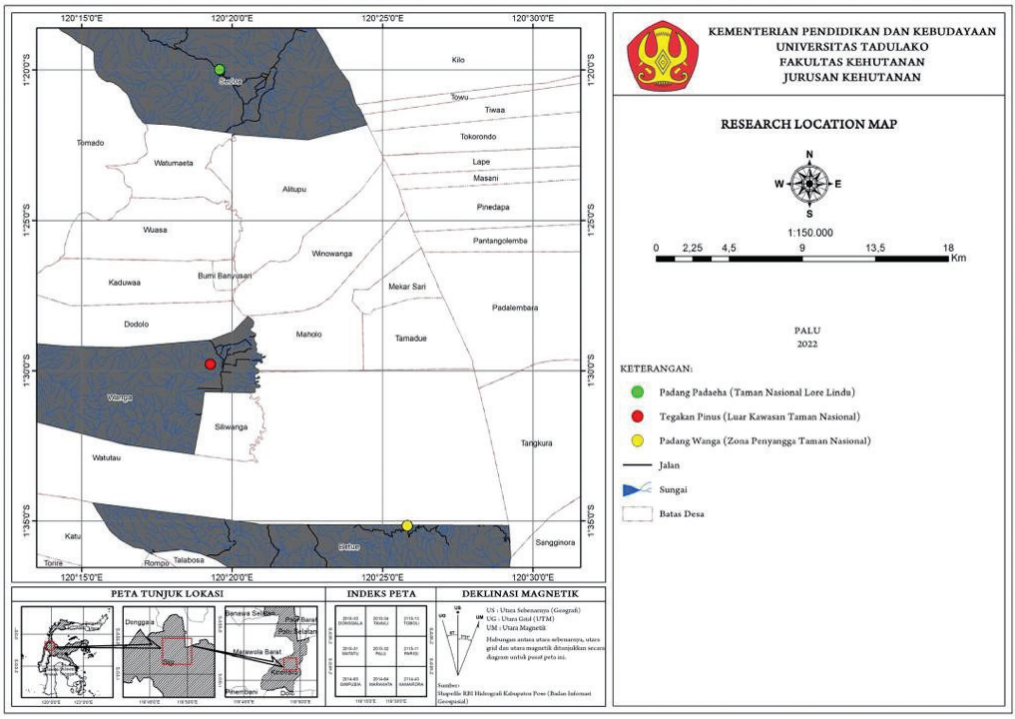


Fig. 2. Map of research locations.

Sample collection of *Nepenthes* spp. and other plants in the research location

In the field, initial identification was carried out to determine the species of *Nepenthes*. Its samples that differed morphologically from other plant species found in the study area were also collected and brought to the Celebence Herbarium (UPT, Sumberdaya Hayati Sulawesi), Tadulako University, Palu, Indonesia, for further identification.

Collection of *Nepenthes* spp. roots and rhizospheric soil samples

Collection of rhizosphere soil samples and *Nepenthes* roots was carried out in May–July 2022. Root and rhizosphere soil samples were collected from each *Nepenthes* species. From each study location, the roots were taken from 15 *Nepenthes* clumps that grew randomly. To represent the heterogeneous environmental conditions at each study location, the distance taken between them and others was approximately 25 m. Because *Nepenthes* is protected and is in a conservation area, the roots were taken carefully by digging up on one side to keep the plants from dying. The roots that appear were cut slowly and cleaned of soil or other impurities, then stored in a plastic box filled with water. After the roots were cleaned, the fine roots were selected and carefully cut into approximately 1 cm in size. These roots were then put into a plastic bottle containing 10 % ethanol. The root samples in these bottles were then taken to the laboratory to be analysed for AMF infection.

At each study location, 100 g of rhizosphere soil samples were collected from each *Nepenthes* clump at a depth of approximately 10 cm. In order to represent the heterogeneous environmental conditions of the study locations, soil samples were randomly collected from 25 *Nepenthes* clumps without differentiating species with a distance of approximately 25 m between the clumps. Soil samples from each *Nepenthes* clump were collected and composited to obtain one sample from each study location. Soil samples were collected separately in plastic bags, labeled, then brought to the laboratory and stored at room temperature.

Colonisation of *Nepenthes* root by arbuscular mycorrhizal fungi

The percentage of colonisation was calculated based on the 'presence and absence' of mycorrhizal structures (McGonigle et al. 1990) after the roots were stained with Trypan blue modified by Phillips and Hayman method (1970).

AMF colonisation enumeration used the infected root length method (Giovannetti and Mosse 1980). The roots that had been treated with Trypan blue were taken and arranged in a Petri dish, then observed carefully. Any field of view which showed colonisation was marked with (+) and (-) if there was no colonisation. There were four categories based on O'Connor et al. (2001), namely: not colonised (0 %), low (10 %), moderate (10–30 %), high (>30 %). Formula (1) is for calculation of AMF infection percentage (Brundrett et al. 1996).

$$\text{AMF infection} = \frac{\sum \text{infected roots}}{\text{Total of observed roots}} = 100, \%. \quad (1)$$

Spore isolation

The technique used in spore extraction was the pour-filter technique followed by centrifugation (Pacioni 1992). The working procedure for the pour-strain technique was to weigh 100 g of soil sample, then add enough water to make 1 L and stir for 1 min, then let it stand for ±15 min to precipitate. Then the soils were sieved in a set of sieves with a size of 325 µm, 50 µm and 40 µm sequentially from top to bottom. The top filter was removed and the second filter was sprayed with tap water. After the second filter was removed, the amount of residual soil left on the bottom filter was transferred into a measuring cup and then poured into a centrifuge tube.

Spore extraction was followed by centrifugation (Brundrett et al. 1996). The filter results were put into the centrifuge twice, the first at 2500 rpm for 5 min and the second at 1200 rpm for 2 min with the addition of 60% glucose solution to a third of the tube. Then the supernatant was washed in the third filter using running water for ±15 s to remove the glucose solution in the sample. The precipitate remaining on the filter was poured into a Petri dish and then observed under a microscope to identify any spores present. The spore density was calculated by the formula (2).

$$\text{Spore density} = \frac{\text{Number of spores}}{15\ \text{g soil}}. \quad (2)$$

Furthermore, spore preparations were made using Melzer dye. After counting the number of spores obtained from extraction and grouping them by type, they were placed on the preparation glass and then given PVLG and Melzer solutions. The spores were broken carefully by pressing the cover slip of the preparation using the tip of the needle. Spore color change in Melzer's solution was an indicator to determine the type of spore present. Identification of AMF spores was carried out based on The International Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM 2022).

Determination of AMF species was based on the characteristics shown by the spores (color, shape, size, hyphae, spore ornament) and grouping them into genera that have these characteristics. Observation of spores was carried out under an Axio Imager microscope with a magnification of 200X.

Data analysis

Data on the percentage of colonisation by AMF were analysed using analysis of variance with the SPSS software package (IBM SPSS Statistics 25, Armonk, NY, USA), and comparisons between study sites using the 5% honestly significant difference test.

Results and Discussion

Characteristics and species of *Nepenthes* spp. and the dominant plants at the research site

The results showed that only two species of *Nepenthes* were found in Padang Padaeha and pine stands, namely *N. tentaculata* Hook.f. and *N. maxima* Reinw. ex Nees, while in Padang Wanga three species were found, namely *N. tentaculata*, *N. maxima* and *N. mirabilis* (Lour.) Druce. In addition, several dominant plant species associated with *Nepenthes* were also found, such as *Imperata cilindrica* L., *Eucalyptus deglupta* Blume, *Selaginela plana* (Desv. ex Poir.) Hieron., *Mallotus* spp. and *Pinus merkussi* Jungh. et de Vriese. The characteristics of study locations are presented in Table 1.

Table 1. Characteristics and species of plants at each research location.

No	Study sites	Altitude, m a.s.l.	Temperature / Humidity	Dominant plants
1	Padang Padaeha (inside)	1607	25.8 °C/39 %	1. *Nepenthes tentaculate* 2. *Nepenthes maxima* 3. *Imperata cilindrica* *4. Eucalyptus deglupta* *5. Selaginela plana*
2	Padang Wanga (buffer zone)	1524	27.3 °C/53 %	1. *Nepenthes tentaculata* 2. *Nepenthes maxima* 3. *Nepenthes mirabilis* 4. *Imperata cilindrica* *5. Eucalyptus deglupta* *6. Selaginela plana*
3	Pine stand (outside)	1524	25.6 °C/40 %	1. *Nepenthes tentaculata* 2. *Nepenthes maxima* 3. *Pinus merkusii* *4. Imperata cilindrica* *5. Selaginela plana* *6. Mallotus* spp.

Based on the exploration results, it was found that three species were spread in the study locations, namely *N. tentaculata*, *N. mirabilis* and *N. maxima*. *N. tentaculata* and *N. maxima*, can be found in the three study locations. Both species were found living terrestrially on the ground, among reeds and without shade. This is in accordance to the results of Mansur (2006) and Khalid et al. (2015) that both have the same place to grow because they prefer open places with high light intensity and cold (humid) temperatures and can grow terrestrially on rocky soil and also between shrubs, ferns or other plants. Both can also interact with surrounding trees to support their growth because their length can reach 5–6 m (Khalid et al. 2015).

N. mirabilis was only found in Padang Wanga at an altitude of ±1524 m a.s.l. The location where this species was found is a field of reeds essentially a basin with water flow so that the soil tends to be moist and wet. Mansur (2006) explained that *N. mirabillis* has high adaptability to its environment, so that this species can live in various habitats in wet and dry places. Usually found in wet clearings and swamps, it also lives in clearings on roadside cliffs, riverbanks, secondary forest edges, and lakeshores and generally grows in red-yellow podzolic soils. The three species of *Nepenthes* found in the three study locations can be seen in Figure 3.

Spore density, colonisation and species of AMF that have symbiosis with *Nepenthes* spp.

The results of AMF colonisation level are presented in Table 2 and showed that the highest percentage was found in *Nepenthes* roots from Padang Padaeha, namely 16.2 % (medium category), followed by *Nepenthes* roots collected from Padang Wangga, namely 15.9 % (also in the category medium) while the lowest percentage was found in *Nepenthes* roots from

a) b) c)

Fig. 3. Species of *Nepenthes* found in three research sites: a) *N. mirabilis*, b) *N. tentaculata*, c) *N. maxima*

Table 2. AMF spore density, AMF species and their colonization on *Nepenthes* spp. roots.

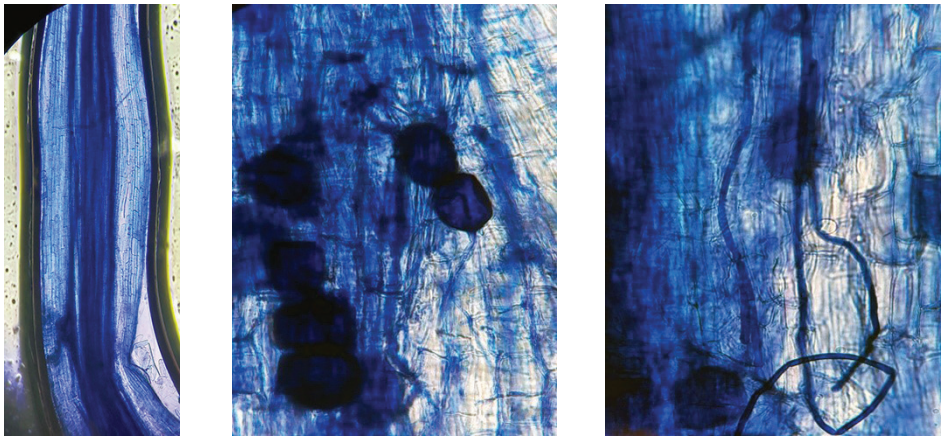
No	Study sites	Type of AMF	Spore density, per 15 g soils	Colonisation, %	Category
1	Padang Padaeha	*Gigaspora* spp.	4 ±1.73	16.2 ±0.85 a	Moderate
		Glomus sp. 1	4 ±2.0		
		Glomus sp. 2	60 ±3.60		
		Glomus sp. 3	5 ±1.0		
2	Padang Wanga	*Glomus* sp. 1	9 ±1.73	15.9 ±1.8 a	Moderate
3	Pine stand	-	-	2.4 ±0.3 b	Low

Note: Each value of AMF colonisation is the mean of three replications, ±Standard Deviation, means followed by same letter within a column are not significant at $p \leq 0.05$ level (Tukey's honestly significant test).

pine stands, namely 2.4 % (low category). The AMF structures found in colonised *Nepenthes* roots were in the form of hyphae and vesicles (Fig. 4). These results indicate that there are AMF that can be associated with *Nepenthes* roots.

Several species of *Nepenthes* are reported to have symbiosis with AMF, for example *N. madagascarensis* Poir. from Eastern Madagascar littoral forests (Ducousso et al. 2008) and *N. sanguinea* Lindl. from Corydon, Indiana, USA (Santiago and Darnowski 2012). Differences in the degree of AMF colonisation on host plant roots are caused by many factors. According to Permanasari et al. (2016), the number of spores is one of the factors that affect mycorrhizal colonies on plant roots. The higher the number of mycorrhizal spores, the higher the number of mycorrhizal colonies on plant roots in the soil. Furthermore, according to Yurisman et al. (2015), apart from being influenced by the number of spores, mycorrhizal colonisation is also influenced by the host plant roots. *Nepenthes* has slow and limited root development (Adamec 1997).

The results of this study showed four



a) b) c)

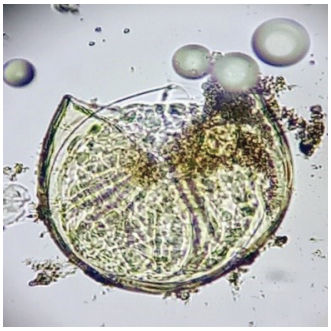
Fig. 4. Root cell structure without AMF infection a) and root cell structure with AMF infection such as hyphae, vesicles and spores b) and c).

AMF morpho-species found at the study site, namely *Glomus* sp. and *Gigaspora* sp. (Table 3, Fig. 5). The genus *Glomus* sp. has a spore development process that starts from the tip of the hyphae which enlarges to the maximum size and forms spores. Its spores are in the form of globos, subglobos, ovoid or obovoid with more than one layer of spore walls. Spores were found in loose aggregate form (Mukerji 1996, Patriyasari 2006). *Glomus* spores were round and oval or oval in shape with various colours, brown, yellowish brown to dark brown. AMF spores are formed as a result of swelling of one or more subtending hyphae in the soil or in roots (Brundrett et al. 1996). The spore shape is generally round to oval with vari-

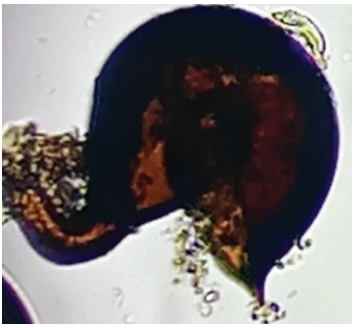
Table 3. Description of arbuscular mycorrhizal fungi spores found in study locations.

Species	Description	Location
Gigaspora sp.	Spores of the genus *Gigaspora* are translucent in colour, diameter is about 150 µm, there are boulbus suspensors, spores form spore-walls and germinal-walls.	A
Glomus sp. 1	*Glomus* genus with a distinctive character in the form of a spore-wall layer that connects what is found in the spore and the attached hyphae. The spore diameter is about 100 µm with a dark brown spore colour.	A, B
Glomus sp. 2	Spores of the genus *Glomus* are light yellow in colour with a diameter of more than 150 µm. Spores are characterised by the presence of several layers of spore-wall. The lipid-dropped spore can be seen very clearly.	A
Glomus sp. 3	Spores of the genus *Glomus* are bright yellow in colour with a diameter of 100–150 µm. Spores are characterised by the presence of a spore-wall layer that is continuous with attachment hyphae.	A

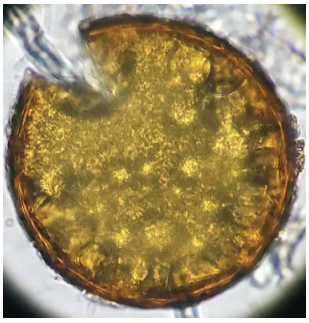
Note: A – Padang Padaeha; B – Padang Wanga (there were no spores in the pine stands).



Gigaspora **sp.**



Glomus **sp. 1**



Glomus **sp. 2**

Glomus **sp. 3**

Fig. 5. Morpho-species of AMF found in *Nepenthes* spp. rhizosphere.

ous diameters. Each spore is bounded by one or more spore walls which have their own thickness. The colour varies from clear, yellow to black. The spore contains fat, cytoplasm and many nuclei. When environmental conditions allow, the spores will germinate by releasing hyphae which directly penetrate the spore wall or form a special germination structure (Nusantara et al. 2012). The development of AMF begins when it is in the soil in the form of spores so that it can infect plant roots (Solaiman and Hirata 1995).

Glomus was found the most because it has the widest distribution area and is the most tolerant of soil salinity conditions. The same results were obtained by Yusran et al. (2022), who found that it has more symbiosis with the plant of *Diospyros celebica* Bakh. compared to *Acaulospora*. The high presence of *Glomus* spores is because this genus has more species than other genera. According to Mukerji (1996), it is the largest genus with about 90 species.

Gigaspora sp. was only found in Padang Padaeha. The spores found were round in shape with a yellowish colour. This is in agreement with Alayya and Budi (2022) who stated that it spores are large, round in shape, creamy yellow, brownish yellow to blackish brown and have a rounded suspensor. *Gigaspora* sp. is a genus of AMF belonging to the family Gigasporaceae. This genus has distinctive features, including spores that are produced singly in the soil; they possess no inner spore wall layer and are globose or

sub-globose in shape, cream to yellow in colour, and 125–600 µm in size (Bentivenga and Morton 1994, INVAM 2022).

Soil condition is a factor that may influences the development and density of the number of AMF spores from the three soil samples. At the research sites in Padang Padaeha and Padang Wanga, *Glomus* sp. and *Gigaspora* sp. spores were found while in the pine stands there were no spores.

The results of physical and chemical properties analysis of the soil at the three study locations can be seen in Table 4.

Table 4. Physical and chemical properties of soil types in study sites.

No	Parameters	Study sites		
		A	B	C
1	Permeability, $cm \cdot h^{-1}$	5.36 ±0.04	1.12 ±0.10	0.49 ±0.03
2	Bulk density, $g \cdot cm^{-3}$	1.59 ±0.20	1.51 ±0.05	1.59 ±0.01
3	Porosity, %	39.92 ±0.40	43.09 ±0.20	40.09 ±0.04
4	Sand, %	76.4 ±0.15	22.3 ±0.40	45.7 ±0.26
5	Silt, %	18.7 ±0.20	50.0 ±0.19	26.0 ±0.10
6	Clay, %	4.9 ±0.20	27.7 ±0.20	28.3 ±0.20
7	pH	5.21 ±0.03	6.36 ±0.03	4.93 ±0.01
8	Organic C, %	0.48 ±0.02	4.49 ±0.02	2.79 ±0.01
9	Total N, %	0.05 ±0.001	0.38 ±0.03	0.17 ±0.01
10	CEC, cmol (+)·kg^{-1}	11.72 ±0.04	22.91 ±0.02	15.22 ±0.02
11	P_2O_5, ppm	4.51 ±0.02	5.12 ±0.10	3.32 ±0.01

Note: Value is the average from three replications, ±Standard Deviation; A = Padang Padaeha, B = Padang Wanga, C = pine stand.

Environmental conditions such as soil factors greatly determine the types of mycorrhiza that are able to develop (Hamel 2007, Helgason and Fitter 2009). Based on the results of soil analysis, the pH of soils ranged from 4.93–6.39 based on the criteria for assessing the chemical properties of the soil. pH 4.93 is classified as strongly acidic. According to Samsi et al. (2017), generally mycorrhizae are resistant to changes in soil pH so that even in alkaline or very acid soils they can be found but the amount depends on the adaptability of each AMF species. Maas and Nieman (1978) further explained that the optimum pH for the development of AMF varies. According to Tuheteru (2003) the optimum pH for AMF development ranges from 5.6–7 for *Glomus*, and pH 4–6 for *Gigaspora*.

The results of soil C-organic analysis showed that its content in Padang Padaeha was very low (0.48 %), in Padang Wanga was high (4.49 %), and in pine stands was moderate (2.79 %). C-organic is the content of organic matter in the soil which plays a role in the mineralisation process. The results of this mineralisation will produce inorganic compounds that can be directly absorbed by plants, so that nutrient needs are met. AMF spores were most commonly found in soil samples with very low organic C content, on the other hand very few in soils with high organic C content and were not found in soils with moderate organic C content. It is suspected that if there is a lot of organic matter in the soil, it will affect soil moisture so that the

AMF sporulation process is lower so that the number of spores will also be small (Burhanuddin 2012, Samsi et al. 2017). C-organic content of the soil in this study did not consistently affect the spore density in each study location. Besides C-organic, many factors affect the number of AMF spores in the soil, for example soil physico-chemical characteristics (Sivakumar 2013), host plant (Eom et al. 2000), season (Silva-Flores et al. 2019) and elevation (Melo et al. 2019).

According to Puspitasari et al. (2012), the number of spores is influenced by the interaction of several factors, including: the mycorrhiza itself, the host vegetation and soil chemical conditions such as pH, organic C and available P. Host vegetation has an influence on the formation of fine roots in the soil, where fine roots facilitate the occurrence of colonies by mycorrhizae.

Based on the results of the study, it was shown that *Glomus* sp. was the most common type of mycorrhizal spore. This shows that it has a higher level of adaptation to the environment than the genus *Gigaspora* sp. Further, explained by Alayya and Budi (2022) and Puspitasari et al. (2012), *Glomus* sp. is a mycorrhizal genus that has a wide distribution and high adaptation to environmental conditions. The physical and chemical properties of the soil that affect the AMF innoculant to infect plant roots are pH, macro- and micronutrients availability, texture, organic matter, moisture and temperature regime (Drouillon and Merckx 2005, Sjoberg 2005). When soil pH, the content of P and C-organic elements increases, the number and types of AMF fungi will increase (Muzakkir 2011).

The results of this study also support Harikumar's research (Harikumar 2013) which reported that carnivorous plants, namely *Drosera burmanii* and *D. indica*, are also in symbiosis with arbuscular mycorrhizal fungi. A fungal structure characteristic of AMF colonisation was found in both plants although the colonisation rate was low (less than 50 %). Root colonisation by AMF and spore density in the rhizosphere were higher in *D. burmanii*. Edappic factors such as soil pH and organic C content positively affect fungal development while soil moisture and P content have a negative effect.

Conclusions

Three species of *Nepenthes* were found in three study locations, namely *N. mirabilis*, *N. maxima* and *N. tentaculata,* spread of 2 species in Padang Padaeha, namely *N. mirabilis* and *N. tentaculata*, 2 species in the pine stand, namely *N. mirabilis* and *N. tentaculata*, and 3 species in Padang Wanga, namely *N. mirabilis, N. tentaculata* and *N. maxima*. There were 4 species of arbuscular mycorrhizal fungi which had symbiosis with *Nepenthes* spp., namely *Glomus* sp. 1, *Glomus* sp. 2, *Glomus* sp. 3 and *Gigaspora* sp. with the level of colonisation between 2.4 % to 16.2 %. The spore density of arbuscular mycorrhizal fungi also varied between 4–60 spores per 15 g of soil. These results imply that *Nepenthes* spp. is symbiotic with AMF and imparts useful information in the conservation management of these species in the future.

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Scrub vegetation in the Slivenska Planina Mt, Bulgaria

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Abstract

This study deals with the diversity of scrub vegetation in Slivenska Planina Mt (Eastern Stara planina Mts). On the basis of analisys of 29 relevés, the scrub phytocoenoses were classifyied into 1 association, 2 variants and 4 plant communities. They have been identified and characterized in the article. The studied communities belong to the the class *Crataego-Prunetea*, order *Paliuretalia* with alliance *Buxo-Syringion* and in order *Prunetalia spinosae* with the alliances *Berberidion vulgaris* and *Brachypodio pinnati-Juniperion communis*. The phytocoenoses belong to 4 habitat types of EUNIS, 2 habitat types of Annex I of the Habitat Directive and 3 habitat types of Red Data Book of Bulgaria, v. 3, Natural habitats.

Key words: Braun-Blanquet approach, scrub habitats, southeastern Bulgaria, syntaxonomy.

Introduction

Slivenska Planina Mt is a part of Eastern Stara Planina Mts. Any detailed study of the scrub vegetation of Slivenska Planina Mt as a separate geographical unit, have not been carried out until now. The gathering of detailed information about the characteristic and successional trends in the scrub vegetation would contribute for their more sustainable management, as well as for some prerequisite for their conservation and sustainable use. It is a part of the study on the biodiversity in the region, which is an important part of these activities related also to their environmental protection. However, the regional vegetation studies are relevant in the context of the observed climatic changes, sustainable developement and nature conservation (Alexandrova et al. 2020).

The earliest studies of the vegetation of Slivenska Planina Mt had a generally descriptive nature or followed the method of the Russian phytocoenological school. They were part of national (Stoyanov 1941, Bondev 1991, Apostolova and Slavova 1997) or regional studies on the territory of the ‘Sinite Kamani’ Natural Park (Andreev 1981, Stoeva 2004).

The studied territory is located in the eastern part of the Balkan Range phytoclimatic region (Stoyanov 1941), where the specific patterns of the vegetation is expressed in the presence of Mediterranean species and also presence of some Euxinian floristic elements. The

former studies of this region have been mainly focused on the forest vegetation (Andreev 1981, Bondev 1991, Sopotlieva et al. 2016, Alexandrova et al. 2020). There is some information for the scrub vegetation but only from small areas (Stoeva 2004) or for certain communities/ habitat types (Tashev 2013). For a first time, the communities which belong to the habitat 5130 of *Juniperus communis* on limestone, have been found in the protected area by Tashev (2013). Kunev and Tzonev (2019) published data for habitat F3.1d Balkan-Anatolian submontane genistoid scrub included in the European Red List of Habitats (Janssen et al. 2016), which includes the communities dominated by *Genista lydia* also from the studied region. Also, Kunev et al. (2020) have studied the scrub communities of *Genista lydia* in the Southeast Balkans and published 4 new associations. One of them *Galio flavescentis-Genistetum lydiae* Kunev et al. 2020 is found from the area of 'Sinite Kamani' Nature Park.

The aim of present study was to investigate the scrub vegetation on the territory of Slivenska Planina Mt according to the approach of Braun-Blanquet's phytocoenological school (Braun-Blanquet 1964).

Object and Methods

The studied area – Slivenska Planina Mt is located in southeastern Bulgaria, at the most western part of the Eastern Stara planina Mts (Balkan range) (Steffanov 2002). The borders of the mountain are: on the north – the valley of Luda Kamchia river which separates it from Kotlenska Mt, on the south – the valley of Assenovska river and the Slivenska Plain, on the west the border goes along the Vratnik Pass which divides it from the Eleno-Tvardishka Mt, and on the east – Icherenski Pass between Slivenska Planina Mt and Stidovska Planina Mt. The southeastern part of the Slivenska Planina Mt belongs to the Grebenets Ridge, whose borders on north and east, are along the valleys of the Ovcharitsa and Marash rivers, while on the south it's borders with the Slivenska Plain (Fig. 1). The total area of the Slivenska Planina Mt is 212.267 km^2. The physico-geographical division of Bulgaria assignes the Slivenska Planina Mt to the Southern low-mountainous sub-region of the Forebalkan-Eastern Balkan Mountains. The bedrocks of the study area are predominantly lower Cretaceous sandstones and marls, which pass into upper Cretaceous and Paleogene flysch formations (Yordanova et al. 2002). According to the climatic division of Bulgaria (Velev 2002), the study area falls into the transitional-continental climatic region and the Eastern Balkan Range climatic region.

This climate is characterized by warm and slightly arid summer and mild winter, with small annual temperature amplitudes, two maximums (July and November) and two minimums (August and February) of the annual rainfalls, with an average of 600 mm. According to the hydrological division of Bulgaria (Yordanova 2002), the study area falls into the zone with Mediterranean climate influence on the outflow and in the Eastern Balkan region. It is characterized by increased aquifer, poorly regulated groundwater runoff and relatively deep and shallow water, which corresponds to a relatively large tide of waters. The soils in the lower parts of the studied area (below 500 m a.s.l.) are included in the Eastern Balkan province of the Carpathian-Danube soil region, while in the higher part are in the Balkan Mountains

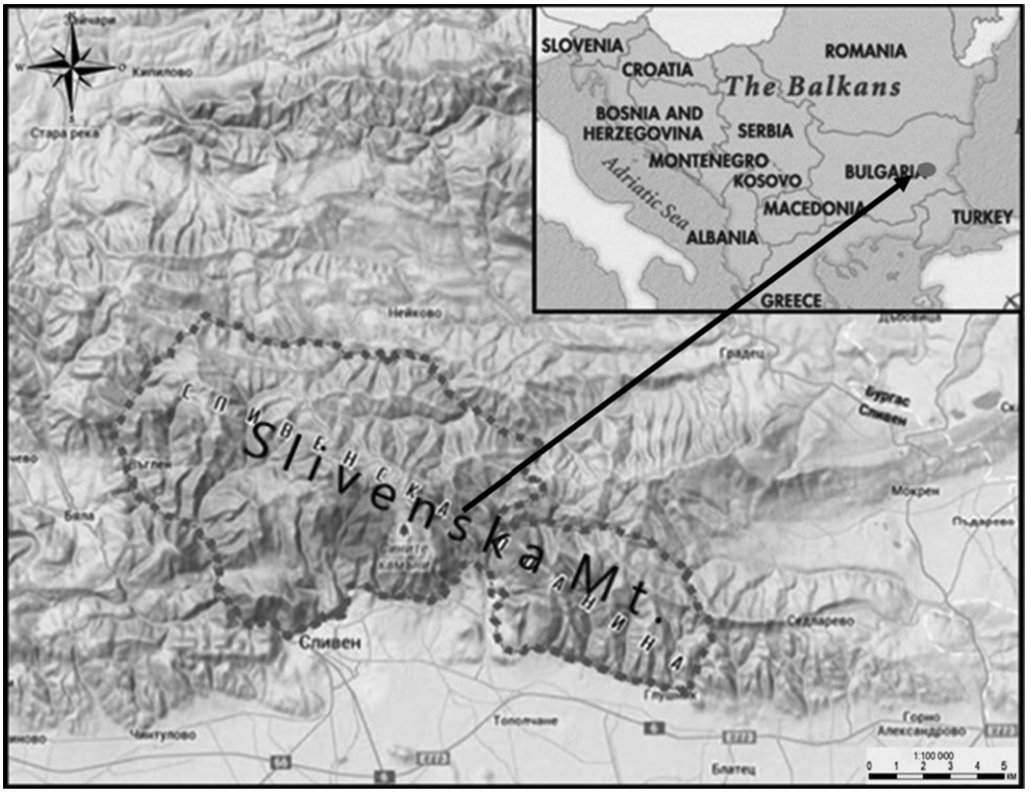

Fig. 1. Slivenska Planina Mt's map.

of the Mediterranean soil region (Ninov 2002). According to the geobotanical division of Bulgaria (Bondev 2002), the Slivenska Mt belongs to the European Broadleaved Forest Area, Illyrian (Balkan) Province and Kotlensko-Preslavski District.

Seven protected areas are located in Slivenska Planina Mt: part of 'Sinite Kamani' Nature Park and 'Kutelka' Reserve; protected areas of 'Lale Bair', 'Orlite', and 'Trakiiski kiln'; natural monuments of 'Halkata' and 'Zmeevi dupki'. Three NATURA 2000 sites party or completely are overlaped with the studied area: BG000016 Sinite Kamani and BG0000420 Grebenets (declared under the Habitat Directive) and BG0002058 Sinite Kamani – Grebenets (declared under the Bird Directive). One Important Plant Area (IPA) site (BGIPA101 Sinite Kamani), covering an area of 122.80 km^2 within the object of study (Alexandrova et al. 2020).

The field studies were conducted during the vegetation seasons of 2014–2016. A total of 29 relevés were collected from scrub vegetation, following the Braun-Blanquet approach (Braun-Blanquet 1964). The relevés were taken in homogeneous parts of scrub stands. Size of the relevés was in the range 100 m^2 (Chytrý and Otýpková 2003). For each relevé, complete floristic composition of vascular flora was recorded. Total vegetation cover, as well as the cover of scrub and herbaceous layers were also recorded. The abundance of each species was estimat-

ed by its cover in percents, which was later transformed into 7-grade Braun-Blanquet scale (Braun-Blanquet 1964). If one species is found in several layers in the relevé, it was recorded in each of them. The data for topographic and ecological peculiarities field (e.g. elevation, aspect, slope) were also collected, in the, whereas basic soil data (e.g. soil type and some soil characteristics as a soil reaction, soil depth and humidity) was exctracted from the Forest Management Plans of State Forestry Units Sliven and Kotel (Agrolesproject 2012, 2014). All relevés were digitalized in Excel format and stored in the Balkan Vegetation database using in TURBOVEG format (Vassilev et al. 2016).

The taxonomic position of vascular plants is standardized according to the Euro+Med PlantBase (Euro+Med 2006–2020). Taxa identified with different taxonomic precision were merged to species 'sensu lato' or species aggregates.

All relevés were exported in JUICE 7.0 software (Tichý 2002). Agglomerative method for numerical analysis (PC-ORD, McCune and Mefford 1999) was used. Square root transformation of species cover/abundance and minimum number of 2 relevés by cluster were applied. Relative Sorensen index was used as distance measure and the similarity was calculated by Flexible beta (ß = -0.25). The phi-coefficient of established groups was applied as a measure of fidelity (Sokal and Rohlf 1995, Chytrý et al. 2002), and all clusters were standardized to equal size (Chytrý et al. 2006). Only the statistically significant phi-coefficient values evaluated by Fisher's exact test ($p < 0.05$) were considered. For each differentiated group the diagnostic, constant and dominant species were determined by 'Analysis of constancy columns in synoptic table' function in the JUICE software. The threshold value for a species to be considered as diagnostic was set up at phi-coefficient ≥ 0.3. The list of constant species includes species with presence at least 40 % in relevés in a cluster, whereas the species with cover more tham 20 % were considered as dominants. Finally, in the 'Results and discussion' section, the value of phi-coefficient for each species per group was presented in parentheses multiplied by 100. Respectively, the values in parentheses for a constant species signify their percentages constancy per group, while for the dominant species the parentheses present the percentage ratios of relevés of the target group where the dominant species cover was above the defined threshold. The final synoptic table presents both species percentage frequency and fidelity (Appendix A).

The syntaxonomic nomenclature on the alliance level followed the concept of Mucina et al. (2016). The classification of established vegetation units is determined after a comparative analysis of the diagnostic, constant and dominant species groups with those indicated in the cited referance (Kojić et al. 1998, Borhidi et al. 2012, Chytrý 2013, Biondi et al. 2014, Coldea et al. 2015). Some of phytocoenoses were not detrmined as belonging to any association and were assigned as 'community types' because of their local occurrence or limited data for their presence in other regions of Bulgaria.

The assignment of communities to some natural habitats is done according to the EUNIS classification scheme (EEA 2019), Red Data Book of the Republic of Bulgaria, v. 3, Natural Habitats (Biserkov et al. 2015) (RB) and Directive 92/43/EEC.

Results and Discussion

The scrub communities on the territory of Slivenska Planina Mt after the cluster analysis (Fig. 2) were separated into 6 groups, presented in the synoptic Table A1 (Appendix A). They belong to 1 class, 2 orders, 2 alliances, 1 association, 2 variants and 4 plant communities.

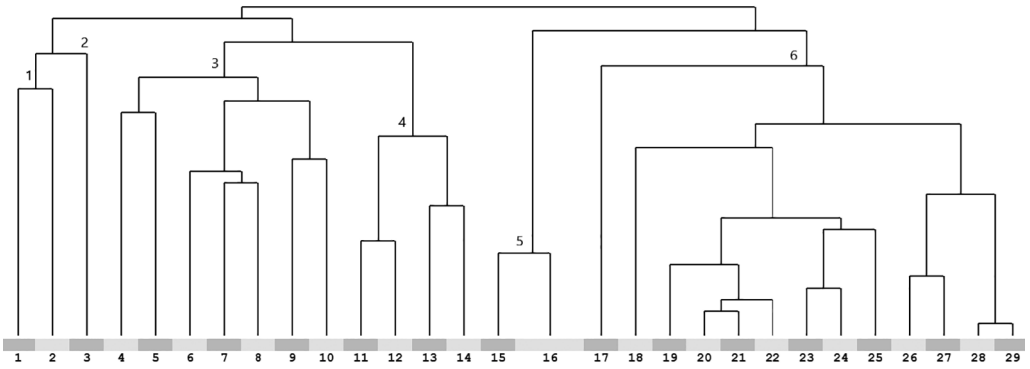

Fig. 2. Classification dendrogram of scrub vegetation on Slivenska Planina Mt.
Note: The identified scrub vegetation groups' number was showen on respective dendrogram separation, while the numbers on the botton of dendrogram corresponded to relevé number.

Syntaxonomical scheme of recorded phytocoenoses

Class *Crataego-Prunetea* Tx. 1962
Order *Prunetalia spinosae* Tx. 1952
Alliance *Brachypodio pinnati-Juniperion communis* Mucina in Mucina et al. 2016
Juniperus communis comm.
Alliance *Berberidion vulgaris* Br.-Bl. ex Tx. 1952
Association *Crataego-Prunetum spinosae* Hueck 1931
variant *Lamium maculatum*
variant *Thymus pulegioides*
Order *Paliuretalia* Trinajstić 1978
Alliance *Buxo-Syringion* P. Fukarek ex Diklić 1965
Rosa spinosissima comm.
Syringa vulgaris comm.
Syringa vulgaris and *Quercus pubescens* comm.

Class *Crataego-Prunetea* Tx. 1962

The scrub vegetation of Slivenska Mt belongs to the class *Crataego-Prunetea*, which includes scrub and mantle vegetation, among or on the margins of broad-leaved forests in the temperate zone and sub-Mediterranean regions of Europe (Mucina et al. 2016). These communities have an intrazonal distribution. The described scrub phycoenoses belong to two orders and three alliances.

Order *Prunetalia spinosae* Tx. 1952

The order *Prunetalia spinosae* includes scrubs on the margins of deciduous forests or serial scrub communities formed in abandoned fields or pastures. Their species composition is very similar to those of forest phytocoenoses from *Carpino-Fagetea* class. Alliance *Brachypodio pinnati-Juniperion communis* Mucina et al. 2016 includes lowland thermophilous and calciphilous Common juniper scrubs in Europe, but the alliance *Berberidion vulg*aris – southern temperate and sub-Mediterranean thermophilous broad-

leaved scrubs in southern and central Europe (Mucina et al. 2016). These communities are of secondary origin, as a result of human activities and have not yet been described in detail both on the territory of Slivenska Planina Mt and also on the territory of Bulgaria.

***Juniperus communis* comm. (figure 2, relevés 1-2; annex A, group 1)**

Diagnostic species: *Juniperus communis* (92.2).

Consant Species: *Viola mirabilis* (50), *Thymus zygioides* (50), *Teucrium chamaedrys* (50), *Tephroseris papposa* (50), *Stachys officinalis* (50), *Sesleria latifolia* (50), *Scabiosa triniifolia* (50), *Sanguisorba minor* (50), *Rubus caesius* (50), *Rosa canina* (50), *Primula veris* (50), *Potentilla erecta* (50), *Polygala major* (50), *Poa pratensis* (50), *Poa compressa* (50), *Piptatherum virescens* (50), *Pilosella officinarum* agg. (50), *Nardus stricta* (50), *Luzula luzuloides* (50), *Leontodon crispus* (50), *Holcus lanatus* (50), *Helianthemum nummularium* (50), *Galium pseudaristatum* (50), *Fragaria viridis* (50), *Filipendula vulgaris* (50), *Filago germanica* (50), *Dianthus pinifolius* (50), *Cytisus hirsutus* (50), *Carlina acanthifolia* (50), *Carex humilis* (50), *Campanula persicifolia* (50), *Avenella flexuosa* (50), *Asperula cynanchica* (50), *Anthyllis vulneraria* (50), *Agrostis capillaris* (50), *Achillea millefolium* agg. (50), *Achillea grandifolia* (50).

Dominant species: *Juniperus communis* (100), *Sesleria latifolia* (50), *Nardus stricta* (50).

In a cluster 2, the relevés are separated with the dominant participation of *Juniperus communis*. They occur in the range of 920–1020 m a.s.l., on the not steep slopes (about 5 degrees), with north and south-west aspect. The soils are *Dystric Cambisols*, nutrient-poor, dry, compacted, medium stony. The rocks are most commonly quartz porphyries and sandstones and less limestones. The scrub layer is dominated by the scrubs or groups of *Juniperus communis*, with fragments in between dominated by herbaceous species such as *Carex humilis, Agrostis capillaris, Nardus stricta, Anthyllis vulneraria, Carlina acanthifolia, Avenella flexuosa*, etc. In the past, these areas were occupied by forests that were cut down to provide territories for grazing. Their formation is secondary, and after abandonment of grazing, the juniper begans to increase in the the area.

Until now, no detailed studies of this type of phytocoenoses have been done and the literature sources are limited. Bondev (1991) determined them as Juniper Scrubs (*Juniperus communis*) mainly on the place of former forests of *Quercus petraea, Fagys sylvatica, Pinus nigra, P. sylvestris*, etc. Redžić (2007) has described the association *Juniperetum communis* Fukarek 1969 from Vranica Mts in Bosnia, and refered it to the alliance *Juniperion communis* Fukarek 1969, order *Pteridio-Juniperetalia* Lakušić 1978, class *Rhamno-Prunetea* Rivas Goday et Borja Carbonell 1961. The specified order is now accepted as a synonym of *Vaccinio-Juniperetalia communis* Passarge 1972 which includes the juniper communities of the class *Calluno-Ulicetea* distributed in the temperate Atlantic regions of Europe. Firstly, only the juniper calciphilous scrubs in Western and Central Europe were referred to the allance *Brachypodio pinnati-Juniperion communis* (Mucina et al. 2016). In the current list of habitats and plant communities in Europe (FloraVeg. EU 2022–2023), the range of the alliance includes also the territory of Bulgaria, and in its ecological characteristics the accent is not on the calciphilous preferences of communities. This is the reason to accept

that the communities of *Juniperus communis* on the territory of Slivenska Planina Mt belong to this alliance. Moreover, the habitat 5130, which includes communities of *Juniperus communis* on limestone areas already has been found on this territory (Tashev 2013).

This community belongs to the following types of natural habitats – EUNIS: S314 Sub-Mediterranean common juniper thickets; RB (2015): 17F3 Common juniper (*Juniperus communis*) scrub; Directive 92/43: 5130 *Juniperus communis* formations on heaths or calcareous grasslands.

Association *Crataego-Prunetum spinosae* Hueck 1931 (figure 2, relevés 15-30; annex A, groups 5-6)

Diagnostic species: *Agrimonia eupatoria* (79.9), *Lychnis coronaria* (71.2), *Crataegus monogyna* (66.8), *Prunus spinosa* (66.7), *Prunus cerasifera* (66.7), *Dorycnium pentaphyllum* s.l. (66.7), *Brachypodium pinnatum* (66.7), *Rosa canina* (63.4), *Vicia tenuifolia* s.l. (61.9), *Veronica chamaedrys* (61.9), *Salvia pratensis* (61.9), *Festuca dalmatica* (60.5), *Clinopodium vulgare* (59.2), *Pyrus communis* s.l. (57.4), *Thymus pulegioides* (56.9), *Rubus canescens* (56.9), *Galium verum* (55.5), *Fragaria vesca* (54.2), *Stachys germanica* (51.6), *Pteridium aquilinum* (51.6), *Cota tinctoria* (51.6), *Hypericum perforatum* (47.1), *Eryngium campestre* (42.0).

Constant species: *Euphorbia cyparissias* (62), *Dactylis glomerata* (62), *Teucrium chamaedrys* (44), *Achillea millefolium* agg. (44).

Dominant species: *Prunus spinosa* (31), *Crataegus monogyna* (31), *Rosa canina* (25), *Pteridium aquilinum* (6), *Prunus cerasifera* (6).

The phytocoenoses of this association are found in the altitudinal range 500–1000 m. The bedrocks are varied – marls, sandstones, shales, quartz porphyries and limestones, and the soils are *Dystric Cambisols*, *Chromic Luvisols* and *Rendzic Leptosols*, medium nutrient-rich, dry to fresh, with exposed rocks. The aspects are diverse, predominatly with eastern and southern components. The slopes are between 5 and 20 degrees. Dominants in the scrub layer are *Crataegus monogyna, Prunus spinosa*, *Rosa canina*, *Prunus cerasifera* and also *Pyrus communis* participates. The herbaceous layer is well developed and a great diversity of species is observed. It includes *Brachypodium pinnatum, Clinopodium vulgare, Dactylis glomerata, Dorycnium herbaceum, Eryngium campestre, Euphorbia cyparissias, Filipendula vulgaris, Hypericum perforatum, Lychnis coronaria, Teucrium chamaedrys, Thymus pulegioides, Vicia dalmatica,* etc. The territories occupied by these communities were used for grazing in the past, but they were subsequently abandoned, which led to the increase of scrubs in them.

The association is published from Romania in the alliance *Berberidion*, order *Prunetalia spinosae*, class *Crataego-Prunetea* (Coldea et al. 2015) and also from Vranica Mt in Bosnia (Redžić 2007). In Czech Republic, the association *Pruno spinosae-Ligustretum vulgaris* Tüxen 1952 is determined as belonging to the alliance *Berberidion vulgaris* Br.-Bl. ex Tüxen 1952 (Chytrý 2013). The same association is reported for Slovenia as well under *Prunus spinosa* community (Šilc and Čarni 2012). The association *Pruno spinosae-Crataegetum* (Soo 1927) Hueck 1931 has been published for Serbia in the alliance *Prunion spinosae* Soo 1940, order *Prunetalia spinosae*, class *Quercо-Fagetea* Br.-Bl. et Vlieg. 1937 (Kojić et al. 1998). In Hungary, the same association is classified to the alliance *Berberid-*

ion, order *Prunetalia spinosae* and class *Crataego-Prunetea* (Borhidi et al. 2012). All names of the mentioned associations are asscepted as synonyms by Chytrý (2013) and Coldea et al. (2015).

The recent studies of the class *Crataego-Prunetea* in Bulgaria were performed by Vassilev et al. (2020). They have published the distribution of 2 associations (*Corno-Ligustretum* Horvat ex Trinajstić & Z. Pavletić 1991, *Pruno spinosae-Ligustretum vulgaris* Tüxen 1952) and 1 community (*Elytrigia repens-Crataegus monogyna* community) belonging to the allance *Berberidion vulgaris*. Sopotlieva et al. (2016) has found the association from the 'Kutelka' Reserve.

The association belongs to the following natural habitat – EUNIS: S357 Subcontinental and continental deciduous thickets.

Two variants are differentiated floristically and ecologically within the association.

Variant *Lamium maculatum* (figure 2, relevés 15-17; annex A, group 5)

Diagnostic species: *Bromus arvensis* (95.6), *Prunus spinosa* (81.6), *Lamium maculatum* (79.1), *Galium aparine* (73.7).

Constant species: *Rosa canina* (67), *Cynosurus echinatus* (67).

Dominant species: *Prunus spinosa* (100).

The communities of this variant are the most xerophytic ones. The soils are dry and with exposed rocks. A typical characteristic is the layer of *Prunus spinosa* in all relevés.

Variant *Thymus pulegioides* (figure 2, relevés 18-30; annex A, group 6)

Diagnostic species: *Agrimonia eupatoria* (90.6), *Crataegus monogyna* (81.3), *Lychnis coronaria* (80.8), *Dorycnium pentaphyllum* s.l. (75.6), *Brachypodium pinnatum* (75.6), *Festuca dalmatica* (71.5), *Clinopodium vulgare* (71.4), *Vicia tenuifolia* s.l. (70.2), *Veronica chamaedrys* (70.2), *Pyrus communis* s.l. (69.7), *Galium verum* (65.8), *Fragaria vesca* (65.8), *Thymus pulegioides* (64.5), *Rubus canescens* (64.5), *Euphorbia cyparissias* (58.6), *Stachys germanica* (58.5), *Pteridium aquilinum* (58.5), *Cota tinctoria* (58.5), *Hypericum perforatum* (58.2), *Rosa canina* (54.5), *Eryngium campestre* (53.5), *Potentilla argentea* (52.0), *Galium album* (52.0), *Prunus cerasifera* (49.9), *Potentilla pedata* (46.9), *Origanum vulgare* (46.9), *Anthoxanthum odoratum* (46.9), *Dactylis glomerata* (46.5), *Salvia pratensis* (43.4), *Filipendula vulgaris* (36.7).

Constant species: *Teucrium chamaedrys* (54), *Achillea millefolium* agg. (54).

Dominant species: *Crataegus monogyna* (38), *Rosa canina* (31), *Prunus spinosa* (15), *Pteridium aquilinum* (8), *Prunus cerasifera* (8).

The communities of this variant are more mesophytic and represent the main characteristics of the association.

Order *Paliuretalia* Trinajstić 1978

The order is represented by thermophilous scrubs in the periphery of the oak forests of the sub-Mediterranean region of southeastern Europe. The sub-Mediterranean alliance *Buxo-Syringion* includes thermophilous scrubs in the continental part of the Northern and Central Balkans (Mucina et al. 2016).

***Rosa spinosissima* comm. (figure 2, relevé 3; annex A, group 2)**

Diagnostic species: *Viscaria vulgaris* (100), *Viola dacica* (100), *Stellaria alsine* (100), *Rosa spinosissima* (100), *Quercus petraea* agg. (100), *Poa nemoralis* (100), *Glechoma hederacea* (100), *Geranium macrorrhizum* (100), *Festuca nigrescens* (100).

Constant species: *Scleranthus annuus* (100), *Avenella flexuosa* (100).

Dominant species: *Rosa spinosissima* (100).

One relevé is separared in this cluster, where *Rosa spinosissima* (90%) has a dominant role in the first layer, but the herbaceous layer is poorly developed. The community is located in the 'Kutelka' reserve, at 1058 m a.s.l. The soils are *Dystric Cambisols*, medium nutrient-rich, fresh, loose and medium rocky. The slope is 15 degrees, with north aspect. On the basis on only one relevé, it is not possible to attribute it to a certain association. Čarni et al. (2009) and Čarni and Mucina (2015) have refered *Rosa pimpinellifolia* (syn. *Rosa spinosissima*) as the diagnostic species for the alliance *Buxo-Syringion* P. Fukarek ex Diklić 1965.

The community belongs to the following types of natural habitats – EUNIS: S2573 Balkano-Hellenic deciduous thickets; Directive 92/43: 40A0* Subcontinental peri-Pannonian scrub.

***Syringa vulgaris* comm. (figure 2, relevés 4-10; annex A, group 3)**

Diagnostic species: *Muscari botryoides* (62.0), *Syringa vulgaris* (61.3), *Vicia hirsuta* (50.0), *Verbascum humile* (50.0), *Sedum acre* (50.0), *Ranunculus illyricus* (50.0), *Melica ciliata* (50.0), *Lathyrus sphaericus* (50.0), *Colchicum autumnale* (50.0), *Clinopodium alpinum* (50.0), *Arenaria serpillifolia* agg. (50.0), *Allium guttatum* (50.0), *Achillea clypeolata* (50.0), *Crupina vulgaris* (44.5).

Constant species: *Koeleria nitidula* (57), *Euphorbia cyparissias* (57), *Achillea millefolium* agg. (57), *Thymus striatus* (43), *Poa bulbosa* (43), *Orlaya grandiflora* (43), *Jasminum fruticans* (43), *Festuca valesiaca* (43).

Dominant species: *Syringa vulgaris* (86), *Pyrus communis* s.l., (14), *Jasminum fruticans* (14), *Festuca valesiaca* (14).

The communities of this group occupy terrains at an altitude between 500 and 1000 m a.s.l., on sunny aspects – with a predominance of eastern and southern components. The soils are *Chromic Cambisols* and *Chromic Luvisols*, medium rich in nutrient, dry to fresh, compacted, medium to highly skeletal. The vertical structure comprises two layers, the first one is dominated by *Syringa vulgaris* (cover between 30 and 90 %). In the herbaceous layer, the floristic composition is a diverse and includes species such as *Euphorbia cyparissias, Jasminum fruticans, Koeleria nitidula, Muscari botryoides, Orlaya grandiflora, Thymus striatus*.

Syringa vulgaris communities are represented on the territory of Serbia by 4 associations – *Syringetum vulgaris* Knapp 1994, *Eryngio-Syringetum vulgaris* Diklić 1965, *Syringetum vulgaris serpentinicum* Vukić. et Jov. 1979 and *Syringetum vulgaris silicicolum* Jov. et Vukić. 1982 (Kojić et al. 1998), which are similiar to the communities in Slivenska Planina Mt. However, the small number of relevés and the various ecological characteristics do not provide enough reasons to refer them to any of the already described associations.

These communities belong to the following types of natural habitats – EUNIS: S35732 Moesian lilac thickets; RB (2015): 20F3 Moesian lilac (*Syringa vulgaris*) thickets.

***Syringa vulgaris* and *Quercus pubescens* comm. (figure 2, relevés 11-14; annex A, group 4)**

Diagnostic species: *Quercus pubescens* (100.0), *Hypericum aucheri* (84.5), *Cyanus thirkei* (84.5), *Carpinus orientalis* (84.5), *Sideritis montana* (75.5), *Ajuga chamaepitys* s.l. (75.5), *Dactylis glomerata* (69.2), *Fraxinus ornus* (68.3), *Securigera varia* (67.4), *Linum austriacum* (67.4),

Geranium purpureum (67.4), *Astragalus spruneri* (67.4), *Arum maculatum* (67.4), *Paliurus spina-christi* (66.2), *Festuca valesiaca* (62.3), *Tanacetum vulgare* (61.3), *Paeonia peregrina* (56.8).

Constant species: *Syringa vulgaris* (75), *Orlaya grandiflora* (75), *Achillea millefolium* agg. (75), *Thymus striatus* (50), *Poa pratensis* (50), *Poa bulbosa* (50), *Jasminum fruticans* (50), *Erysimum diffusum* (50), *Centaurea stoebe* (50), *Ajuga laxmannii* (50).

Dominant species: *Syringa vulgaris* (25), *Carpinus orientalis* (25).

The communities are distributed in the range of 400–500 m a.s.l., on eastern aspects. The slopes are steep – between 15 and 30 degrees. The rocks are marls, and the soils are *Chromic Luvisols*, nutrient-poor, dry, skeletal, with erosion processes observed in places. Typical to this group is the domination of *Syringa vulgaris*, and in some releves *Carpinus orientalis* and *Quercus pubescens* have also found. The herbaceous layer is well developed, with species characteristic to the group 3 and the alliance *Buxo-Syringion* (*Ajuga chamaepytis* s.l., *Centaurea thirkei, Festuca valesiaca, Hypericum aucheri, Jasminum fruticans, Orlaya grandiflora, Sideritis montana, Thymus striatus*). However, the species characteristic of the alliance *Syringo-Carpinion orientalis* Jakucs (1959) 1960 and the association *Syringo-Carpinetum orientalis* Jakucs 1959 (Coldea et al. 2015) (*Dactylis glomerata, Carpinus orientalis, Fraxinus ornus, Quercus pubescens*) also appear, which emphasized the transitional position of this group between these two alliances. Additional researches are needed in the area and also in other parts of Bulgaria to clarify the group‘s affiliation to some specific association.

The communities belong to the following types of natural habitats – EUNIS: S35732 Moesian lilac thickets; RB (2015): 20F3 Moesian lilac (*Syringa vulgaris*) thickets.

Conclusions

The scrub vegetation occupies a small part of the studied area (about 5%). Its distribution is fragmentary, mainly in the ridge of the mountain. However, the scrub vegetation has increased in the area in the recent years, probably due to the abandonment of grazing in the meadows. This is again the result of anthropogenic activity and subsequent successional processes. Probable successional series, part of which are established scrub communities, occur in several variants, most likely of which are: on carbonate soils – associations *Galio pseudaristati-Fagetum sylvaticae* Tzonev et al. 2006 or *Genisto januensis-Quercetum pubescentis* (Jackus 1961) Tzonev et al. 2019 → *Syringa vulgaris* and *Quercus pubescens* comm. → *Syringa vulgaris* comm. → *Rosa spinosissima* comm.; on silicate soils – association *Plantagini subulatae-Agrostietum capillaris* Pedashenko et al. 2013 → *Juniperus communis* comm.; associations *Quercetum frainetto-cerris* (Rudski 1949) Trinajstić et al. 1996 or *Carpino-Fagetum* Paucă 1941 → association *Crataego-Prunetum spinosae* Hueck 1931 → association *Galio flavescentis-Genistetum lydiae* Kunev et al. 2020 → community of *Koeleria nitidula.* Depending on the directions of antropogenic and zoogenic activity, they can proceed in the opposite order.

Some more detailed studies on the scrub communities are necessary also to make more precise analisys of the associations and successional processes. It will be also with practical needs, be-

cause of right management of seminatural ecosystems.

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Appendix A

Table A1. Synoptic table of scrub vegetation on Slivenska Mt

Group number	1	2	3	4	5	6
Group name	*Juniperus communis* comm.	*Rosa spinosissima* comm.	*Syringa vulgaris* comm.	*Syringa vulgaris* and *Quercus pubescens* comm.	Ass. *Crataego-Prunetum spinosae* variant *Lamium maculatum*	Ass. *Crataego-Prunetum spinosae* variant *Thymus pulegioides*
Number of relevés	**2**	**1**	**7**	**4**	**3**	**13**
Diagnostic taxa of groups						
Juniperus communis	100$^{92.2}$	0^{---}	14^{---}	0^{---}	0^{---}	0^{---}
Quercus petraea agg.	0^{---}	100$^{100.0}$	0^{---}	0^{---}	0^{---}	0^{---}
Viscaria vulgaris	0^{---}	100$^{100.0}$	0^{---}	0^{---}	0^{---}	0^{---}
Stellaria alsine	0^{---}	100$^{100.0}$	0^{---}	0^{---}	0^{---}	0^{---}
Poa nemoralis	0^{---}	100$^{100.0}$	0^{---}	0^{---}	0^{---}	0^{---}
Geranium macrorrhizum	0^{---}	100$^{100.0}$	0^{---}	0^{---}	0^{---}	0^{---}
Viola dacica	0^{---}	100$^{100.0}$	0^{---}	0^{---}	0^{---}	0^{---}
Glechoma hederacea	0^{---}	100$^{100.0}$	0^{---}	0^{---}	0^{---}	0^{---}
Rosa spinosissima	0^{---}	100$^{100.0}$	0^{---}	0^{---}	0^{---}	0^{---}
Festuca nigrescens	0^{---}	100$^{100.0}$	0^{---}	0^{---}	0^{---}	0^{---}
Muscari botryoides	0^{---}	0^{---}	43$^{62.0}$	0^{---}	0^{---}	0^{---}
Syringa vulgaris	0^{---}	0^{---}	100$^{61.3}$	75^{---}	33^{---}	0^{---}
Vicia hirsuta	0^{---}	0^{---}	29$^{50.0}$	0^{---}	0^{---}	0^{---}
Ranunculus illyricus	0^{---}	0^{---}	29$^{50.0}$	0^{---}	0^{---}	0^{---}
Colchicum autumnale	0^{---}	0^{---}	29$^{50.0}$	0^{---}	0^{---}	0^{---}
Lathyrus sphaericus	0^{---}	0^{---}	29$^{50.0}$	0^{---}	0^{---}	0^{---}
Verbascum humile	0^{---}	0^{---}	29$^{50.0}$	0^{---}	0^{---}	0^{---}
Melica ciliata	0^{---}	0^{---}	29$^{50.0}$	0^{---}	0^{---}	0^{---}
Sedum acre	0^{---}	0^{---}	29$^{50.0}$	0^{---}	0^{---}	0^{---}
Clinopodium alpinum	0^{---}	0^{---}	29$^{50.0}$	0^{---}	0^{---}	0^{---}
Arenaria serpyllifolia agg.	0^{---}	0^{---}	29$^{50.0}$	0^{---}	0^{---}	0^{---}
Allium guttatum	0^{---}	0^{---}	29$^{50.0}$	0^{---}	0^{---}	0^{---}
Achillea clypeolata	0^{---}	0^{---}	29$^{50.0}$	0^{---}	0^{---}	0^{---}
Crupina vulgaris	0^{---}	0^{---}	43$^{44.5}$	25^{---}	0^{---}	0^{---}
Quercus pubescens	0^{---}	0^{---}	0^{---}	100$^{100.0}$	0^{---}	0^{---}
Hypericum aucheri	0^{---}	0^{---}	0^{---}	75$^{84.5}$	0^{---}	0^{---}
Cyanus thirkei	0^{---}	0^{---}	0^{---}	75$^{84.5}$	0^{---}	0^{---}
Carpinus orientalis	0^{---}	0^{---}	0^{---}	75$^{84.5}$	0^{---}	0^{---}

Group number	1	2	3	4	5	6
Sideritis montana	0---	0---	14---	75$^{75.5}$	0---	0---
Ajuga chamaepitys s.l.	0---	0---	14---	75$^{75.5}$	0---	0---
Dactylis glomerata	0---	0---	0---	100$^{69.2}$	0---	77$^{46.5}$
Fraxinus ornus	0---	0---	29---	75$^{68.3}$	0---	0---
Arum maculatum	0---	0---	0---	50$^{67.4}$	0---	0---
Sherardia arvensis	0---	0---	0---	50$^{67.4}$	0---	0---
Astragalus spruneri	0---	0---	0---	50$^{67.4}$	0---	0---
Linum austriacum	0---	0---	0---	50$^{67.4}$	0---	0---
Geranium purpureum	0---	0---	0---	50$^{67.4}$	0---	0---
Securigera varia	0---	0---	0---	50$^{67.4}$	0---	0---
Paliurus spina-christi	0---	0---	0---	75$^{66.2}$	33---	0---
Festuca valesiaca	0---	0---	43---	75$^{62.3}$	0---	0---
Tanacetum vulgare	0---	0---	0---	50$^{61.3}$	0---	8---
Convolvulus cantabrica	0---	0---	14---	75$^{60.5}$	33---	0---
Satureja montana	0---	0---	14---	50$^{56.8}$	0---	0---
Paeonia peregrina	0---	0---	14---	50$^{56.8}$	0---	0---
Stipa pulcherrima s.l.	0---	0---	14---	50$^{56.8}$	0---	0---
Bromus arvensis	0---	0---	0---	0---	100$^{95.6}$	8---
Prunus spinosa	0---	0---	0---	0---	100$^{81.6}$	38---
Lamium maculatum	0---	0---	0---	0---	67$^{79.1}$	0---
Galium aparine	0---	0---	0---	0---	67$^{73.7}$	8---
Agrimonia eupatoria	0---	0---	0---	0---	0---	85$^{90.6}$
Crataegus monogyna	0---	0---	14---	25---	0---	100$^{81.3}$
Lychnis coronaria	0---	0---	0---	0---	0---	69$^{80.8}$
Dorycnium pentaphyllum s.l.	0---	0---	0---	0---	0---	62$^{75.6}$
Brachypodium pinnatum	0---	0---	0---	0---	0---	62$^{75.6}$
Festuca dalmatica	0---	0---	14---	0---	0---	69$^{71.5}$
Clinopodium vulgare	0---	0---	0---	25---	0---	77$^{71.4}$
Vicia tenuifolia s.l.	0---	0---	0---	0---	0---	54$^{70.2}$
Veronica chamaedrys	0---	0---	0---	0---	0---	54$^{70.2}$
Pyrus communis s.l.	0---	0---	29---	0---	0---	77$^{69.7}$
Galium verum	0---	0---	14---	0---	0---	62$^{65.8}$
Fragaria vesca	0---	0---	0---	25---	0---	69$^{65.8}$
Thymus pulegioides	0---	0---	0---	0---	0---	46$^{64.5}$
Rubus canescens	0---	0---	0---	0---	0---	46$^{64.5}$
Euphorbia cyparissias	0---	0---	57---	0---	0---	77$^{58.6}$
Stachys germanica	0---	0---	0---	0---	0---	38$^{58.5}$
Cota tinctoria	0---	0---	0---	0---	0---	38$^{58.5}$
Pteridium aquilinum	0---	0---	0---	0---	0---	38$^{58.5}$
Hypericum perforatum	0---	0---	29---	0---	0---	62$^{58.2}$
Rosa canina	50---	0---	0---	25---	67---	100$^{54.5}$
Eryngium campestre	0---	0---	14---	25---	0---	62$^{53.5}$
Potentilla argentea	0---	0---	0---	0---	0---	31$^{52.0}$

Group number	1	2	3	4	5	6
Galium album	0---	0---	0---	0---	0---	31$^{52.0}$
Prunus cerasifera	0---	0---	0---	0---	33---	54$^{49.9}$
Potentilla pedata	0---	0---	14---	0---	0---	38$^{46.9}$
Anthoxanthum odoratum	0---	0---	14---	0---	0---	38$^{46.9}$
Origanum vulgare	0---	0---	14---	0---	0---	38$^{46.9}$
Salvia pratensis	0---	0---	0---	0---	33---	46$^{43.4}$
Filipendula vulgaris	50---	0---	0---	0---	0---	46$^{36.7}$
Other taxa						
Geum urbanum	0---	0---	0---	0---	0---	15---
Clematis vitalba	0---	0---	0---	0---	0---	15---
Rumex tuberosus	0---	0---	0---	0---	33---	0---
Sesleria latifolia	50---	0---	29---	0---	0---	8---
Centaurea scabiosa	0---	0---	0---	0---	0---	8---
Trifolium campestre	0---	0---	29---	0---	0---	23---
Achillea millefolium agg.	50---	0---	57---	75--	0---	54---
Inula oculus-christi	0---	0---	14---	0---	0---	8---
Thymus zygioides	50---	0---	0---	0---	0---	0---
Dianthus pinifolius	50---	0---	14---	0---	0---	0---
Cynosurus echinatus	0---	0---	14---	0---	67---	15---
Geranium sanguineum	0---	0---	29---	0---	33---	38---
Digitalis lanata	0---	0---	0---	0---	0---	15---
Carex humilis	50---	0---	0---	0---	0---	0---
Anthyllis vulneraria	50---	0---	14---	0---	0---	0---
Euphorbia myrsinites	0---	0---	29---	25---	0---	0---
Festuca rupicola	0---	0---	14---	0---	0---	0---
Centaurea stoebe	0---	0---	29---	50---	0---	31---
Carex spicata	0---	0---	0---	0---	33---	0---
Orlaya daucoides	0---	0---	14---	0---	0---	0---
Luzula luzuloides	50---	0---	0---	0---	0---	0---
Agrostis capillaris	50---	0---	0---	0---	0---	23---
Poa bulbosa	0---	0---	43---	50---	33---	8---
Thymelaea passerina	0---	0---	14---	0---	0---	0---
Poa compressa	50---	0---	14---	0---	0---	15---
Sorbus torminalis	0---	0---	0---	0---	0---	15---
Briza media	0---	0---	0---	0---	0---	23---
Linum tenuifolium	0---	0---	14---	0---	0---	0---
Minuartia verna	0---	0---	14---	0---	0---	0---
Scleranthus annuus	0---	100---	14---	0---	0---	0---
Petrorhagia prolifera	0---	0---	14---	0---	0---	8---
Ballota nigra s.l.	0---	0---	0---	0---	33---	0---
Orlaya grandiflora	0---	0---	43---	75--	33---	23---
Seseli rigidum	0---	0---	14---	0---	0---	0---
Odontarrhena muralis	0---	0---	14---	0---	0---	0---

Group number	1	2	3	4	5	6
Plantago lanceolata	0---	0---	14---	0---	0---	15---
Rubus caesius	50---	0---	0---	0---	0---	23---
Stachys officinalis	50---	0---	0---	0---	0---	23---
Teucrium polium	0---	0---	14---	0---	0---	0---
Vicia tetrasperma	0---	0---	0---	0---	0---	8---
Sambucus ebulus	0---	0---	0---	0---	0---	8---
Leontodon crispus	50---	0---	14---	25---	0---	0---
Asperula cynanchica	50---	0---	29---	0---	0---	8---
Brachypodium sylvaticum	0---	0---	0---	0---	0---	8---
Aremonia agrimonoides	0---	0---	0---	0---	0---	8---
Quercus frainetto	0---	0---	0---	0---	0---	8---
Alliaria petiolata	0---	0---	0---	0---	33---	0---
Arabis turrita	0---	0---	0---	0---	33---	0---
Geranium pyrenaicum	0---	0---	0---	0---	0---	15---
Daucus carota	0---	0---	0---	0---	0---	8---
Vicia narbonensis	0---	0---	14---	0---	0---	0---
Trifolium ochroleucon	0---	0---	0---	0---	0---	8---
Avenella flexuosa	50---	100---	0---	0---	0---	0---
Geranium robertianum	0---	0---	0---	0---	0---	15---
Heracleum sphondylium s.l.	0---	0---	0---	0---	0---	8---
Nepeta cataria	0---	0---	14---	0---	0---	0---
Clinopodium acinos	0---	0---	29---	0---	0---	8---
Knautia macedonica	0---	0---	0---	0---	0---	23---
Lathyrus laxiflorus	0---	0---	0---	0---	0---	15---
Poa pratensis	50---	0---	14---	50---	0---	31---
Inula hirta	0---	0---	0---	0---	0---	8---
Astragalus glycyphyllos	0---	0---	0---	0---	0---	23---
Campanula persicifolia	50---	0---	0---	0---	0---	0---
Quercus cerris	0---	0---	0---	0---	0---	8---
Lapsana communis	0---	0---	0---	0---	0---	23---
Seseli peucedanoides	0---	0---	0---	0---	0---	23---
Trifolium pannonicum	0---	0---	0---	0---	0---	8---
Viola hirta	0---	0---	0---	25---	33---	8---
Xeranthemum cylindraceum	0---	0---	0---	0---	0---	8---
Torilis japonica	0---	0---	14---	25---	33---	8---
Geranium dissectum	0---	0---	0---	0---	0---	8---
Trifolium pratense	0---	0---	0---	0---	0---	8---
Geranium molle	0---	0---	14---	0---	0---	0---
Cruciata glabra	0---	0---	0---	0---	0---	15---
Euphorbia amygdaloides	0---	0---	0---	0---	0---	8---
Jasminum fruticans	0---	0---	43---	50---	0---	0---
Nardus stricta	50---	0---	0---	0---	0---	0---
Thymus striatus	0---	0---	43---	50---	0---	0---

Group number	1	2	3	4	5	6
Dianthus giganteus	0---	0---	0---	0---	0---	23---
Anagallis foemina	0---	0---	14---	0---	0---	0---
Cornus mas	0---	0---	0---	25---	0---	23---
Sanguisorba minor	50---	0---	29---	25---	0---	31---
Tragopogon pratensis	0---	0---	14---	0---	0---	0---
Rorippa lippizensis	0---	0---	0---	0---	0---	8---
Nepeta nuda	0---	0---	0---	0---	0---	15---
Salvia verticillata	0---	0---	0---	0---	0---	8---
Piptatherum virescens	50---	0---	0---	25---	0---	0---
Calamagrostis arundinacea	0---	0---	0---	0---	0---	15---
Poa trivialis s.l.	0---	0---	0---	0---	0---	15---
Medicago falcata	0---	0---	14---	0---	0---	0---
Hylotelephium maximum	0---	0---	14---	0---	0---	0---
Sedum ochroleucum	0---	0---	14---	0---	0---	0---
Fragaria viridis	50---	0---	0---	0---	0---	0---
Odontites vernus	0---	0---	0---	0---	0---	8---
Agrostis stolonifera	0---	0---	0---	0---	0---	8---
Teucrium chamaedrys	50---	0---	29---	25---	0---	54---
Lotus corniculatus	0---	0---	0---	0---	0---	23---
Trifolium arvense	0---	0---	14---	0---	33---	15---
Centaurium erythraea	0---	0---	14---	0---	0---	15---
Cirsium vulgare	0---	0---	14---	0---	33---	0---
Ochlopoa annua	0---	0---	14---	0---	0---	0---
Lathyrus aphaca	0---	0---	14---	0---	0---	0---
Trifolium pallidum	0---	0---	14---	0---	0---	0---
Achillea grandifolia	50---	0---	0---	0---	0---	0---
Poa alpina	0---	0---	14---	0---	0---	0---
Arrhenatherum palaestinum	0---	0---	14---	0---	0---	0---
Conium maculatum	0---	0---	0---	0---	0---	8---
Galium pseudaristatum	50---	0---	14---	0---	0---	0---
Scabiosa triniifolia	50---	0---	14---	0---	0---	31---
Viola mirabilis	50---	0---	0---	0---	0---	0---
Marrubium peregrinum	0---	0---	14---	0---	0---	0---
Tulipa hungarica	0---	0---	0---	25---	0---	0---
Saponaria glutinosa	0---	0---	0---	25---	0---	0---
Potentilla recta s.l.	0---	0---	14---	0---	33---	8---
Torilis arvensis	0---	0---	0---	25---	0---	0---
Lathyrus pratensis	0---	0---	0---	0---	0---	8---
Melampyrum arvense	0---	0---	0---	0---	0---	8---
Holcus lanatus	50---	0---	0---	0---	0---	15---
Cruciata laevipes	0---	0---	0---	0---	0---	8---
Carlina vulgaris	0---	0---	14---	25---	0---	8---
Campanula bononiensis	0---	0---	0---	0---	0---	8---

Group number	1	2	3	4	5	6
Noccaea kovatsii	0---	0---	14---	0---	0---	0---
Ranunculus acris	0---	0---	0---	0---	0---	8---
Cuscuta europaea	0---	0---	0---	0---	0---	8---
Myosotis sylvatica	0---	0---	0---	0---	0---	8---
Xeranthemum annuum	0---	0---	14---	0---	0---	8---
Veronica officinalis	0---	0---	0---	0---	0---	8---
Crucianella angustifolia	0---	0---	0---	0---	0---	15---
Vicia grandiflora	0---	0---	14---	0---	0---	0---
Cytisus hirsutus	50---	0---	0---	0---	0---	0---
Mentha longifolia	0---	0---	0---	0---	0---	8---
Anisantha sterilis	0---	0---	14---	0---	0---	0---
Ferulago campestris	0---	0---	0---	0---	0---	8---
Carlina acanthifolia	50---	0---	0---	0---	0---	8---
Allium flavum	0---	0---	14---	0---	0---	0---
Pilosella officinarum agg.	50---	0---	0---	0---	0---	0---
Viola tricolor	0---	0---	0---	25---	33---	23---
Anisantha tectorum	0---	0---	14---	25---	0---	0---
Carduus candicans s.l.	0---	0---	14---	0---	0---	0---
Bromus squarrosus	0---	0---	29---	25---	0---	8---
Ajuga laxmannii	0---	0---	14---	50---	0---	15---
Potentilla erecta	50---	0---	0---	0---	0---	8---
Veronica austriaca s.l.	0---	0---	29---	25---	0---	0---
Linaria genistifolia	0---	0---	0---	25---	0---	15---
Ficaria verna	0---	0---	0---	25---	0---	0---
Dipsacus laciniatus	0---	0---	0---	0---	0---	8---
Tephroseris papposa	50---	0---	0---	0---	0---	0---
Buglossoides arvensis	0---	0---	14---	0---	0---	0---
Verbena officinalis	0---	0---	0---	0---	0---	8---
Primula veris	50---	0---	0---	0---	0---	15---
Lactuca quercina	0---	0---	14---	0---	0---	0---
Elytrigia repens	0---	0---	0---	0---	33---	15---
Cotinus coggygria	0---	0---	0---	25---	0---	0---
Euphorbia nicaeensis	0---	0---	14---	0---	0---	0---
Phleum phleoides	0---	0---	14---	0---	0---	0---
Arrhenatherum elatius	0---	0---	0---	0---	0---	15---
Fumana procumbens	0---	0---	0---	25---	0---	0---
Polygala major	50---	0---	0---	0---	0---	0---
Carex leporina	0---	0---	0---	0---	0---	8---
Buglossoides purpurocaerulea	0---	0---	0---	25---	0---	0---
Schedonorus giganteus	0---	0---	0---	25---	0---	0---
Milium effusum	0---	0---	14---	0---	0---	0---
Prunus tenella	0---	0---	0---	25---	0---	0---
Euphorbia esula	0---	0---	0---	0---	0---	15---

Group number	1	2	3	4	5	6
Vicia sepium	0---	0---	0---	0---	0---	8---
Muscari comosum	0---	0---	14---	0---	0---	0---
Digitalis viridiflora	0---	0---	0---	0---	0---	8---
Ranunculus millefoliatus	0---	0---	14---	0---	0---	0---
Torilis leptophylla	0---	0---	14---	0---	0---	0---
Chrysopogon gryllus	0---	0---	0---	0---	0---	8---
Calamagrostis epigejos	0---	0---	0---	0---	0---	15---
Chaerophyllum byzantinum	0---	0---	0---	0---	0---	8---
Scorzonera hispanica	0---	0---	29---	25---	0---	8---
Erysimum diffusum	0---	0---	14---	50---	0---	8---
Luzula forsteri	0---	0---	0---	0---	0---	23---
Vicia cassubica	0---	0---	0---	0---	0---	8---
Trifolium alpestre	0---	0---	0---	0---	0---	23---
Filago germanica	50---	0---	14---	0---	0---	0---
Helianthemum nummularium	50---	0---	0---	0---	0---	0---
Drymocallis rupestris	0---	0---	14---	0---	0---	0---
Phleum montanum	0---	0---	14---	0---	0---	0---
Koeleria nitidula	0---	0---	57---	0---	0---	38---
Milium vernale	0---	0---	14---	0---	0---	0---
Apera spica-venti	0---	0---	0---	0---	0---	8---
Trifolium hybridum	0---	0---	0---	0---	0---	15---
Dioscorea communis	0---	0---	0---	25---	0---	0---
Lactuca muralis	0---	0---	0---	25---	0---	0---
Alyssum alyssoides	0---	0---	14---	0---	0---	0---
Euphorbia agraria	0---	0---	14---	0---	0---	0---
Allium paniculatum	0---	0---	14---	0---	0---	0---
Cerastium glomeratum	0---	0---	14---	0---	0---	0---
Avenula pubescens	0---	0---	14---	0---	0---	0---
Scilla bifolia	0---	0---	14---	0---	0---	0---
Astragalus angustifolius	0---	0---	0---	25---	0---	0---
Crepis sancta	0---	0---	0---	25---	0---	0---
Alyssum hirsutum	0---	0---	0---	25---	0---	0---
Ononis pusilla	0---	0---	0---	25---	0---	0---
Dasypyrum villosum	0---	0---	0---	25---	0---	0---
Scutellaria orientalis	0---	0---	0---	25---	0---	0---
Odontarrhena tortuosa	0---	0---	14---	0---	0---	0---
Reseda lutea	0---	0---	0---	25---	0---	0---
Acer platanoides	0---	0---	0---	0---	0---	8---
Mentha spicata	0---	0---	0---	0---	0---	8---
Silene dichotoma	0---	0---	14---	0---	0---	0---
Acer pseudoplatanus	0---	0---	0---	0---	0---	15---
Cerastium gracile	0---	0---	14---	0---	0---	0---

Group number	1	2	3	4	5	6
Jacobaea vulgaris	0^{---}	0^{---}	0^{---}	0^{---}	0^{---}	8^{---}
Agrostis castellana	0^{---}	0^{---}	0^{---}	0^{---}	0^{---}	8^{---}
Agrostis gigantea	0^{---}	0^{---}	0^{---}	0^{---}	0^{---}	8^{---}
Potentilla astracanica	0^{---}	0^{---}	0^{---}	25^{---}	0^{---}	0^{---}
Iris reichenbachii	0^{---}	0^{---}	14^{---}	0^{---}	0^{---}	0^{---}
Viola kitaibeliana	0^{---}	0^{---}	14^{---}	0^{---}	0^{---}	0^{---}
Carex liparocarpos	0^{---}	0^{---}	14^{---}	0^{---}	0^{---}	0^{---}
Scleranthus perennis	0^{---}	0^{---}	14^{---}	0^{---}	0^{---}	0^{---}
Astragalus depressus	0^{---}	0^{---}	14^{---}	0^{---}	0^{---}	0^{---}
Calystegia sepium	0^{---}	0^{---}	14^{---}	0^{---}	0^{---}	0^{---}
Hypericum umbellatum	0^{---}	0^{---}	14^{---}	0^{---}	0^{---}	0^{---}

Note: The frequency of each species is presented by percentage. The fidelity was presented by their phi-coefficient per group multiplied by 100 (in superscript). The group numbers correspond to the syntaxa numbers described in the text. The frequency and fidelity of diagnostic taxa for the groups are colored in gray.

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Structure of a riparian forests in Western Greece

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Abstract

Purpose of this research was to study the structure of the riparian forests of Trichonida Lake in the Western Greece (a protected area of Natura 2000 network with Code GR2310009). The research recorded four different types of structure consisting of mixed stands of *Platanus orientalis and Salix alba* and pure stands of *Platanus orientalis* or *Salix alba*. The mixed stands of *Platanus orientalis-Salix alba* were at the stage of the medium to thick trunks. They were dominated by 80 % plane trees and belonged to habitat 92C0. In the mixed stands of *Salix alba-Platanus orientalis, Salix alba* (60 % of the trees) appeared on the upper and middle storey and was absent from the under storey, where only *Platanus orientalis* occurred. In this type of structure there were also a few stems of *Salix alba* and *Platanus orientalis* in the aging phase with DBH> 120 cm. *Platanus* dominated at the upper and middle storey and was absent from the understorey, which was dominated by *Salix*. Mixed willow-plane stands resulted from penetration of willows into original plane stands, which were at their eventual dominance due to the rapid growth of willows. These stands were classified as habitat 92A0. The pure stands of *Platanus* were stable, multi-storieyed and at the stage of medium-sized stems. The pure stands of *Salix alba* were very stable (slenderness index 39) two-storieyed stands (without understorey). *Platanus* was the pioneer tree species, while *Salix* colonized the old *Platanus* stands and participated in all tree storeys. The intense human presence in the area has had a negative impact on the structure and dynamic of the riparian forest, leading to the risk of possible change of habitat types. It is proposed to draw up immediately a management plan, which should set limitations of human pressures, rehabilitation, restoration and protection of the structure and habitats of the forest Trichonida's Lake with the appropriate silvicultural measures.

Key words: Lake Trichonida, plane forest, protected area, softwood riparian forest.

Introduction

According to Baker (2002) the term 'riparian areas' that describes areas adjacent to surface freshwater bodies, first appeared in the scientific literature in the early 1970's (Zaimes 2007). Since then, it has been officially accepted by the scientific community with hundreds of articles and dozens of books being published, related to riparian areas. Riparian forests act as ecological corridors with significant benefits for biodiversity (Sitzia et al. 2018). From ancient times they served as migration routes of flora and fauna contributing to the exchange of biogenetic material (Gerken 1988, Kuhn 1991). They are complex ecosystems affected

by variable flood regimes, altitude climate changes and mountain influences. These factors put the plant community under a wide range of competitive pressures, resulting in a high level of biodiversity (Naiman et al. 1993, Tabacchi et al. 1998, Steiger et al. 2005). The great biodiversity of riparian forests relies on the functioning of riparian areas. Erosion, transport and deposition of materials, the uprooting of riparian vegetation from one area and its establishment in another during flooding are the basic functions of a river system (Mandžukovski et al. 2021).

Riparian forests are considered to be among the most dynamic ecosystems with a very high biodiversity (Efthimiou et al. 2016) They contain mainly hydrophilic plant species, resistant to mechanical pressure, since they have to deal with a soil full of water and strong river flows (Zogaris et al. 2007). According to Mandžukovski et al. (2021), the riparian forests are very rich and dynamic ecosystems. They are periodically flooded by surface or underground water for a time interval of a few days to a few weeks (Efthimiou 2000).

In a riparian ecosystem, water and vegetation are in constant and mutual interaction. The function of the flood contributes to the dynamics of riparian forests, the colonization of islets and banks, the reappearance of species in new locations or in areas that had been displaced by other species. Flooding often limits or displaces species that cannot withstand inundation. First, the willow colonizes the newly formed riparian soils and stabilizes them. In the second year poplar enters, and as the fastest growing species (Hohenberger 1989), surpasses the other trees and dominates the upper floor.

These ecosystems are characterized also by rich fauna and many different habitat types despite the fact of their occurrence mainly on sandy soils. The rich biological activity makes riparian forests very similar to rainforests (Efthimiou 2001).

Depending on their composition, structure, dynamics and ecological conditions, two large groups of riparian forest communities are distinguished: riparian forests growing in places with direct influence of water and hardwood forests with less, indirect influence of water (Efthimiou 2000, 2001).

Riparian forests are a key element of Europe's natural heritage with high value due to their high biodiversity (Tsitsonis 2009). According to Cierjacks et al. (2010), these ecosystems play an important role in the storage of organic carbon. However, knowledge about the spatial patterns of organic matter distribution of riparian ecosystems is still insufficient.

Dafis (1993) characterized the riparian forests as ecosystems with great internal stability and balance as long as they remain unaffected by any human activity and intervention. But they are threatened with immediate collapse of destruction after all sorts of external disruptions. According to Efthimiou (2017), the lack of management of riparian forests led these ecosystems to their degradation, shrinkage and often their extinction. The intense grazing of the riparian forests as well as their intensive logging with the aim of converting these areas into monocultures, (mainly poplar), limited their surface to one third (1/3) during the 18th century (Dafis 1993). Therefore, there is an urgent need for their conservation. Unfortunately, the knowledge of most European countries about the importance of these forests is still insufficient. The natural riverside forests in Greece are found around the rivers Nestos, Spercheios, Pinios, Axios, Acheloos, Evinos and in the lakes Volvi, Doirani and Trichonida.

An ecologically important riparian forest of Western Greece is found around Lake Trichonida. The riparian forest receives very intense human activity but we have very little knowledge (almost non-existent research) about its structure. The originality of this research is that it is the first attempt to characterize the structure of the riparian forest in a lake in Western Greece which belongs to the European ecological network Natura 2000. According to Dister and Drescher (1987), Heller (1963, 1969), the knowledge of forest structure and dynamic is a necessary precondition of the forest's knowledge in general and of forestry capacity as well as decision making regarding their management in particular (Efthimiou 2012). According Chianucci et al. (2016) its effective management is hampered by the complexity of the interactions of mixed tree species and the influence of environmental factors on plant diversity.

A riparian forest is all the closer to nature when it is made up of a spatial mosaic of various types of structure, depending on the phase of development and consists of various overlapping floors with abundance of climbing species. The description of the remaining natural riparian forests in Europe combines the above characteristics (Knapp 1971, Carbiener 1974).

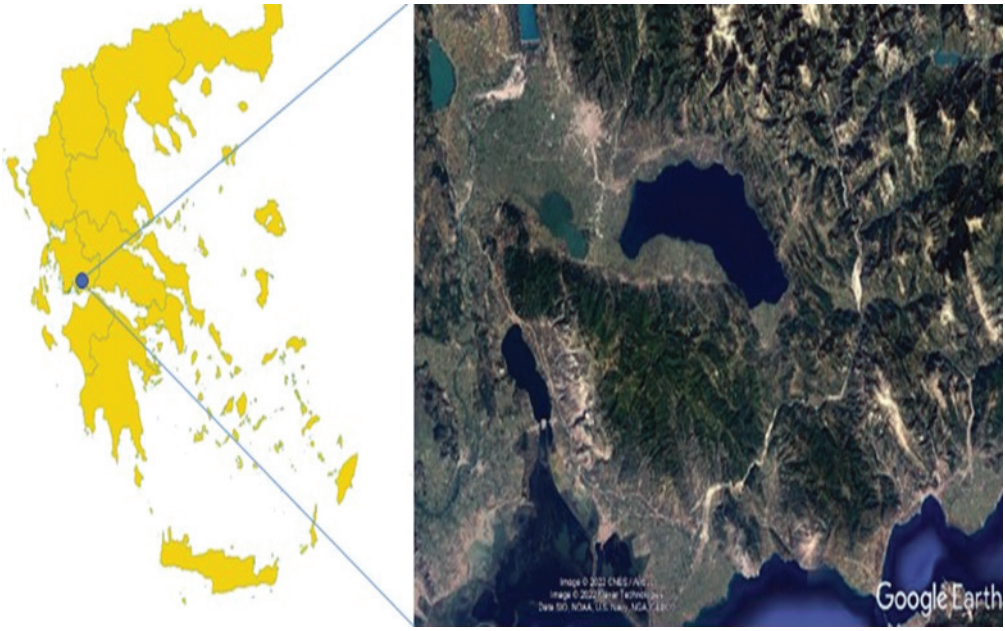
According to Assmann (1961), the slenderness index characterized the stability of stand. Low degrees of slenderness show constant conditions, while high degrees are characteristic for stands of non-constant structure (Röhle 1982). More specifically, when a tree has mechanical stability H:D > 100 it is characterized as very unstable, H:D = 80–100 unstable, H:D < 80 stable, H:D < 0.45 very stable (Apatsidis 2013).

The main objectives of this research were to answer the questions: a) what are the structures of softwood riparian forests found around Lake Trichonida? What are the biotic and abiotic factors that threatens them? Answering of these questions could help the close-to-nature management of these forests in the protected area.

Materials and Methods

Lake Trichonida (Fig. 1) is located in the southeastern part of the Prefecture of Aitoloakarnania between the coordinates 38°28' and 38°40' north latitude and 21°20' and 21°40' east longitude, in Western Greece. It is 20 km away from the city of Agrinio and is one of the most important lakes in Western Greece as it has great aesthetic, ecological and fishing value. It hosts a large number of rare and endangered plant and animal species. It has been included in the European Ecological Network 'NATURA 2000' with code GR2310009 (EEA 2021) as a Special Area of Conservation (SAC).

Lake Trichonida is the largest and deepest lake in Greece (Seguin et al. 2020). It is a warm monomictic lake of karstic origin with an area of 98 km^2, a maximum depth of 58 m and a water volume of about $2.8 \cdot 10^9$ m^3 (Doulka 2010). According to Zacharias et al. (2003) the hydrological basin of Lake Trichonida has an area of almost 400 km^2 and is characterized as lowlands to semi-mountainous. It is bounded north by the peaks of Mount Panetoliko (1924 m), southwest by Mount Arakinthos (<900 m) and east by Mount Harama (<700 m). According to recent studies by Kehayias and Doulka (2014), Lake Trichonida is classified as an oligotrophic lake with a trend of increasing eutrophication, i.e., there is a movement towards the prevalence of mesotrophic



a b

Fig. 1. The case study (a) (Papathanassiou 2016–2022) and Lake Trichonida (b) (Network of Cities with Lakes 2021).

conditions.

For the study of the structure of riparian vegetation around Lake Trichonida, a total of 5 representative sample plots consisting of *Platanus orientalis* L. and *Salix alba* L. were established. These two species are found in four different stand types (ST) differing in the composition of the dominant arboreal vegetation (Athanasiadis 1985, 1986; Efthimiou 2000).

On each sample plot, all trees with a diameter of 4 cm or above were measured as well as height measured with the help of Haga altitude.

Tree individuals were classified according to the IUFRO classification system (Dafis 1966, Dister and Drescher 1987, Smiris 1987, Efthimiou 2012, Efthimiou et al. 2015) according to their social status. Their social status is characterized by Classes of height in tree storeys (Upper storey: Middle storey, Under storey):

a) 100 Upper storey: individuals with a height greater than 2/3 of the upper height of the stand.

b) 200 Middle storey: stems whose height varies from 1/3–2/3 of the upper height.

c) 300 Under storey: stems less than 1/3 height of the upper height.

The structure data were processed statistically and the diagrams were drawn with the help of Excel. The parameters of descriptive statistics for diameter and height were calculated for each type of structure in total, by type and by storey. The total basal area per hectare ($m^2 \cdot ha^{-1}$) was also calculated in total and by species for each surface. The mechanical stability was calculated as (H:D) = total tree height divided by DBH both overall for each surface and by type and storey.

Results

The research revealed that the riparian forests consist of riparian tree species in pure or mixed stands. The vegetation recorded in each type of structure studied refers to the dominant vegetation. Finally, the understorey was dominated by the species *Nerium oleander* L., *Rubus* sp, *Vitex agnus-castus* L., *Agrostis vinealis* Schreber. Individuals of *Juncus acutus* L. were also observed around the perimeter of the survey area. A large part of the understorey was occupied by *Rubus* sp. and *Hedera helix* L.

The four types of structure found and measured in the survey area were:

(i) pure stand of *Platanus orientalis* (ST1);

(ii) mixed stands of *Platanus orientalis* and *Salix alba* (ST2);

(iii) mixed stands of *Salix alba* and *Platanus orientalis* (ST3);

(iv) pure stand of *Salix alba* (ST4).

The detailed results for each type of structure are analyzed below.

1. Pure stand of *Platanus orientalis* (ST1)

It was a pure stand of *Platanus orientalis*, which appeared in all classes with a diameter of 15 to 70 cm (Fig. 2). The total density of the stand was 338 stems·ha^{-1}, with average diameter 40.11 cm, average height 25.71 m, total basal area 48.97 m^2·ha^{-1} and slenderness index 64. All these parameters characterize a fixed stand (Table 1).

About 69 % of the total basal area was produced from the upper storey. Also, 55 % of stems were in the upper-storey, 34 % in the middle-storey and 11 % in the under-storey.

The tallest tree was 44 m. Most stems appeared in the 34 and 38 cm DBH classes which, in terms of maturity phase, are classified in the stage of moderate trunks (Fig. 2), while a significant number of stems were classified in the phase of mature trunks (classes of diameter 54 and 58 cm). The distribution of height showed a multi-storey stand with distinct storeys with four maximum in steps 19, 23, 29 and 35 m (Fig. 3).

2. Mixed stand of *Platanus orientalis-Salix alba* (ST2)

It is a mixed stand of *Platanus orientalis* and *Salix alba* with a total density of 332 stems·ha^{-1}, an average diameter of 39.3 cm, an average height of 29.40 m, a total basal area of 47.02 m^2·ha^{-1} and the slenderness index is 75. That shows that these are fixed stands (Table 2). Total 94 % of the total basal area was produced by the upper storey.

The tallest tree was *Platanus orientalis* with a height of 37 m. The largest num-

Table 1. Stand parameter statistics of *Platanus orientalis* pure stand (ST1).

Stand parameter	Upper storey	Middle storey	Under storey	Total
DBH, cm	45.23	34.47	31.50	40.11
DBH [min–max], cm	23–70	15–52	21–40	15–70
Height (H), m	32.74	19.21	10.00	25.71
H [min–max], m	24–44	15–23	7–12	7–44
Slenderness Index (H:D)	72	56	32	64
Total basal area (G), m^2·ha^{-1}	33.89	12.03	3.05	48.97
Density, ha^{-1} (share, %)	187 (55)	115 (34)	36 (11)	338

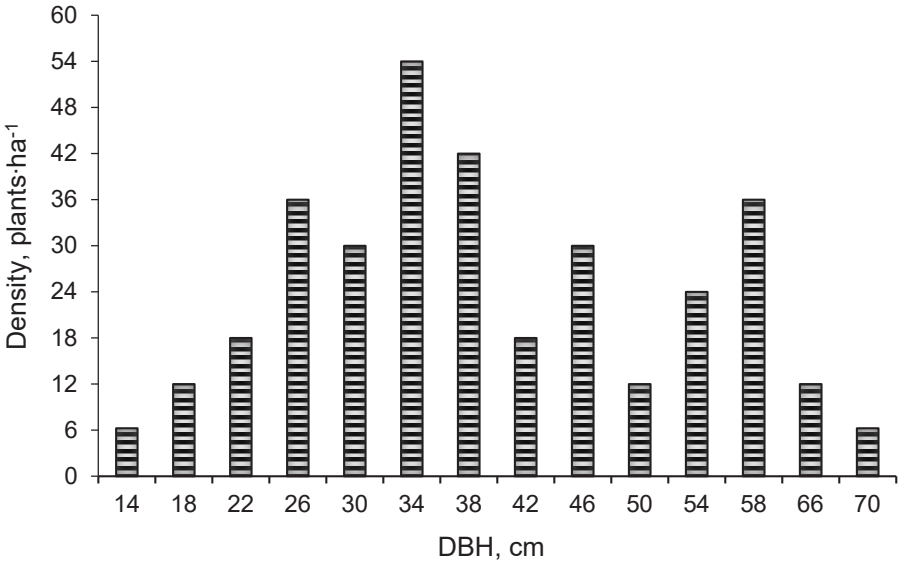


Fig. 2. Diameter distribution of *Platanus orientalis* pure stand (ST1).

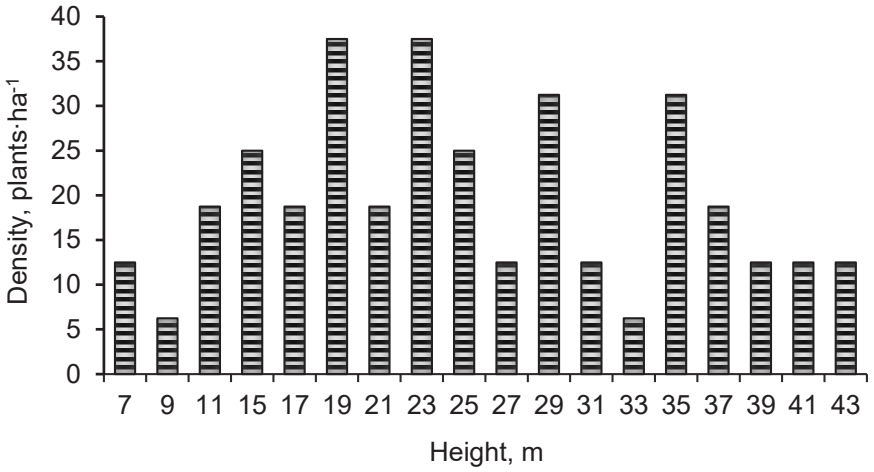


Fig. 3. Height distribution of *Platanus orientalis* pure stand (ST1).

ber was found in diameter classes o of 50 and 58 cm, which classified to the maturity stage of the moderate to thick logs (Fig. 4). The height distribution shows a storey stand with maximums at heights of 31 and 35 m (Fig. 5).

Total 75 % of *Platanus orientalis* individuals are in the upper storey, 25 % in the middle storey while *Salix alba* appeared equally only in the upper storey and under storey (50 % are in the upper storey and 50 % in the under-storey) and was completely absent from the middle storey (Table 3).

Table 2. Stand parameter statistics of *Platanus orientalis-Salix alba* mixed stand storeys (ST2).

Stand parameter	Upper storey	Middle storey	Under storey	Total
DBH, cm	48.00	20.00	17.00	39.30
DBH [min–max], cm	30–58	16–24	16–18	16–58
Height (H), m	34.29	24.00	6.35	29.40
H [min–max], m	31–37	19–29	6–8	6–37
Slenderness Index (H:D)	71	120	37	75
Total basal area (G), $m^2 \cdot ha^{-1}$	44.08	2.18	0.76	47.02
Density, ha^{-1} (share, %)	233 (71)	66 (20)	33 (9)	332

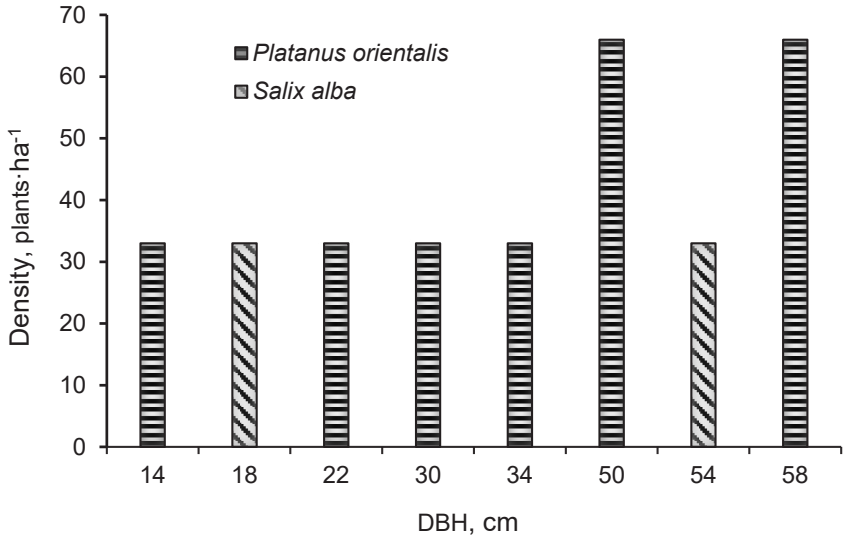


Fig. 4. Diameter distribution of *Platanus orientalis-Salix alba* mixed stand (ST2).

Table 3. Stand parameter statistics of *Platanus orientalis-Salix alba* mixed stand species (ST2).

Stand parameter	*Platanus orientalis*				*Salix alba*			
	Upper storey	Middle storey	Under storey	Total	Upper storey	Middle storey	Under storey	Total
DBH, cm	47.17	20.00	-	40.38	53.00	-	17.00	35.00
DBH [min–max], cm	30–58	16–24	-	16–58	53	-	16–18	16–53
Height (H), m	34.00	24.00	-	32.00	31.00	-	6.35	24.00
H [min–max], m	31–37	19–29	-	19–37	30–36	-	6–8	6–36
Slenderness Index (H:D)	72	120	-	79	58	-	37	68
Total basal area (G), $m^2 \cdot ha^{-1}$	36.3	2.18	-	38.91	7.35	-	0.76	8.11
Density, ha^{-1} (share, %)	200 (75)	66 (25)	-	266	33 (50)	-	33 (50)	66

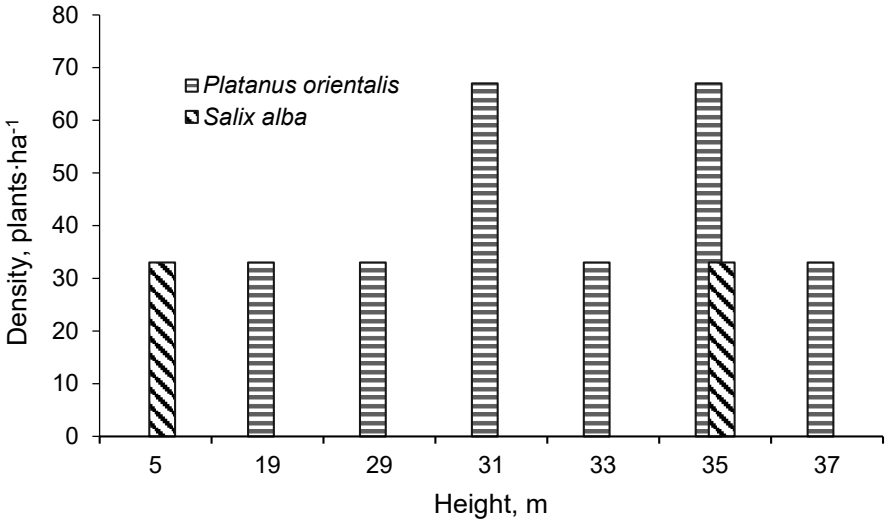


Fig. 5. Height distribution of *Platanus orientalis-Salix alba* mixed stand (ST2).

3. Mixed stand of *Salix alba* and *Platanus orientalis* (ST3)

The mixed stand with *Salix alba* and *Platanus orientalis* had a total density of 75 stems·ha^{-1}, average diameter of 33 cm, average height of 15 m, total basal area 15.20 m^2·ha^{-1} and the slenderness index 69. These results indicate that the stand is stable or fixed (Table 4). Total 96 % of the basal area was produced by the upper storey.

Salix alba dominates with 45 stems per hectare and is followed by *Platanus orientalis* with 30 stems per hectare. For *Salix alba* 78 % of the stems were in the upper storey, and 22 % in the middle storey, while for *Platanus orientalis* 50 % were in the upper storey, 33 % in the middle storey and 17 % on the under storey (Table 5).

The tallest tree was 30 m (*Platanus orientalis*). The largest number of trees were found in the diameter classes of 14 and 22 cm which means they are at the stage either of maturity or thin logs (Fig. 6). Height distribution showed a two storied stand with maximum number of stems at 13 and 17 m (Fig. 7).

Table 4. Stand parameter statistics of *Salix alba-Platanus orientalis* mixed stand stories (ST3).

Stand parameter	Upper storey	Middle storey	Under storey	Total
DBH, cm	39.00	19.50	6.00	33.00
DBH [min–max], cm	8–140	13–28	6	6–140
Height (H), m	17.20	12.50	2	15.00
H [min–max], m	13–30	6–18	2	2–30
Slenderness Index (H:D)	76	60	33	56
Total basal area (G), m^2·ha^{-1}	14.54	0.65	0,01	15.20
Density, ha^{-1} (share, %)	50 (67)	20 (27)	5 (6)	75

Table 5. Stand parameter statistics of *Salix alba-Platanus orientalis* mixed stand species (ST3).

Stand parameter	*Salix alba*				*Platanus orientalis*			
	Upper storey	Middle storey	Under storey	Total	Upper storey	Middle storey	Under storey	Total
DBH, cm	33.00	19.00	-	26.00	59.30	21.00	6.00	28.80
DBH [min–max], cm	14–120	14–23	-	14–120	8–140	13–28	6	8–140
Height (H), m	16.40	12.00	-	14.20	19.00	13.00	2	11.30
H [min–max], m	6–18	6–23	-	6–23	11–30	6–20	2	2–30
Slenderness Index (H:D)	92	43	-	76	71	59	33	54
Total basal area (G), $m^2 \cdot ha^{-1}$	5.90	0.28	-	6.18	8.08	0.37	0.01	8.46
Density, ha^{-1} (share, %)	35 (78)	10 (22)	-	45	15 (50)	10 (33)	5 (17)	30

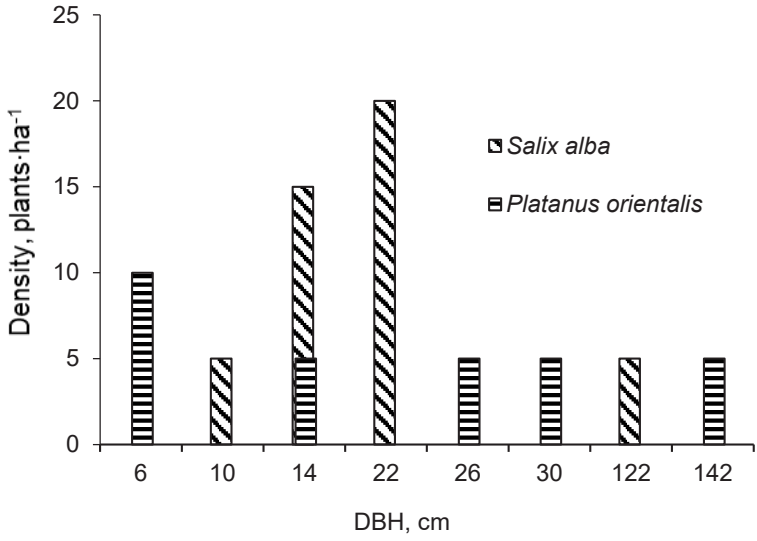


Fig. 6. Diameter distribution of young stands *Salix alba-Platanus orientalis* mixed stand ST3.

Figures 4 and 6 indicate that certain diameter classes are missing. These could have been illegally logged, which is confirmed by the many stumps observed in the study area during fieldwork.

4. Pure stand of *Salix alba* (ST4)

The pure stand of *Salix alba* had a total density 67 stems·$ha^{-1,}$ average diameter of 31 cm, average height of 12 m, total basal area of 5.56 $m^2 \cdot ha^{-1}$ and slenderness index 39, which show, that these are very high stability stands (Table 6). About 92 % of the total basal area was produced by the upper storey. About 66 % of stems were in the upper storey, and the remaining 34 % – in the middle storey.

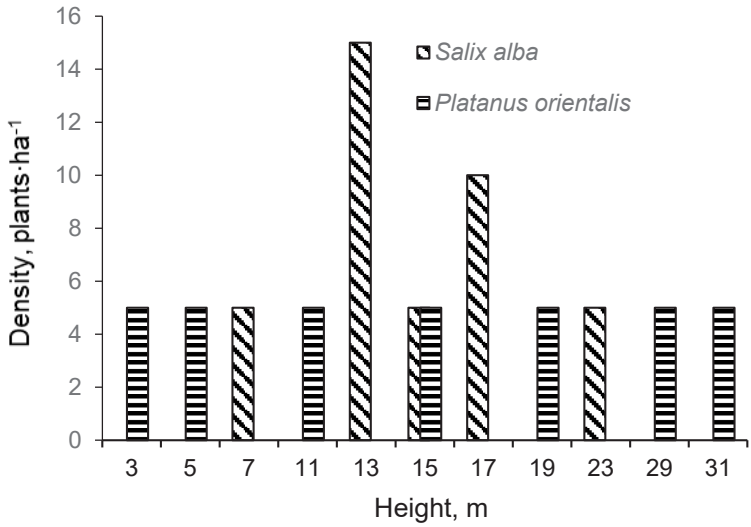


Fig. 7. Height distribution of young stands *Salix alba-Platanus orientalis* mixed stand ST3.

Table 6. Stand parameter statistics of *Salix alba* pure stand (ST4).

Stand parameter	Upper storey	Middle storey	Under storey	Total
DBH, cm	38.00	31.50	-	31.00
DBH [min–max], cm	30–43	13–18	-	13–43
Height (H), m	15.00	10.00	-	12.00
H [min–max], m	12–17	4–7	-	4–17
Slenderness Index (H:D)	39	32	-	39
Total basal area (G), $m^2 \cdot ha^{-1}$	5.13	0.43	-	5.56
Density, ha^{-1} (share, %)	44 (66)	23 (34)	-	67

The tallest tree was 17 m. The largest number of trees was found in the diameter class of 42 cm, and therefore, the stand can be classified in the stage of medium trunks (Fig. 8). The distribution of height shows a multi-storey structure (Fig. 9).

Discussion

Lake Trichonida belongs to the European ecological network NATURA 2000 with code GR2310009. In the study area, riparian forests with two dominant forest species, *Platanus orientalis* and *Salix alba*, were recorded around the lake.

The mixed stands willows dominated numerically (by 50 % of plane) on the two upper floors while they were absent from the basement. Mixed willow-sycamore stands resulted from the entry of willows into original sycamore stands and their eventual dominance due to the rapid growth. These clusters should be listed in habitat 92A0.

The pure stands of *Platanus orientalis* (ST1) are fixed stands. They were found on all storeys and in all size classes with a greater appearance of trees in the stage of medium and old age trunks. There is mod-

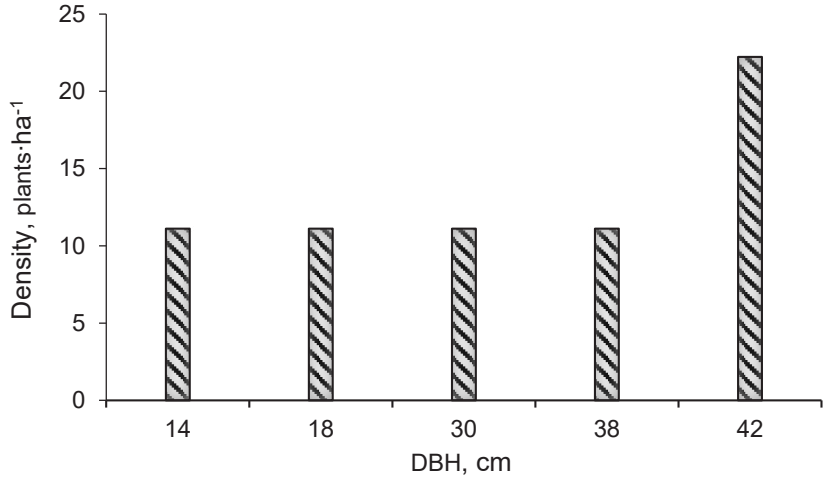


Fig. 8. Diagram of diameter distribution of *Salix alba* pure stand (ST4).

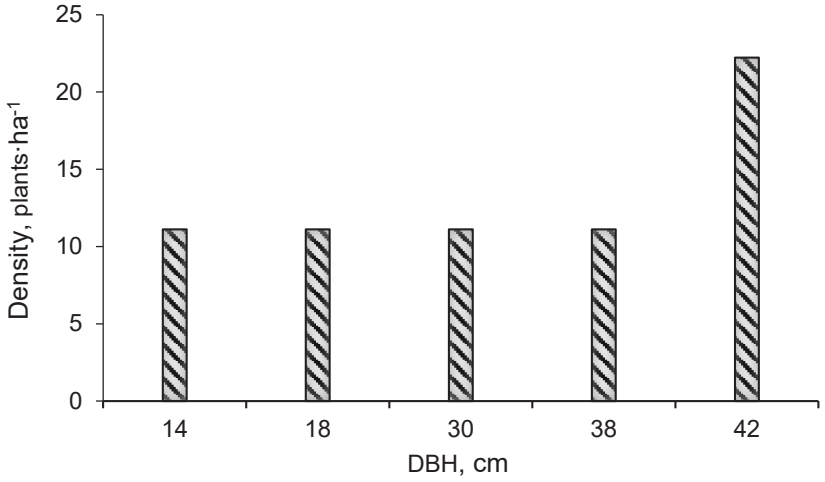


Fig. 9. Height distribution of *Salix alba* pure stand (ST4).

erate natural regeneration despite intense human activity. A similar type of structure with pure stands of *Platanus orientalis*, has been studied in central Greece, around Pinios River (Pipinis 2003). There the rebirth of *Platanus orientalis* does not exist at all from spermoblasts but only a few premoblasts that came from illegal timbering.

The mixed stands *Platanus orientalis-Salix alba* (ST2) was the main and most frequent variant of the riparian forest of Lake Trichonida. The absence of *Salix* from the middle storey indicates their possible logging or grazing in previous years. The absence of *Platanus* from the under storey and entrance of *Salix* shows a dynamic tendency of the riparian forest to be dominated by the *Salix*, if they are

not protected as to ensure their natural evolution.

The mixed stands *Platanus orientalis-Salix alba* (ST2) are characterized as stands with marginal stability, according to the classification of the stability of a stand (Apatsidis 2013). According to Navratil and MacIsaac (1993), the high value of the slenderness index is an indication that there is competition between trees. Consequently, the high value of the slenderness index, 120 on the middle storey, shows that the trees of the middle storey, which are only *Platanus*, show a strong competitiveness with each other (tables 2 and 3).

According to Efthimiou et al. (2015) a similar type of structure of mixed riparian stands with *Platanus orientalis*, *Populus alba*, *Salix alba* is found, in the riparian forest of the river Richios where *Platanus orientalis* and *Salix alba* appear in the middle storey and in the under storey, while the upper storey consists of all three species. In Peneus River *Platanus orientalis* (Pipinis 2003), forms mixed stands with *Salix alba* in which the two species appear on all floors of the stand. Mixed forests of *Platanus orientalis* and *Salix alba*, with a similar type of structure are found in southern Bulgaria and specifically in Struma, Mesta, Ardas, Vacha, Chaia rivers and their tributaries Dimitrov et al. (2018). Research in a mixed riparian stand *Platanus mexicana* Moric. and *Alnus acuminata* Kunth, on the banks of the Xaltatempa River, in the State of Puebla, Mexico, reports that in the 'gallery' forest *Platanus mexicana*, was recorded along the entire length of the river and had the highest values in diameter, height and density than *Alnus acuminata* (Luna 2018).

In the mixed stands of *Salix alba-Platanus orientalis* (ST3), the study of their structure showed that *Salix* colonizes the old stands of *Platanus orientalis* and gradually appears on the upper two storeys, probably due to the intensity of pressures from human activities.Mixed stands of *Salix alba-Platanus orientalis* are the dominated riparian forest type around Lake Trichonida. These are young mixed stands in which *Salix alba* had greater competitiveness (higher slenderness index values) than *Platanus orientalis* especially on the upper storey (Table 5). *Salix alba* was absent in the under storey. This dynamic is captured by the analysis of structure elements in mixed willow-plane stands. In the research of Efthimiou et. al. (2016) on Richios River (Macedonia), pure riparian stands of *Platanus orientalis*, *Populus alba* and *Salix alba* were recorded in which the *Salix alba* penetrated into old stands of *Platanus orientalis* and appears on all three storeys.

In the mixed stands of *Platanus orientalis-Salix alba*, the study of their structure shows that the *Salix* colonizes in old stands of *Platanus orientalis* and gradually appears on all storeys or in some depending on the intensity and time of pressures from human activities. This dynamics in mixed stands of *Platanus* has been confirmed by similar structure research in mixed stands *Platanus-Populus-Salix* in deciduous riparian forest in SW United States and northern Mexico, in which the *Platanus* is a pioneer tree species (Stromberg 2002).

In both of the structure's types of mixed stand with plane and willow, the willow participates in the upper storey. If it is considered that the sycamore disease has been detected in the area (verbal communication with Management Body) and given the 'aggressive invasion' of the willow in the riparian forest, the 92A0 habitat is likely to dominate in the future. This must be considered seriously for the man-

agement of the riparian forest.

Pure stands of *Salix alba* (ST4) are stands with high stability. These are two-storied stands with very high stability (slenderness index 39) while trees are absent from the under storey at the stage of maturity of the medium logs (Table 6).

The study of Sitzia et al. (2018), on the genetic diversity and structure of *Salix alba* populations along fragmented riparian corridors in northeastern Italy (on the Po Plain) on the middle of the river Brenta, showed high genetic similarity among different structures. The magnitude of genetic diversity in each structure and genetic differentiation among them suggested that these bases are dynamic, capable and adaptive to possible changes in their humid environment. A study by Piton et al. (2020), on the semi-natural banks of rivers in the French Alps (Isère, France), showed that the restoration of the degraded bank of the river using willow planting techniques, improved both protection against erosion and riparian ecological functions. These techniques are often based on the establishment of Salicaceae species and sometimes Tamaricaceae species that can be strongly affected by the appearance of a beaver. In our country there are no such surveys.

The research on the structure of the riparian forest of Lake Trichonida, which is the first study of the structure of the riparian forest, showed that in recent years its existence is directly threatened not only by the intense human activity but also by the fungus *Ceratocystis platani* (J.M. Walter) Engelbr. & T.C. Harr. A parallel can be seen with the northern part of 'Bosco Fontana' area of Italy, where the mixed deciduous forest is considered one of the most endangered ecosystems in Europe.

Conclusions

In conclusion the riparian forest of Lake Trichonida is dominated by *Platanus* and *Salix* either in pure or mixed stands. Willow invasion into sycamore stands created mixed stands, in which willow appears with a potential to dominate the upper floors in the future. This shows a dynamic of predominance of 92A0 habitats against of 92C0. A big problem for the riparian forest of Trichonida is the absence of trees in the lower floor (absence of regeneration), absence of intermediate diameter classes mainly due to intense human activities. These pressures are changing the structure, undermining the natural dynamics and the future of the structure and composition of the riparian forest. As a result of the intense human presence, the current riparian forest that exists in Lake Trichonida, in western Greece, is noticeably shrunk and degraded. This is especially evident in mixed *Salix-Platanus* stands (ST3) where many diameter classes are absent between young and very old trees. The existence of the riparian forest maintains the rich biodiversity of the area, which gives it great ecological value and makes it imperative to immediately protect, recover and restore it with new plantings of riparian forest species (*Platanus*, *Salix*, *Populus*) in selected public areas around the lake. The problems identified in the structure and dynamics of the riparian forest must be the basis for the immediate preparation of a management plan, which provides for its demarcation, the limitation of human pressures (eig. illegal logging in site), for the recovery, restoration and preservation of the structure, dynamics and of the biotopes of the riparian forest of Lake Trichonida in western Greece with appropriate silvicultural measures.

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A brief study on the Grey wolf (*Canis lupus*) diet on the Northern slopes of Central Stara Planina Mts, Bulgaria

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Abstract

The diet of the Grey wolf (*Canis lupus* Linnaeaus, 1758) in the mountainous region of central Bulgaria was studied. Based on 200 excrements collected and analyzed, it was found that among wild ungulates, remains of red deer *Cervus elaphus* were the most frequent (20.9 %), followed by roe deer *Capreolus capreolus* (15.6 %) and wild boar *Sus scrofa* (12.6 %). The most preferable prey for wolf was the horse *Equus caballus* (33.5 %), and domestic ungulates consumption included also cattle (7.28 %). In the studied region, the consumption of horse biomass (34.3 %) was also the greatest, followed by that of roe deer (19.2 %) and red deer (18.0 %). Rodents, sheep, hares and wild cats are considered additional food sources for the species. It can be concluded that the studied near-natural habitat offers the wolf a diverse food base and that it benefits approximately equally from wild and domestic ungulates.

Key words: excrements, food, livestock, predator, victims, wild ungulates.

Introduction

The Grey wolf (*Canis lupus* Linnaeus, 1758) is the second largest predator found in Bulgaria, primarily in mountainous regions, which comprise one-third of the country's territory. Although the species has faced intensive hunting in the past, it has not been severely eradicated due to conflicts with humans (Mihaylov and Stoyanov 2012). According to the IUCN Red List, the Grey wolf is considered Least Concern and can be hunted throughout the year. The study of its feeding behaviour is crucial for understanding its role as a regulator of prey populations and resolving conflicts with gamekeepers and farmers. The wolf primarily attacks domestic animals in areas with developed animal husbandry (Vos 2000, Sidorovich et al. 2003, Migli et al. 2005, Nowak et al. 2005, Russo et al. 2014). This behaviour is often linked to the abundance of its natural prey, such as wild ungulates (Sidorovich et al. 2003, Zlatanova et al. 2014). The availability of food and migra-

tion patterns of its natural prey influences the wolf's behaviour towards livestock (Nelson et al. 2016). The wolf is an opportunistic species that takes advantage of the most available food sources to survive (Meriggi and Lovari 1996). It also relies on carrion from large ungulate game, such as American bison in North America (Jung 2011), cows, and domestic pigs in Europe (Jędrzejewski et al. 2002). The wolf is considered a facultative scavenger occupying the food resource found (Wirsing and Newsome 2021). It is well known that in the specific conditions of Bulgaria, the wolf is hunted by attracting with carrion, especially in the Eastern Rhodopes Mountains (Mts), Strandzha Mountain (Mt) and Sakar Mt.

Its feeding behaviour in Stara Planina Mts is poorly studied. The current study relies on fecal sample analysis, a non-invasive tool in line with modern ecological trends. Information on the wolf's diet in Bulgaria is limited to the Osogovo Mt (Dolapchiev 2019) and the Western Rhodopes Mts (Georgiev et al. 2008).

The aim of the present study was to investigate the feeding behaviour of the Grey wolf (*Canis lupus*) in a near-natural habitat – the central part of Stara Planina Mts, Bulgaria.

Material and Methods

Study area

The study was conducted on the northern slopes of the Central Stara Planina Mts (Fig. 1), centered around N 42°49'59.83'' E 25°00'20.74'', 582 m a.s.l., covering an area of 784 km^2. It included the State

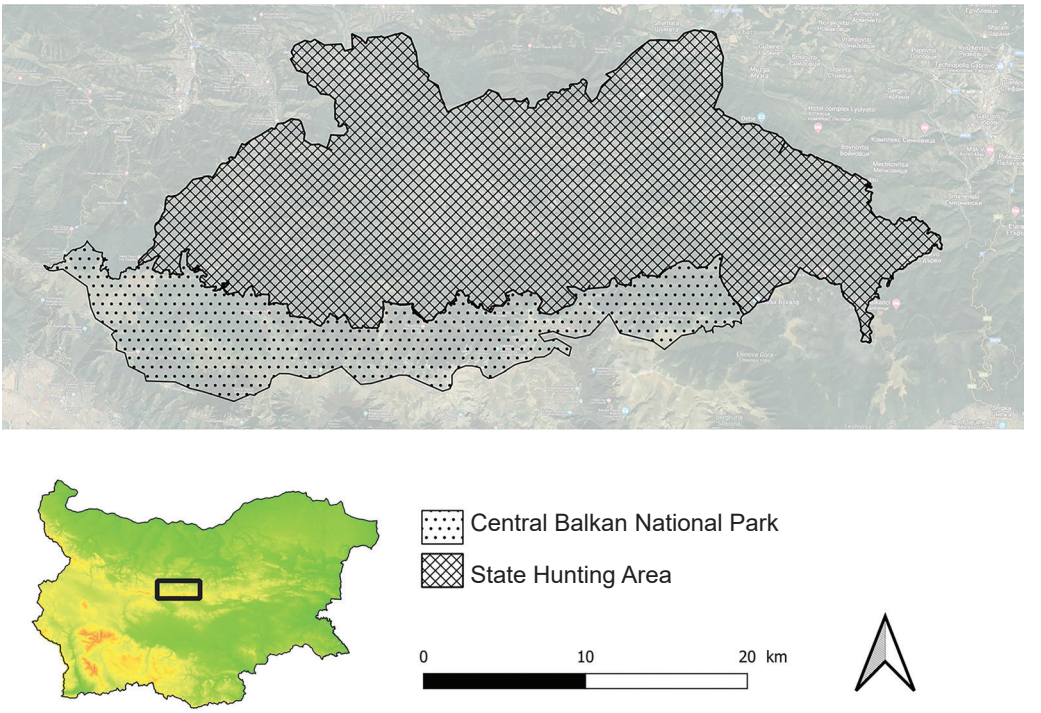

Fig. 1. Study area.

Hunting Enterprises 'Rusalka' and 'Rositsa' to the north and a part of the Central Balkan National Park to the south.

Big game, including red deer (*Cervus elaphus* L.), roe deer (*Caprolus capreolus* L.), wild boar (*Sus scrofa* L.), and in the highest parts, wild goat (*Rupicapra rupicapra* L.) is keeping on the territory. The area above both game preserves and lying on the main mountain ridge constitutes the Central Balkan National Park, where hunting is prohibited and the animals are not disturbed. Most of this area (above 1300 m a.s.l.), consists of meadows used for grazing by cattle (*Bos primigenius taurus* L.), horses (*Equus ferus caballus* L.) and sheep (*Ovis aries* L.) from May to September. The latter are guarded by shepherds and dogs, while some cattle herds are left unguarded by their owners and the horses are completely free-ranging. During spring, when cubs are born and reared, the wolf occupies isolated areas (Mech and Boitani 2003, Spassov 2007, Spiridonov and Spassov 2015). At that time, various activities, such as logging and game management, are carried out in the State Hunting Enterprises. At the beginning of summer, most wild ungulates are concentrated in this so called 'calm zone', around the upper boundary of the forest.

Sample collection and processing

One of the most widely applied non-invasive methods for determining the carnivore's diet is the analysis of undigested plant and animal components in their excrements (Putman 1984). The accuracy of the obtained results for each prey species increases significantly when more than 200 feces are collected as a representative sample for the wolf's diet (Marucco et al. 2008).

A total of 200 wolf excrements were collected between October 2017 and October 2020 along forest roads and animal paths in their habitats. The transects were cleared out of already deposited excrements before the first sampling, in order to start collection of fresh samples after a month. The feces were sampled every month during the study period. The morphology and specific odor of the samples were taken into account to determine species affiliation. Laboratory procedures followed those described by Reynolds and Aebischer (1991) and Ciucci et al. (1996). The samples were heat-treated in autoclave for 90 min, washed under running water through a 2 mm sieve, and then air-dried at room temperature. Electronic scales were used to weigh each food component up to 0.01 g for estimating biomass consumed. Undigested food items were hand separated, as it is widely used in quantitative fecal analysis (Ciucci et. al 2004). Undigested remains of mammalian bones, teeth, hoof, and hairs were identified by referring to collections of specimens from the study area deposited in the Museum of Natural History and Trakia University, Bulgaria. Most of the food items found originated from small number well distinguishable large ungulates by hairs' structure. For this reason a microscopic analysis of hairs wasn't applied. The help of a specialist (E.R.) in hairs' discrimination at a species level was used. The macroscopic hairs' characteristics (thickness, shape and color) were estimated under a 10x lens. Unspecified elements did not exceed 2 % of the total amount and were excluded from the analysis. The relative frequency of occurrence (RFO %) of each food component was calculated by dividing the number of occurrences of a particular food component by the total number of all food components. Components with

RFO values of up to 1 % in the samples were excluded from the analysis. The importance of easily digestible food items however, may be underestimated by this method (Klare et al. 2011). To complement this method (Okawara et al. 2018), the weight ratio of food components was also calculated, using the following regression equation: $y = 0.439 + 0.008x$, where y is the air-dry weight of prey remains in each excrement sample and x is the sought ingested and digested biomass (kg), as described by Weaver (1993).

It was possible to distinguish between young individuals (juveniles) and those over 1 year old of roe deer by the size of hooves found in the samples (Fig. 2).

Results and Discussion

Losses of game, livestock or crops could be of great economic value. Revealing feeding behaviour of medium-sized and large carnivores is useful for understanding their 'economic food niche' (Litvaitis 2000).

A total of 194 samples wolf's feces (97 %) contained a single component and 6 samples (3 %) contained 2 components. Of all two-component samples, only one contained traces of a third species (hedgehog fur), which was most likely ingested with the stomach contents of a first-order victim, and was thus excluded from the analysis. This is consistent with the trend found by Wagner et al. (2012). Smaller animals are often swallowed whole by predators (Jędrzejewski et al. 2002), thus untypical food components could be ingested.

Horses (RFO = 33.5 %) were the primary food source for wolves in the Stara Planina Mts, followed by red deer (RFO = 20.9 %), roe deer (RFO = 15.6 %), and wild boar (RFO = 12.6 %). Cattle, rodents and sheep represented an additional food source (Fig. 3). The wolf consumed hares and wild cats as an exception.

When dividing the wolf's food into

a) **b)**

Fig. 2. Remains of Roe deer: juvenile (a) and adult (b) found in wolf excrements in the Central Stara Planina Mts, Bulgaria.

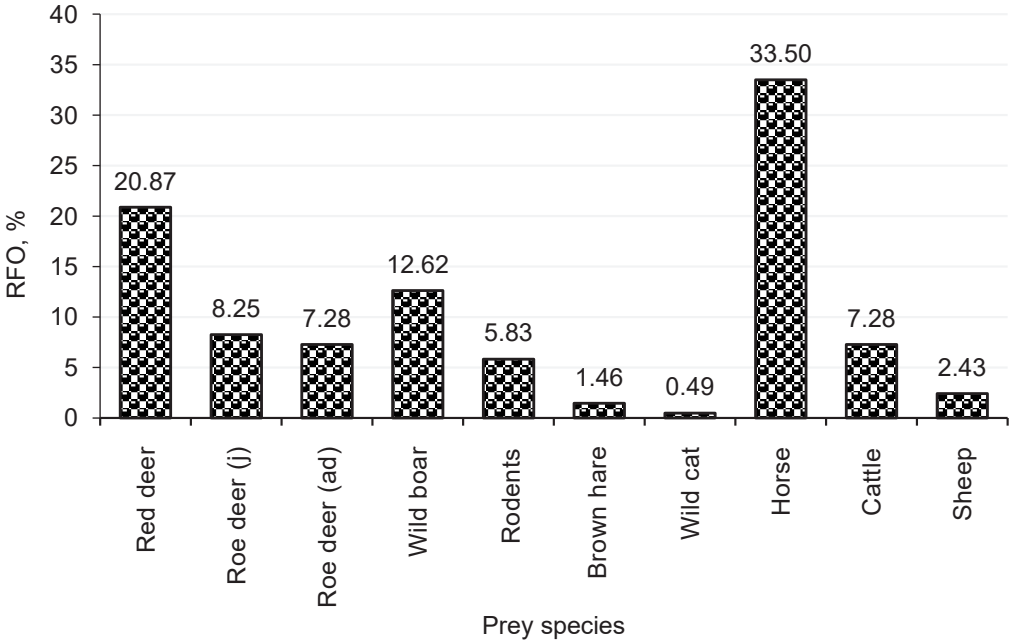

Fig. 3. Relative frequency of occurrence of prey species detected in the excrements of wolves in the Central Stara Planina Mts, Bulgaria.

natural and anthropogenic sources, the following ratio was obtained: 56.9 % natural and 43.1 % anthropogenic (mainly livestock). In southern Europe, red deer consumption is similar to that of roe deer and wild boar in the wolf's diet, when all three species are numerous (Meriggi et al. 2011, 2015). If the wild boar population is reduced due to African swine fever (ASF), the wolf switches to cervids (Klich et al. 2021). In Sikhote-Alin, the wolf's diet primarily consists of wild ungulates, mainly *Cervus elaphus xanthopygos* (Gromov 1979).

Roe deer were found in lower relative frequency of occurrence (15.6 %) in the wolf's diet compared to red deer, with juveniles predominance (Fig. 3), a trend already described for cervids by Wagner et al. (2012). In the Western Rhodopes Mts, the wolf preys on roe deer as red deer are scarce, (Genov et al. 2008, Georgiev et al. 2008). Similar feeding strategy has been reported for the Osogovo Mt (FO = 72 %; Stancheva 2004). In other Bulgarian mountains, roe deer consumption is significantly lower: in the Pirin Mt – 18.4 % (Dutsov et al. 2022), in the Kraishte Mt – 11 % (Dutsov et al. 2004), and in the Western Rhodopes Mts – 38.5 % (Georgiev et al. 2008, Genov 2015). In central and western Poland, the species occurs in the wolf's diet at a high rate, 42.8 % (Nowak et al. 2011), reaching 56 % in southeastern Estonia (Valdmann et al. 1998). In this research, the yearlings' remains were prevalent (RFO = 8.3 %) among those of roe deer (RFO = 7.3 %) found in the wolf's diet. A similar trend for both roe deer and red deer was described by Wagner et al. (2012).

The wild boar was the third most common wild ungulate found in the samples (RFO = 12.6 %; Fig. 3). This is probably

due to the wolf's preference for sufficiently large victim at lower risk, such as young roe deer and red deer. Wild boar and red deer are of different importance in the food of the wolf in Italy depending on a particular year (Marucco et al. 2008). Wild boar was the main food resource for the wolf in the Bulgarian part of the Osogovo Mt located in the western Bulgaria (Dolapchiev et al. 2022). The wild boar forms noisy herds, which are easily detectable by the wolf. It is the main food for the wolf in Italy (Mori et al. 2017), in Anatolia, Turkey (Mengüllüoğlu et al. 2019) and in other places where it is abundant. In Spain, the boar consumed biomass was found to vary greatly – 3–31 %, with offspring being the predominant prey. However, the wolf's contribution to reducing the number of this problematic ungulate species is considered insignificant compared to hunting (Nores et al. 2008). Predation by the wolf is thought to mitigate human-wild boar conflict (Mengüllüoğlu et al. 2019). The less frequent consumption of wild boar in the study area is likely due to the outbreak of African swine fever during the sampling period. The origin of the wild boar remains found in the wolf feces in Bulgaria is equivocal, as it could have come from active predation or scavenging. It is common for remains from hunter harvests to be left in Bulgarian nature.

The horses were found to be the most prevalent prey in the wolf diet in the Central Stara Planina Mts, both in terms of occurrence and ingested biomass (figs 3 and 4). Newborns and foals make up the majority of the predator's food during the snow-free period of the year, due to their availability (as per B. Koychev's personal observation). Wolves tend to be attracted to horses when the two species coexist. In northern Portugal wolves feed primarily on horses when herds graze freely in the mountains (Vos 2000). Similarly, in central Asia (Altai), horses are consumed more frequently than cows (Bondarev 1985), as seen in Mongolia (Hovens and Tungalaktuja 2005, Nakazawa et al. 2008, van Duyne et al. 2009), consistent with the findings in this study.

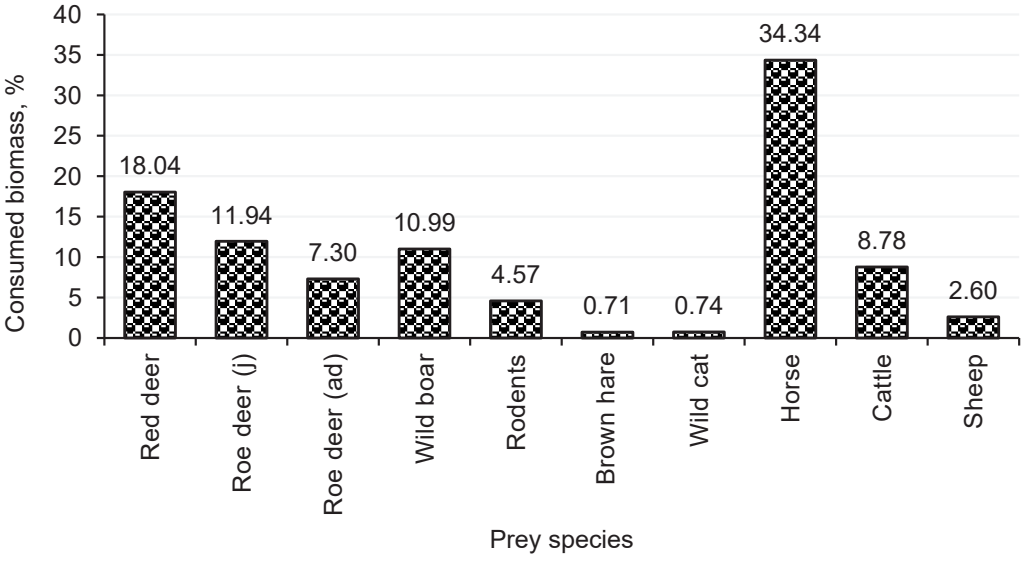

Fig. 4. Relative proportion of wolf's prey according to biomass consumed.

Cattle were reported to be attacked less frequently and mostly served as a buffer prey, along with medium-sized mammals (Sidordvich et al. 2003, Velázquez et al. 2022), consistent with our results (figs 3 and 4). In the study area, cattle are herded to farms at the foot of the mountain for the winter, making them a buffer food resource with temporary access. However, in cases of year-round pasturing, their occurrence in the predator's diet increases (Merkle et al. 2009). Sheep are scarce in the study area, as they are traditionally bred in the lowlands. Medium-sized mammals such as hares, wild cats, and rodents, are not of a particular nutritional value for the wolf and were rarely found in excrements (Fig. 3) with the lowest biomass ingested (Fig. 4). In the Western Rhodopes Mts, the wolf mainly feeds on wild ungulates, both in terms of frequency of occurrence and ingested biomass (Georgiev et al. 2008).

The wolf's diet in our study consisted of more than 40 % livestock (Fig. 5). The predator attacks primarily the freely grazing foals on the highland meadows, while rarely calves. Sheep are accidental prey for the wolf as herds are accompanied by shepherds and dogs during the day, and herded into pens at night. This foraging strategy has been described in previous studies in Bulgaria (Spassov et al. 1999, Spassov 2007). In comparison, wolves in Kazakhstan consumed 79.3 % livestock (Grachev 1979), and in Mongolia, 70 % (Hovens and Tungalaktuja 2005, Nakazawa et al. 2008, van Duyne et al. 2009). In our country, however, the farm animals' consumption is not always a result of ac-

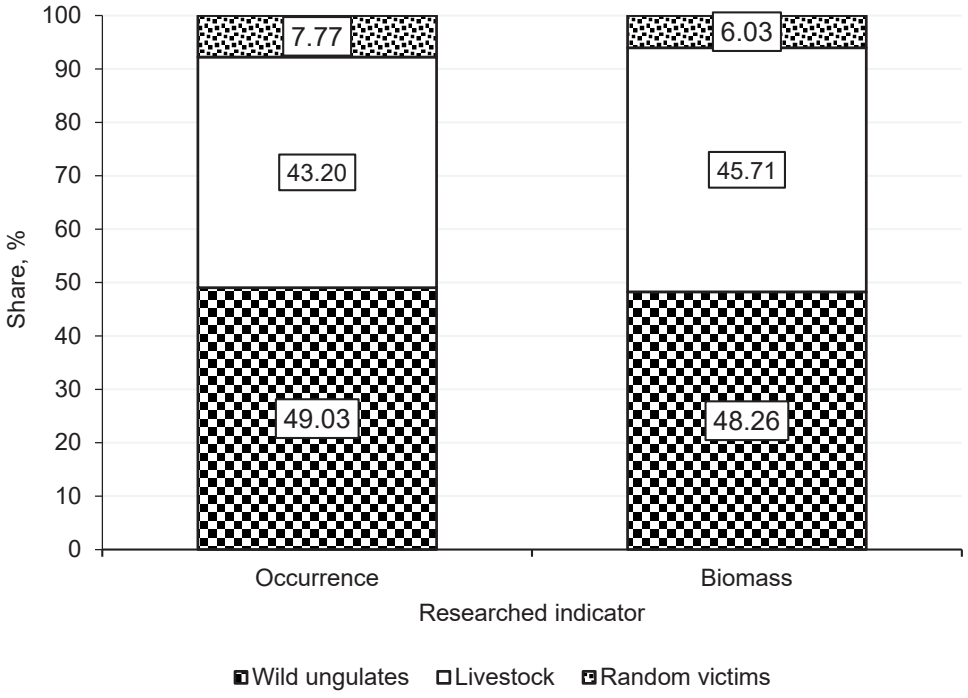

Fig. 5. Main food groups ratio in the wolf's diet in Central Stara Planina Mts, Bulgaria.

tive predation. Commonly, hunters attract wolves for shooting by setting livestock leftovers or whole animal carcasses. Thus, the damage caused by wolves on livestock is difficult to determine through their feces. In North America, cattle make up more than 22 % of the wolf's diet, in places with carcass discarded (Petroelje et al. 2019). It is assumed that the presence of livestock in the wolf's diet in Central Stara Planina Mts is due to the large number of grazing animals in the 'Central Balkan' National Park and the presence of hunting farms nearby. Unlike in North America, where wild prey provides for the wolves' nutritional needs, with waste and domestic animals being consumed only in isolated areas (Peterson and Ciucci 2003), in North Caucasus, domestic animals were found in 94.5 % of samples (Bibikov 1985). In Portugal, 84.7 % of the ingested volume was made up of livestock (Roque et al. 2001) occurrence.

Wild ungulates made up the other half of the wolf's diet in the study area, both in terms of occurrence (49.0 %; Fig. 5) and ingested biomass (48.3 %; Fig. 5). Wolves in Lithuania specialize in preying on wild ungulates (Špinkytė-Bačkaitienė and Pėtelis 2012), and in Italy, the use of this food source by wolves increased from 1976 to 2004, when it became a preferred prey over livestock when it was plentiful (Meriggi et al. 2011). The reason for this may be that wolves try to avoid confrontation with humans and remain unnoticed. In Alpine, Apennine and Mediterranean ecosystems the consumption of wild ungulates reaches 89.4–95.1 % (Capitani et al. 2004). The additional food groups were found at low frequencies and quantities, representing 7.8 % and 6.0 % respectively (Fig. 5). No remains of domestic ungulates were found in the wolf's diet in near-natural habitat in Mongolia. Such habitats are believed to limit predation on livestock due to the presence of diverse wild fauna (Tiralla et al. 2021).

Conclusion

The near-natural character of the Central Stara Planina Mts habitat determines the approximately equal consumption of wild and domestic ungulates by the wolf, known as typical large hypercarnivore mammal.

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Influence of local vegetation on soil properties in riparian habitats along the Iskar River in the region of Sofia, Bulgaria

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Abstract

The aim of the present study is to trace the correlation between major types of local riparian vegetation and soil properties under their slope in a section along the Iskar River. The objects of study were Alluvial Fluvisols spread on the territory between Plana and Lozenska Mountains in the area of Kokalyane village, Sofia region. In order to investigate the influence of vegetation on soil properties, 3 representative sample plots were defined with vegetation in the initial stage, composed respectively of annual grass communities in SP-1, in an advanced stage composed of perennial grass communities in SP-2 and in the final stage – a tree community with a distinct tree floor in SP-3. The obtained data show a significant soil properties alternation depending on the specifics of plant communities. The increase of soil organic matter under the different types of vegetation follows the natural successional order – from an initial stage with annual grass communities to stable tree communities with a clearly defined floor structure. The content of soil organic matter, total N and plant available water capacity are the most reflective indicators showing the importance of plant communities for the formation and changes of soil fertility. The statistically proven differences in the values of these indicators point out the role of vegetation in soil formation processes.

Key words: C:N ratio, Fluvisols, pH, plant available water capacity, plant communities, soil organic matter.

Introduction

Riparian soils and vegetation are important factors influencing the biodiversity and biogeochemical processes of river ecosystems (Zhao et. al. 2020). Vegetation along river banks not only protects soil from erosion, but at once affects both changes in soil and river indicators and properties (Dosskey et al. 2010). Studies of riparian ecosystems in different parts of the world report correlation between soils characteristics and type of vegetation established on them.

Investigation of Rhoades et al. (2001) on Mountain Alder (*Alnus viridis* Chaix subsp. *crispa*) influence on soils in ecosystems of the Agashashok River valley, northwest Alaska, shows that the Alder significantly increases exchangeable soil

N in each ecosystem. Jiao et al. (2014) have registered changes in soil nutrients content and soil structure under different vegetation types in the Yellow River Delta (China). Results from the assessment of changes in vegetation structure and soil properties, following riparian restoration, show that floods redistributed organic matter, with this effect depends on the vegetation also the probability of organic matter retention was positively correlated with groundcover and stem density of plants (Halea et al. 2018). In the study of riparian soils and their response to environmental factors (need space and height of trees, shrubs and grass, distance to river and altitude), Zhao et. al. (2020) found that soil properties like clay content, soil organic carbon and nitrate nitrogen in riparian zones were significantly affected by vegetation type.

Bulgarian studies analysing specifically the correlation between changes in vegetation along river banks and changes in soil indicators are relatively few in number. As a result of a study by Kachova et al. (2020) on the riparian soils in a section of the Iskar River and their correlation with the distribution of *Impatiens glandulifera* Royle localities, 4 types of localities of this invasive alien species have been identified. Research by Georgieva (2021) on the dynamics and growth of *I. glandulifera* in riparian habitats proves the positive correlation between soil organic matter, plant's height and fruit capsule length. More studies are focused on areas with artificial anti-erosion forest stands on eroded terrains along the Arda, Badinska and other Bulgarian rivers, which establish their role in improving soil fertility, soil physical properties and reducing surface water runoff (Velizarova 2008, Pavlova-Traikova et al. 2018).

Intense rainfalls and extreme flooding are very common in the riparian zone of Bulgaria. They have a negative impact on the environment and are also a disaster from a social and economic point of view. Analysing the interactions between soil and vegetation is a basis for determining the possibilities for restoration, protection and improvement of soil fertility and the development of vegetation as a condition for biodiversity protection, increasing the productivity of plant communities and rational use of natural resources.

The aim of the present study is to investigate the correlation between major types of local riparian vegetation and soil properties under their canopy within the area along the Iskar River.

Material and Method

The objects of the study were Alluvial Fluvisols (Penkov et al. 1992) occurring on the territory between Plana and Lozenska Mountains in the Kokalyane village area, Sofia region (Fig. 1).

The average altitude of the studied section is 650 m. The area is characterized by relatively mild winters, low annual temperature amplitudes and two rainfall maxima during the year (Georgieva 2021). The maximum average month temperature in July is 21.5 °C and the minimum in January is 1.5 °C. The predominant direction of the wind is northwest, and winter winds are strongest in December, January, February and March. The average annual rainfall is 590.7 mm with a maximum in May and a minimum in December. A second rainfall maximum is observed in June, and a second minimum in August. The duration of the snow cover is usually from the end of November to the end of March (Sabev and Stanev 1963, Topliyski 2006). Vegetation development

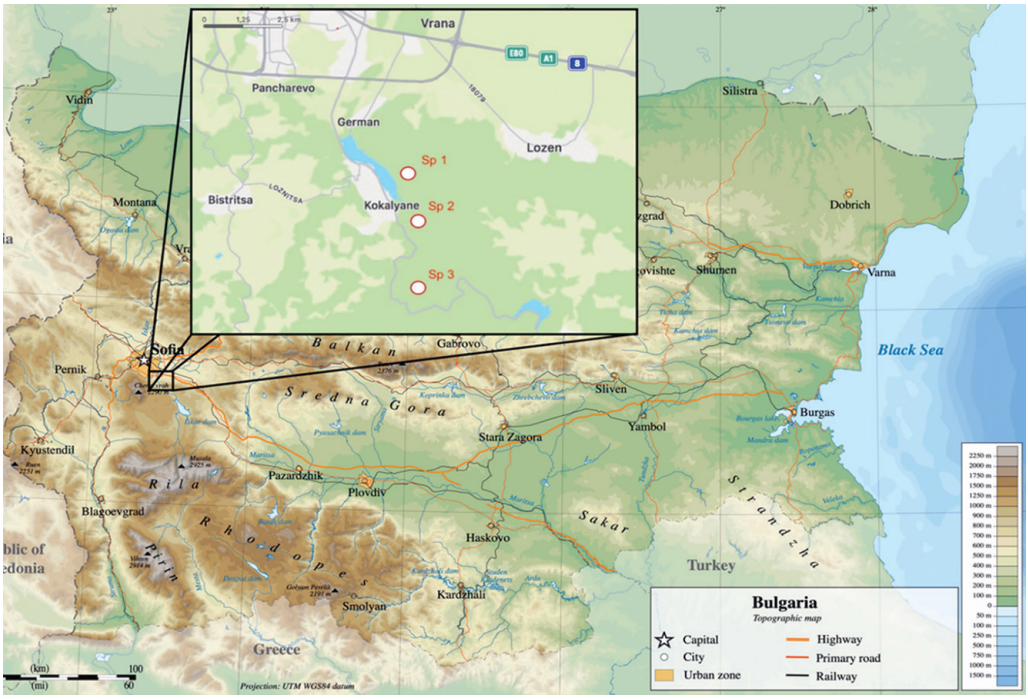

Fig 1. Location of sample plots (SP-1–3) in the study area.

limits surface runoff and erosion processes in the study area.

The characteristics of the soils correspond to those of the soil group Fluvisols in World reference base for soil resources (IUSS … 2015). At the next lower taxonomic level in the same classification model, the main qualifiers for the studied soils are Skeletic and Leptic.

The research site is part of Protected Area BG0000613 under NATURA 2000 and includes habitat 91E0 Alluvial forests with *Alnus glutinosa* (L.) Gaertn. and *Fraxinus excelsior* L. (Glogov 2021).

In order to investigate the influence of vegetation on soil properties, 3 representative sample plots (SP) were defined with vegetation in the initial stage, composed respectively of annual grass communities (AC) in SP-1, in an advanced stage composed of perennial grass communities (PC) in SP-2 and in the final stage – a tree community with a distinct tree floor (TC) in SP-3 (Table 1). The classification of plant communities in the sample plots is in accordance with the results of the studies of Glogov and Georgieva (2021) and Georgieva (2021).

Soil samples have been taken from five profiles in each plot from the layers 0–10 cm and 10–20 cm. The following methods were used to analyse the soil samples:

• Soil organic matter (SOM, %) by the Turin method;

• Total N (%) content, with a modified version of the classic Kjeldahl method;

• C:N ratio – calculation method;

• Soil acidity (pH in water extraction) – measured potentiometrical;

• Plant available water capacity (PAWC, mm), by a laboratory method,

Table 1. Sample plots data.

Sample plot	Geographic coordinates	Altitude, m	Soil type	Type of vegetation	Plant communities
SP-1	42°34'58.674'' N 23°25'39.212'' E	600	Fluvisols	Annual herbaceous communities (AC)	Ass. *Calystegio sepium-Impatientetum glanduliferae* Hilbig 1972, All. *Senecionion fluviatilis* Tüxen ex Moor 1958, Ord. *Convolvuletalia sepium* Tüxen 1950 ex Mucina in Mucina, Grabherr & Ellmauer 1993 and Cl. Galio-Urticetea Passarge ex Kopecký 1969
SP-2	42°33'35.6" N 23°25'21.4" E	650	Fluvisols	Perennial herbaceous communities (PC)	Ass. *Melilotetum albo-officinalis* Sissingh 1950, All. *Dauco carotae-Melilotion* Görs ex Rostański et Gutte 1971, Cl. *Artemisietea vulgaris* Lohmeyer et al. ex von Rochow 1951
SP-3	42°34'04.5" N 23°25'42.7" E	610	Fluvisols	Tree communities (TC)	Ass. *Stellario nemorum-Alnetum glutinosae* Lohmeyer 1957, All. *Alnion incanae* Pawłowski et al. 1928, Ord. *Fagetalia sylvaticae* Pawlowski in Pawlowski et al. 1928 and Cl. *Querco-Fagetea* Br.-Bl. et Vlieger in Vlieger 1937

with the calculation of field capacity and permanent wilting point.

The results of the soil properties study were statistically analysed using Statistica 12 and SPSS programmes. Mean, minimum (Min) and maximum (Max) values, standard deviation (SD) and coefficient of variation (CV) were determined. LSD Post-Hoc analysis (IBM SPSS 26.0 for Mac) was applied to prove statistically significant differences between indicators in individual sample areas. A significance level of $p < 0.05$ was chosen.

Results and Discussion

The field soil survey showed that the soils in all three test areas – with vegetation in the initial stage with annual grass communities (AC), in the advanced stage with perennial grass communities (PC) and in the final stage with tree communities (TC) have, in general, similar morphological structure of the soil profile, which is typical of class Fluvisols (Ilinkin et al. 2017, 2018). Genetic horizons have not been formed due to the periodic deposition of new sediments. The soil profile is composed of separate layers and a poorly formed surface humic horizon, which is in initial phase of development.

It was found that the chemical and physical properties of soils, as well as their morphological features, were changed because of the vital activity of plant communities, and the changes depend on their type. Above all, the formation of an organic O horizon with a low depth (between 2 and 4 cm) is observed in the soil under the tree

community (TC). Two layers were distinguished in its morphological structure – a fresh undecomposed plant litter and a humified layer, which is composed of amorphous humic substances and has a direct correlation to soil fertility, because it determines the content of soil organic matter.

A major source of organic matter in the soil under tree vegetation is the plant litter, which consists of leaves, twigs, bark, etc., and forms the organic O horizon in forest soils. In turn, it contributes to the accumulation of humus in the forest soils. The differences in the content of soil organic matter and total N depending on the nature of the plant cover are clearly expressed, both in the upper (0–10 cm) and in the lower (10–20 cm) soil layer. The mean content of soil organic matter in the soil in annual grass communities (AC) is 1.41 % in soil layer with depth 0–10 cm and 0.88 % in depth 10–20 cm (Table 2).

Table 2. Soil properties.

Sample plot	Depth, cm	Indicator	SOM, %	Total N, %	C:N	pH	Depth, cm	PAWC, mm
AC	0–10	Min	1.24	0.024	20.4	6.06	0–20	-
		Max	1.59	0.044	30.5	7.18		-
		Mean	1.41	0.035	24.4	6.54		-
		SD	0.16	0.008	4.2	0.52		-
		CV	0.11	0.229	0.2	0.08		-
	10–20	Min	0.77	0.016	21.1	6.21		27.0
		Max	0.98	0.027	28.4	7.29		32.0
		Mean	0.88	0.021	25.0	6.77		29.8
		SD	0.10	0.004	3.3	0.53		1.9
		CV	0.11	0.190	0.1	0.08		0.1
PC	0–10	Min	2.92	0.125	8.1	6.98	0–20	-
		Max	3.44	0.240	11.9	7.32		-
		Mean	3.26	0.208	9.2	7.20		-
		SD	0.20	0.048	1.6	0.15		-
		CV	0.06	0.230	0.2	0.02		-
	10–20	Min	1.49	0.092	7.9	6.91		46.0
		Max	2.17	0.135	11.3	7.48		56.0
		Mean	1.77	0.115	9.0	7.08		50.2
		SD	0.25	0.017	1.4	0.23		3.6
		CV	0.14	0.149	0.2	0.03		0.1
TC	0–10	Min	3.42	0.255	7.5	7.14	0–20	-
		Max	4.31	0.316	8.7	7.47		-
		Mean	3.81	0.281	7.9	7.32		-
		SD	0.36	0.023	0.5	0.12		-
		CV	0.09	0.082	0.1	0.02		-
	10–20	Min	2.00	0.120	8.6	6.98		50.0
		Max	3.45	0.210	10.2	7.36		63.0
		Mean	2.40	0.149	9.4	7.24		56.2
		SD	0.61	0.038	0.7	0.15		5.3
		CV	0.25	0.260	0.1	0.02		0.1

Note: SOM – soil organic matter, PAWC – plant available water capacity, AC – annual grass communities, PC – perennial grass communities, TC – tree community.

The mean content of soil organic matter in perennial grass communities (PC) is significantly higher in both layers, 3.26 % and 1.77 %, respectively. The increase in soil organic matter content in sampling site with tree vegetation (TC) is even greater. The mean value reaches 3.81 % in 0–10 cm soil layer and 2.40 % in layer 10–20 cm (Table 2).

The content of total N alters according to soil organic matter content. While in annual grass communities (AC) its mean value is 0.035 % in 0–10 cm soil layer and 0.021 % in 10–20 cm layer, in the perennial grass communities (PC) it increases in both soil layers to 0.208 % and 0.115 %, respectively, and even more increases in the tree community (TC) – 0.281 % and 0.149 %, respectively (Table 2). The predominant cover of *Melilothus officinalis* (L.) Pall., which is a species from the legume family (Fabaceae) is an additional factor of the higher nitrogen content in soils under perennial grass phytocoenoses (PC) compared to annuals (AC) (Van Riper and Larson 2009).

The obtained data show a difference in the ratio of the amount of total carbon to the amount of total nitrogen (C:N) in the soil depending on the type of plant communities. The ratio C:N expresses the degree of soil organic matter decomposition and helps to clarify the nature of the soil processes. Simultaneously high carbon and low nitrogen content is an indicator of slow organic matter mineralization, and conversely, in cases of faster decomposition of organic matter, the carbon content decreases and nitrogen content increases.

The values of the C:N ratio in the tree community (TC) and in the perennial grass communities (PC) were significantly lower compared to the soil in annual grass communities (AC), both in the upper 0–10 cm soil layer and in the lower 10–20 cm layer. The mean values in both soil layers in the tree community (TC) were 7.9 and 9.4 respectively, and in the sample area with perennial grass communities (PC) – 9.2 and 9.0, respectively (Table 2). The higher mean value of the C:N ratio in the lower soil layer in the tree community (TC) indicates that mineralization proceeds more slowly, and at the same time, that the humus is poorer in nitrogen. On the other hand, the insignificant difference of values in both soil layers in the perennial grass communities (PC) might be a consequence of more intensive flushing of the soil profile, in which mobile fulvic acids were transported to depth together with their associated nitrogen-rich compounds (Bogdanov 2022).

The significantly higher values of the C:N ratio in annual grass communities (AC) – 24.4 in 0–10 cm soil layer and 25.0 in 10–20 cm layer (Table 2), are an indicator of the lower intensity of the processes of organic matter mineralisation. This is in conformity with the differences in the microbiological activity of the studied soils in the tree vegetation and perennial grass communities, on the one hand, and in the annual grass vegetation in an initial stage on the other, which are determined by the influence of the microclimate on the vital activity of soil microorganisms.

It could be accepted that the organic matter composition and the basic cations migration are the main factors influencing changes in soil acidity. The lowest pH values were found in the annual grass communities (AC) – 6.54 in 0–10 cm soil layer and 6.77 in 10–20 cm layer. This could be explained by the removal of a part of the basic cations due to more intensive surface and soil runoff in the annual grass communities (AC). In the perennial grass communities (PC) the mean pH values are 7.20 in 0–10 cm soil layer and 7.08

in 10–20 cm layer, and in the tree community (TC) – 7.32 and 7.34, respectively (Table 2).

The plant available water capacity depends on the soil texture and the amount of soil organic matter, which are related to the plant development and type of vegetation. The organic O horizon formation with high moisture content under the tree vegetation further helps to increase the value of plant's available water capacity. This has been confirmed by the results of the present study. The value of this indicator is lowest in the annual grass communities (AC) – 29.8 mm, while in perennial grass communities (PC) it significantly increases to 50.2 mm and is the highest in the tree community (TC) – 56.2 mm (Table 2).

The statistical analysis of the results showed statistically significant differences between the three trial areas in terms of soil organic matter (Fig. 2), total N (Fig. 3) and plant's available water capacity (Fig. 4). Therefore, these are the indicators that reflect the influence of individual plant communities on soil properties to the highest degree.

The statistically proven significant differences of these indicators, which are used to assess the fertility of forest soils (Donov 1993, Bogdanov 2022), emphasize the importance of the vegetation as a factor of soil formation.

The lack of statistical significance in pH changes shows that the influence of vegetation on this indicator is poorer compared to other factors, among which the soil forming rock probably plays a leading role.

The properties of the studied soils changed in a great extent under the influence of vegetation and depend on the type of plant communities. The enrichment of soils with organic substances under different types of vegetation follows the natural successional order – from initial stage with annual grass communities to stable tree communities with a clearly defined floor structure.

Independent of the fact that plant com-

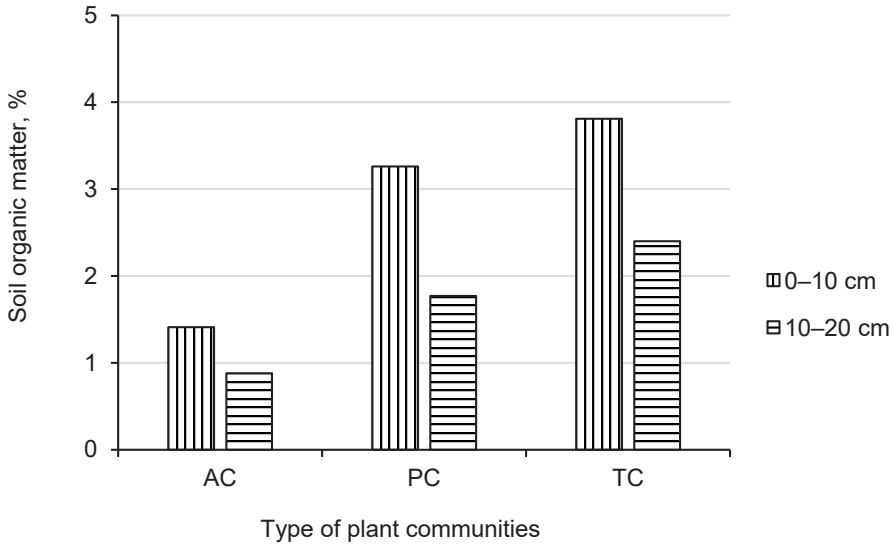

Fig. 2. Statistically significant differences ($p = 0.024 < 0.05$) in content of soil organic matter.

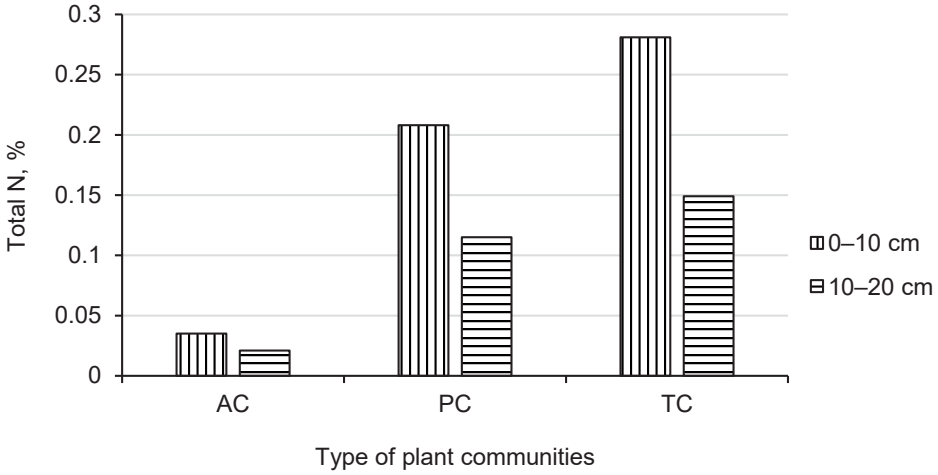

Fig. 3. Statistically significant differences ($p = 0.028 < 0.05$) in content of total N.

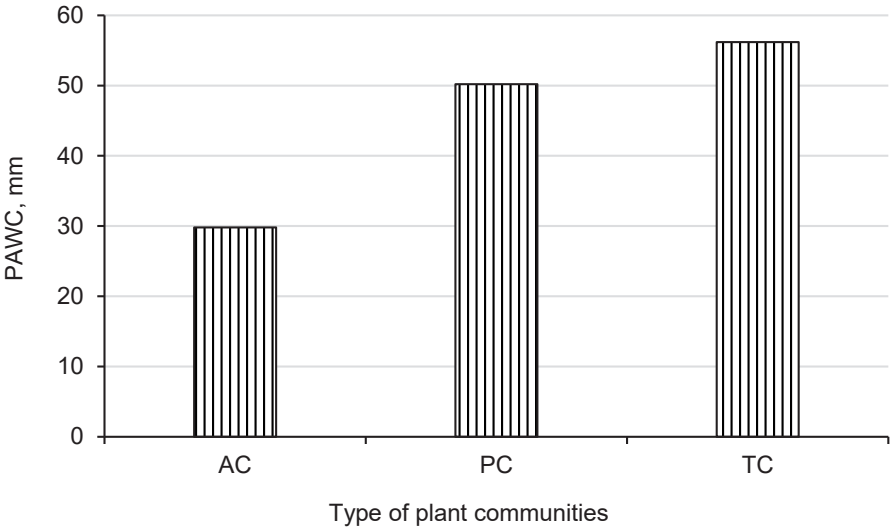

Fig. 4. Statistically significant differences ($p = 0.004 < 0.05$) in plant available water capacity (PAWC).

munities in all three trial areas are relatively young, and a large part of the nutrients entering the soil as a result of the synthesis and mineralisation processes of organic matter are spent on their growth and development, soil fertility improves in accordance with the stage of plant formation development. This largely depends on the organic matter accumulation in the surface horizon under the tree community and its transformation, which provides the reserves of soil organic matter, total N and plant's available water.

The positive influence of vegetation on

microclimate, depending on its characteristics, supports the vital activity of soil microorganisms, as a condition for intensive mineralisation of organic matter and synthesis of humic substances.

The changes in soil acidity (pH) reflect the influence of plant communities in accordance with their coverage and the composition of organic matter that they provide to the soil.

The content of soil organic matter, total N and plant available water capacity are the most reflective indicators showing the importance of plant communities for the formation and changes of soil fertility. The statistically proven differences in the values of these indicators point out the role of vegetation in soil formation processes.

Conclusion

The properties of the studied soils changed to a great extent under the influence of vegetation and depend on the type of plant communities. The enrichment of soils with organic substances under the different types of vegetation follows the natural successional order – from an initial stage with annual grass communities to stable tree communities with a clearly defined floor structure.

Independent of the fact that the plant communities in all three trial areas are relatively young, and a large part of the nutrients entering the soil as a result of the synthesis and mineralisation processes of organic matter are spent on their growth and development, soil fertility improves in accordance with the stage of plant formation development. This largely depends on the organic matter accumulation in the surface horizon under the tree community and its transformation, which provides the reserves of soil organic matter, total N and plant available water.

The positive influence of vegetation on microclimate, depending on its characteristics, supports the vital activity of soil microorganisms, as a condition for intensive mineralisation of organic matter and synthesis of humic substances.

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The content of soil organic matter, total N and plant's available water capacity are the most reflective indicators showing the importance of plant communities for the formation and changes of soil fertility. The statistically proven differences in the values of these indicators point out the role of vegetation in soil formation processes.

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Physical and mechanical properties of *Paulownia tomentosa* (Thunb.) Steud. wood under the conditions of the Western Forest-Steppe of Ukraine

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Abstract

The forest raw material base of Ukraine needs a long-term recovery, caused by current shortage of wood and in the long-term perspective, as well. One of the ways to solve this problem is to create plantations of *Paulownia tomentosa* (Thunb.) Steud., which are able to accumulate in a very short time a significant stock of wood, suitable for industrial use. Determination of the physical and mechanical properties was carried out on samples of standard sizes. It has been established that the density of paulownia wood, grown under the conditions of the Western Forest Steppe of Ukraine, ranges from 329–342 kg·m^{-3} (sapwood, heartwood) to 416 kg·m^{-3} (middle part between the sapwood and heartwood). Under the conditions when only air moisture influences the wood, the moisture content increases up to 12.9 %. Static bending strength, compressive strength parallel to grain, static hardness, modulus of elasticity of sapwood and heartwood parts of the trunk are characterized by minimum values, and the middle part (between the sapwood and heartwood) – by maximum ones. Therefore, in terms of appearance and texture, the wood has the most similarity with the wood of common ash; considering its density value, paulownia wood resembles the wood of Norway spruce and silver fir, but due to lower moisture absorption and the content of tannins, it is more resistant to decay than the mentioned species; the middle part according to some mechanical indicators is close to common pine wood, therefore, it can be widely used in building industry. *Paulownia tomentosa* wood is advisable to be used as a promising material for the production of firewood and wood pellets. The data we have obtained can be taken into account by specialists in the woodworking, furniture and agricultural industries when planning the creation of plantations and the use of its wood.

Key words: modulus of elasticity, moisture content, plantations, porosity, shrinkage, strength, swelling.

Introduction

In terms of the total area of forest resources and wood stocks, Ukraine belongs to the countries with insufficient forest resources. Forests on the territory of the country are situated unevenly – most of them are distributed in Polissia and the

Ukrainian Carpathians (Gensiruk 1992). Due to a number of geographical, historical and economic factors, the restoration of the forest resource base of Ukraine is long-term process. According to the latest data of the State forest cadastre of Ukraine, on the areas, covered by forests, young trees and middle-aged plantations dominate, while share of mature and over-mature ones has only 23–24 % in total. The reason for such distribution by age classes is the excessive felling of stands in recent decades and reforestation on the resulting logs.

The age of the main felling for most forest-forming species (common oak, pine, European (common) beech, Norway spruce and silver fir) is 80–100 years. In this regard, the main problem is not the predominance of young and middle-aged plantations, but a long period for cultivation of autochthonous tree species that causes a shortage of commercial timber now and in the future.

One of the ways to solve this problem is to set up plantations of fast-growing introducers, which are able to accumulate a significant supply of wood suitable for industrial use in a very short time (5–10 years) and do not pose a biological threat to native species and biodiversity. *Paulownia tomentosa* (Thunb.) Steud. is one of such potentially promising introducers for cultivation in Ukraine on an industrial scale.

The plant species is considered an invasive one in some countries and is not recommended for cultivation there (Jakubowski 2022). In Ukraine, since 2023, paulownia is prohibited for use in reforestation (Order No 184 of the Ministry of Environmental Protection and Natural Resources of Ukraine), but not prohibited for growing on plantations.

As a promising trend for the application of raw plant materials of *Paulownia tomentosa* is considered the medical and pharmaceutical industry, in particular as a source of anti-inflammatory and antibacterial herbal drugs (Darmohray and Lysiuk 2021, Sławińska et al. 2023).

Most species of the genus Paulownia are native to East and Southeast Asia – Japan, Korea, China, Laos, Vietnam (Vorobyov 1982, Zayachuk 2014). In Ukraine, *Paulownia tomentosa*, which grows quite quickly and whose wood has high heat transfer rates, can potentially be used as a raw material for the production of sawdust, biofuel, ethanol, etc. The effective duration of the use of its plantations with benefits for the economy and the environment comprises 20–50 years. After each felling of trees, which can be carried out on plantations regardless of the season at intervals of 4–8 years, the trees are regenerated with stump sprouts. With proper care, after 7 years, one can get 240–350 m^3 of high-quality wood from 1 ha, while the wood, which accumulates a large amount of tannins, is resistant to the effects of many pests and diseases.

The physical properties of wood are revealed during its interaction with the external environment and significantly affect the technological processes of wood processing. The main physical properties that are important for various technological processes of wood processing and largely determine its use comprise appearance, density, moisture, drying and swelling, thermophysical properties of wood (Ali et al. 2021, Nowak et al. 2021, Tomec and Kariž 2022).

The properties of *Paulownia tomentosa* wood are described in a limited number of scientific sources and contain rather contradictory data. The majority of scientific works highlight the problem of plantation cultivation and the conditions of culti-

vation of the wood species. Thus, Icka et al. (2016) describe the results of the cultivation of *Paulownia tomentosa* in the forest vegetation conditions of south-eastern Albania, indicating the rapid growth and high adaptability of the species to different climate conditions.

Sobhani et al. (2011) note that paulownia is a promising wood species for production of sandwich panels due to its low density (0.26 $g \cdot cm^{-3}$) and high strength to weight ratio of wood.

While investigating the morphology and anatomy of cells, the physical and mechanical properties of the wood of the new hybrid Green Paulownia, San et al. (2016) recommend the wood of the species as a material for the manufacture of furniture and cellulose production.

The density of hybrid paulownia (Shan Tong) wood, grown in Poland, as determined by Kozakiewicz et al. (2020), in an absolutely dry state is about 250 $kg \cdot cm^{-3}$. Researchers point to the high porosity of Paulownia wood (85 %) and consider it as a good thermal insulation material that has favourable resistance properties, characterized by a modulus of elasticity of 4.05 GPa.

Based on the studies of the physical and mechanical properties of Paulownia COTE−2 wood, grown in Granada (region of Spain), Lachowicz and Giedrowicz (2020) found that Paulownia wood is very light, its density is 270 $kg \cdot cm^{-3}$. The static bending was 38 MPa and the compressive strength parallel to grain was 24 MPa.

Cultivation of *Paulownia tomentosa* on plantations in Hungary has been practiced for the last 10–15 years. Koman et al. (2017) compared *Paulownia tomentosa* wood with poplar wood in terms of basic mechanical properties. These authors, after establishing the low density of paulownia wood (300 $kg \cdot m^{-3}$) and low indicators of its strength (static bending strength 42 MPa, compressive strength parallel to grain 22 MPa, tensile strength 33 MPa, impact strength 1.6 $j \cdot cm^{-2}$), don`t recommend it for the production of structural materials.

Akyildiz and Kol (2010) determined the mechanical properties of *Paulownia tomentosa* wood, grown in Turkey, in particular, the wood's static bending strength (43.56 $N \cdot mm^{-2}$), modulus of elasticity (4281.32 $N \cdot mm^{-2}$), compressive strength parallel to grain (25.55 $N \cdot mm^{-2}$). The authors of the study characterize paulownia wood as relatively light that has an increased resistance to decay, undergoes no deformation, without cracks, and they recommend it for the manufacture of furniture, decoration of cars, airplanes, as well as for the paper industry.

There is no information at all about the characteristics of *Paulownia tomentosa* wood, grown in Ukraine. Mainly, the scientific works of a number of researchers in the field of wood science are devoted to the study of the physical and mechanical properties of the wood of autochthonous and other introduced coniferous and deciduous species in Ukraine.

The study of the properties of paulownia wood, grown in the conditions of Ukraine, would enable to compare it with autochthonous species, give an assessment of quality and determine the prospects for use in the industry.

The object of the research is *Paulownia tomentosa* wood, grown in the conditions of the Western Forest Steppe of Ukraine.

The subject of the study comprises the physical and mechanical properties of *Paulownia tomentosa* wood, grown in the conditions of the Western Forest Steppe of Ukraine.

The purpose of the work is to investi-

gate the physical and mechanical properties of *Paulownia tomentosa* wood, grown in Ukraine, and to make an assessment of its suitability for the industrial uses.

To achieve this goal, the following main research tasks were planned: to determine the density, porosity, moisture content, shrinkage and swelling, strength of wood; make a comparative assessment of the physical and mechanical properties of *Paulownia tomentosa* wood with autochthonous species.

Materials and Methods

The 10-year-old *Paulownia tomentosa* model trees were harvested in the conditions of Lviv district of Lviv region. Wood samples of standard sizes for studying the physical and mechanical properties were prepared from selected model trees of paulownia, in accordance with the requirements of the applicable standards (Ugolev 1965, Leontiev 1970, Borovykov and Ugolev 1989). Test samples of wood were made from three different ridges within the trunk to detect changes in wood properties and selected along the trunk radius to determine wood properties within the diameter.

Determination of the physical and mechanical properties of the wood of *Paulownia tomentosa* was carried out on samples of standard sizes in the wood science laboratory at the Department of Botany, Wood science and Non-wood Forest Products of the Ukrainian National Forestry University on the universal electromechanical testing machine IR 5057-50.

The absolute and relative moisture content of the wood was determined, according to the standard (ISO 3129:2012).

Determination of wood shrinkage was carried out according to standard methods on samples, sizes 20×20 mm, 30 mm high with a tolerance of +1 mm, in which the annual layers are parallel with two margins of the selected samples on the transverse plane and perpendicular to two opposite borders. The selected wood samples were soaked in distilled water at room temperature to reach the saturation limit of the cell membranes for 25 days. Two repeated control measurements were carried out within three days. If the difference in measurements was less than 0.02 mm, the selected wood samples were considered saturated. Then the selected samples were dried for 48 hours at a temperature of 103 ±2 °C in a drying cabinet. Drying of wood samples was stopped when the difference between the control measurements of the samples was less than 0.02 mm. Dried wood samples were cooled in hermetically sealed vessels and their minimum dimensions were measured. The calculation of complete linear shrinkage in the radial, tangential directions and volumetric shrinkage was carried out, in accordance with the standard formulas (Ugolev 1965, Borovykov and Ugolev 1989, Vintoniv et al. 2007).

To determine the density of wood, standard samples of 20×20×30 mm were used, weighed to an accuracy of 0.001 g and their dimensions were measured with a digital caliper with a measurement accuracy of 0.01 mm. Determination of density indicators was carried out, according to the standard formulas (Barnett and Jeronimidis 2003, Vintoniv et al. 2007).

The wood porosity was determined in the basic and completely dry states, in accordance with the standard formulas (Barnett and Jeronimidis 2003, Vintoniv et al. 2007).

Determination of the compressive strength parallel to grain was carried out on a special device, in accordance with

the current standard (ISO 3129:2012). A wood sample measuring 20×20 mm and 30 mm high with a tolerance of +1 mm was uniformly loaded with force at such a speed that the destruction of the wood sample occurred after 0.5–1.5 min after the start of the load. The moment of failure of the test sample was recorded on a digital force gauge. The value of the compressive strength parallel to grain was determined, according to the standard formulas, with the further calculation to moisture content of 12 %.

Determination of the wood modulus of elasticity in static bending was carried out in accordance with the applicable standard. Wooden blocks 20 mm thick × 20 mm wide × 300 mm long were used for research (Poluboyarinov 1976). The samples were placed on two supports, which are symmetrically located relative to the middle of the sample's length. Efforts were directed tangentially to the annual layers. The samples were loaded to 800 N and smoothly unloaded to 300 N six times, measuring for 10 s deflection with an accuracy of 0.01 mm. The modulus of elasticity of wood in static bending is determined by the standard formulas with a conversion to 12 % moisture content.

Determination of the static hardness of wood was carried out on the device of A. H. Pevtsov. Wooden blocks 20 mm thick × 20 mm wide × 150 mm long parallel to grain were used for research (Poluboyarinov 1976). The static hardness of wood was determined on the radial plane of the sample by the size of the imprint through the copy paper from the three-time drop of a steel ball with a diameter of 25 mm from a height of 500 mm. The static hardness of wood was determined, according to the standard formulas, with conversion to 12 % moisture content.

Results and Discussion

The wood of *Paulownia tomentosa* is classified as quite valuable and is used for the manufacture of furniture, musical instruments, in shipbuilding and aviation industry, as well as in many other industries (Komán 2023) where there is a need for lightweight and light-coloured wood, with a beautiful texture (Fig. 1).

Fig. 1. The texture of *Paulownia tomentosa* wood.

As it can be seen from Figure 1, the wood of *Paulownia tomentosa* has a light colour from light coloured honey to steel gray shades with a characteristic silky shine. Among native species, in terms of appearance and texture, common ash (*Fraxinus excelsior* L.) wood is the most similar to *Paulownia tomentosa*.

Due to its relatively low density, *Paulownia tomentosa* wood has a soft surface. This facilitates the mechanical processing

of wood, but leads to the appearance of scratches during exploitation.

On the cross-section of the vessels of the secondary xylem of *Paulownia*, round or elliptical, mostly uniform medullary rays are clearly visible, some of which may have a spindle-like shape and well-developed parenchyma cells. Also, the presence of a small amount of tracheids is a feature of the wood of *Paulownia*. Libriform fibres appear in annual rings at the early stages of development and are arranged in vertical rows 550–1700 μm long (Ates et al. 2008).

We have conducted a number of studies of the physical properties of the *Paulownia tomentosa* wood. The obtained results are shown in Table 1.

Table 1. Physical properties of the *Paulownia tomentosa* wood.

Indicator	*N*, pieces	*M* ±*m*	±σ	*V*, %	*P*, %
Wood density, kg·m^{-3}:					
sapwood	100	329 ±3.99	39.87	12.1	1.2
middle part	100	416 ±7.49	74.88	18.0	1.8
heartwood	100	342 ±5.57	55.72	16.3	1.6
Shrinkage, %:					
tangential	100	3.6 ±0.05	0.46	12.8	1.3
radial	100	2.4 ±0.04	0.42	17.5	1.8
volumetric	100	5.9 ±0.07	0.77	13.0	1.3
Coefficient of shrinkage:					
tangential	100	0.12 ±0.002	0.02	16.7	1.7
radial	100	0.08 ±0.001	0.01	12.5	1.2
volumetric	100	0.20 ±0.004	0.04	20.0	2.0
Wood porosity, %	100	77.65 ±0.90	8.96	11.5	1.1

Note: *M* – average arithmetic value; *m* – error of the average value; σ – mean square deviation, *V* – coefficient of variation, %; *P* – accuracy of the experiment, %.

The data, presented in Table 1, indicate that with a relatively low wood density and a relatively high porosity index, the shrinkage of *Paulownia tomentosa* wood in both the tangential and radial directions is less than for most native timbers. So, according to the index of density, the wood of *Paulownia tomentosa* is similar to the wood of Norway spruce and silver fir, for which it is 410–460 kg·m^{-3}, respectively. However, the tangential shrinkage for these timbers is on average 7.6–7.8 %, and the radial shrinkage is 3.6–3.8 %, while for *Paulownia tomentosa*, according to our data, the tangential shrinkage is 3.6 %, and the radial one – 2.4 %. Practically it means that during drying of *Paulownia tomentosa* wood, it will change its linear dimensions and volume much less, which in turn reduces the manifestation of internal stresses in the wood and the appearance of such typical wood defects as shrinkage cracks and warping. When drying paulownia wood at high temperatures in drying chambers for 24–48 h, the moisture content can reach a level of 10–12 % without visible signs of deformation, warping and twisting of lumber.

We prepared cross-sections of *Paulownia tomentosa* wood and dried them in a drying cabinet at a temperature of 120 °C, as well as by the open-air method directly in the sun. After drying them, we did not observe any signs of warping or shrinkage cracks (Fig. 2).

a **b**

Fig. 2. The appearance of cross-sections of *Paulownia tomentosa* wood after drying: a) in the drying cabinet; b) in the open air.

The wood of deciduous species, in particular *Paulownia tomentosa*, is characterized by such a characteristic as moisture absorption upon repeated exposure to moisture after drying. We conducted a study to check the repeated impact of moisture on the wood of *Paulownia tomentosa* (Table 2).

Table 2. The results of research on the repeated influence of moisture on *Paulownia tomentosa* wood.

Day of observation	Number of samples	Average indicator			
		Initial moisture content of the sample, %	Moisture content of the sample after drying to a completely dry state, %	Moisture content of the sample after repeated wetting, %	
				in a desiccator on a grid over water	in a desiccator in water
1st	50	21.2	0	-	-
10th	50	-	-	11.6	46.2
20th	50	-	-	11.9	48.2
30th	50	-	-	12.9	51.9

As it can be summarized from the data in Table 2, in conditions where wood is affected only by moisture, present in the air, *Paulownia tomentosa* wood behaves atypically, compared to native timber species. During all 30 days of observation, the moisture content of samples, placed in a desiccator on a grid above water, increased only to a value of 12.9 %. Such a percentage is quite acceptable under

the conditions of constant exploitation of wood or products, made from it, and carrying out technological processes of wood processing and indicates the absence of the need for repeated drying of raw materials or products. Under conditions of complete immersion in water, *Paulownia tomentosa* wood, like most timber species, has achieved significant moisture absorption, but its feature is a relatively low indicator of the final value of wood moisture – 51.9 %.

Thus, the results of our research make it possible to recommend *Paulownia tomentosa* wood for using it in industries where the wood needs quick drying and application in conditions of significant influence of moisture in the air.

Paulownia tomentosa wood is recommended as a promising raw material for the production of firewood and pellets (Ryabchuk et al. 1996, Ryabchuk et al. 2000, Matskevych et al. 2019). Important aspects in the drying of firewood comprise the duration of drying and the optimal selection of the drying mode. In order to determine the duration of drying for *Paulownia tomentosa* wood, we selected samples of wood chips from two-year-old paulownia trunks, which were rejected from experimental crops during maintenance, and due to their sizes were suitable for using as a fuel (Fig. 3).

The selected samples were divided into two groups, uniform in quantity and composition and physical size, and dried under different drying modes: the first group – natural drying in an open space in the dried air mode, the second group – the dried air mode with preliminary drying in drying cabinet at a temperature of 90 °C. The results of the research showed that with the dried air mode of drying in sunny, dry weather, *Paulownia tomentosa* wood chips reach the optimal moisture content for burning after 7–9 days, and preliminary dried ones in a drying cabinet – after 3–4 days. The absence of any shrinkage cracks can also be considered a characteristic feature of this type of wood. In combination with the above-described property of wood not to re-absorb mois-

Fig. 3. Samples of *Paulownia tomentosa* wood chips after drying.

ture after drying, it can be considered a promising species for the production of wood fuel. However, its thermophysical properties require further study and clarification.

As it can be concluded considering data, presented in Figure 4, according to its main averaged values of the density, the wood of *Paulownia tomentosa* is close to Norway spruce and silver fir wood, but due to the content of tannins, it is more resistant to decay than the specified species. It should also be noted that the middle part of the trunk of *Paulownia tomentosa* by some indicators is close to the wood of Scots pine, and therefore, it can be widely used in construction.

The mechanical properties of wood by its very nature represent ability to resist the action of external forces that cause temporary or permanent deformations, and at extreme (maximum) loads destroy wood. The main characteristic of such resistance is the strength of wood, which is closely related to the concepts of compression, stretching and bending, the modulus of elasticity. The strength characteristics are necessary indicators for establishing the practical and economical use of wood in various industries.

We have conducted a number of studies of the main mechanical properties of *Paulownia tomentosa* wood, according to typical methods (Ugolev 1965, Poluboyarinov 1976) and the obtained results are given in Table 3.

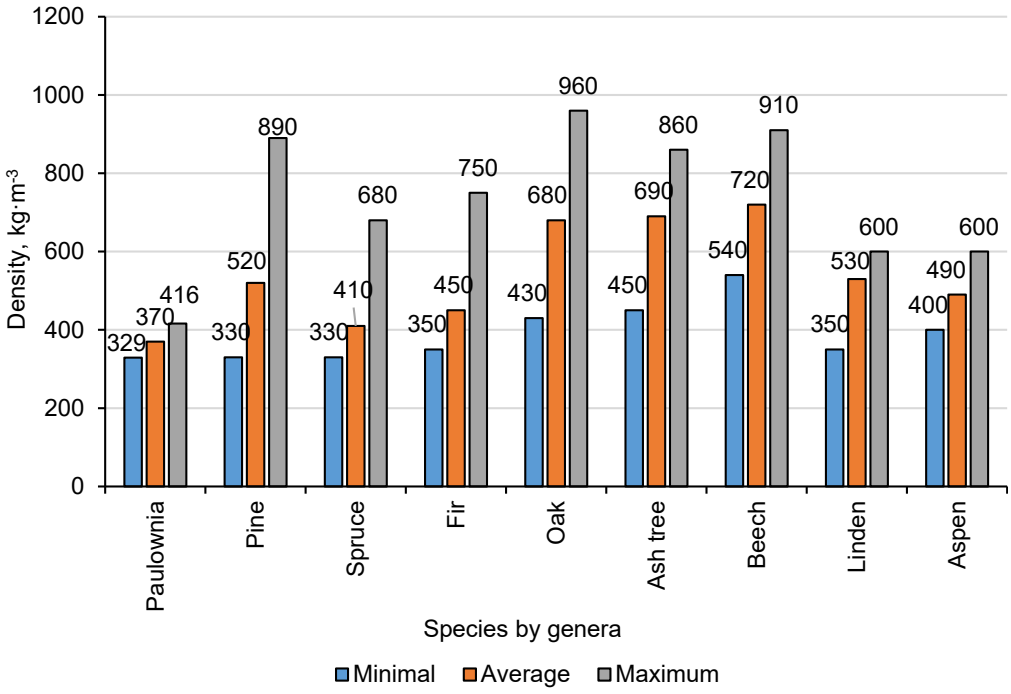


Fig. 4. Comparative data of *Paulownia tomentosa* wood density with other species.

Note: The data for all species, except *Paulownia tomentosa*, are adopted from EN 350:2016 (2016).

Table 3. The main mechanical properties of *Paulownia tomentosa* wood.

Indicator	*N*, pieces	*M* ±*m*	±σ	*V*, %	*P*, %
Compressive strength parallel to grain, N·mm^{-2}:					
sapwood	100	22 ±0.42	4.18	19.0	1.9
middle part	100	29 ±0.32	3.26	11.2	1.1
heartwood	100	18 ±0.29	2.87	15.9	1.6
Static bending strength, N·mm^{-2}:					
sapwood	100	26 ±0.60	5.95	22.9	2.3
middle part	100	72 ±1.79	17.93	24.9	2.5
heartwood	100	24 ±0.31	3.12	13.0	1.3
Static hardness, N·mm^{-2}:					
sapwood	30	11 ±0.54	2.96	26.9	2.7
middle part	30	46 ±1.92	10.53	22.9	2.3
heartwood	30	16 ±0.62	6.42	21.4	2.1
Modulus of elasticity, N·mm^{-2}:					
sapwood	100	5200 ±67.39	673.92	13.0	1.3
middle part	100	11450 ±274.46	2744.56	24.0	2.4
heartwood	100	7300 ±109.43	1094.27	15.0	1.5

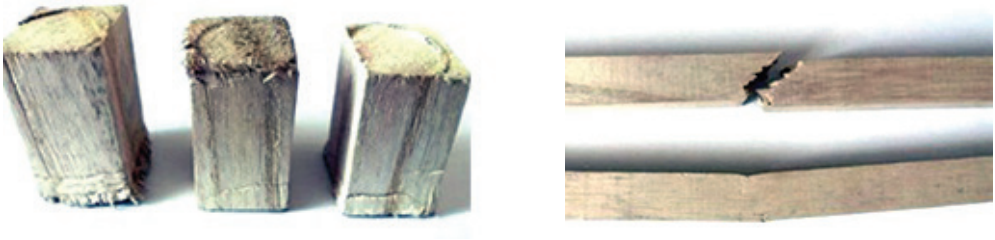
Note: *M* – average arithmetic value; *m* – error of the average value; σ – mean square deviation, *V* – coefficient of variation, %; *P* – accuracy of the experiment, %.

The data presented in Table 3 indicate that the main mechanical indicators of *Paulownia tomentosa* wood are not very high, which can be explained by wide annual growth rings, and as it is known, fast-growing tree species, mainly, have low indicators of the mechanical properties of wood. A characteristic feature of *Paulownia tomentosa* wood is a significant difference in the strength indicators between the sapwood and the heartwood, which are in a close range of values, and the middle part of the trunk, the strength of which is much higher, sometimes, as in the case of the static bending strength and hardness, even by a factor of three. It means that for further practical application it should be considered, that in products, which are subject to significant loads, is advisable to use only the middle part of *Paulownia tomentosa* trunk between the heartwood and the sapwood.

The tendency of a sharp change in the mechanical properties of *Paulownia tomentosa* wood within the diameter of the trunk is clearly visible when the samples are destroyed during tests (Fig. 5). Thus, when tested for static bending, the wood of the heartwood and sapwood parts gives characteristic sharp cracks, but the middle part of the trunk bends, but without sharp cracks, that confirms our recommendations for the practical use of *Paulownia tomentosa* wood in various products and constructions that require strength under static bending.

For more accurate and objective comparison of the properties of *Paulownia tomentosa* wood, we present its characteristics in comparison with native coniferous and deciduous species (Table 4).

Data presented in Table 4 reveal that *Paulownia tomentosa* is characterized by a sharp change in the mechanical properties of wood within the diameter of the trunk. Indicators of the main mechanical



a b

Fig. 5. Characteristics of damages of *Paulownia tomentosa* wood samples: a) compressive, parallel to grain; b) static bending.

Table 4. Comparative characteristics of mechanical properties of *Paulownia tomentosa* wood and autochthonous species.

Indicator	Wood species								
	Paulownia	Pine	Spruce	Fir	Oak	Ash tree	Beech	Linden	Aspen
Modulus of elasticity, $N \cdot mm^{-2}$	7700 (5200–11450) (heartwood – middle part)	12000	12500	11000	13000	13400	16000	9000	7800
Compressive strength parallel to grain, $N \cdot mm^{-2}$	22 (18–29) (heartwood – middle part)	55	44	47	61	52	60	48	33
Static bending strength, $N \cdot mm^{-2}$	46 (24–72) (heartwood - middle part)	100	95	73	88	120	123	98	56
Static hardness, $N \cdot mm^{-2}$	28 (11–46) (heartwood – middle part)	40	32	30	64	65	72	39	22

Note: The data for all species, except *Paulownia tomentosa*, are adopted from EN 350:2016 (2016).

properties of the heartwood and sapwood parts are characterized by minimum values, and of the middle part of the trunk (between the heartwood and sapwood) by maximum ones. So, if in terms of its main averaged characteristics, the wood of *Paulownia tomentosa* is close to the wood of aspen, then the wood of its middle part of the trunk is closer to the wood of conifers, which are widely used in construction.

Conclusions

In terms of appearance and texture, the wood of *Paulownia tomentosa* is the most similar amongst the native species to the wood of common ash. *Paulownia tomentosa* wood, in terms of density, resembles the wood of Norway spruce and silver fir, but due to the content of tannins, it is more resistant to decay than the mentioned species.

During drying, *Paulownia tomentosa* wood changes its linear dimensions and volume much less, which in turn reduces the manifestation of internal stresses in the wood and the appearance of such typical wood defects as shrinkage cracks and warping. The shrinkage of *Paulownia tomentosa* wood in the tangential and radial directions is less than for most native timber species.

When using *Paulownia tomentosa* wood in products that are subjected to significant loads, it is advisable to use only the middle part of the cross-section of the trunk between the heartwood and the sapwood. The middle part of paulownia wood is close to common pine wood, according to some indicators and, therefore, it can be widely used in construction.

The scientific novelty of the obtained research results is that there was no information in the analyzed printed and electronic publications about the physical and mechanical properties of *Paulownia tomentosa* wood grown in Ukraine.

The practical significance of the research results includes the data obtained by us, which can be further taken into account by specialists in the woodworking, furniture and agricultural industries when planning the creation of plantations and the uses of *Paulownia tomentosa* wood.

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Micropropagation of *Neolamarckia macrophylla* (Roxb.) Bosser to support *ex situ* conservation and breeding: multiplication, rooting, and acclimatization

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Abstract

This research aimed at obtaining the appropriate media for *in vitro* multiplication, rooting, and acclimatization, as well as seedling quality, for the development of red jabon through tissue culture technique. Two experimental designs were used in this study: a factorial design for multiplication and rooting stages and a completely randomised design for acclimatization stage and seedling quality. The results showed that Murashige and Skoog (MS) medium, Woody Plant Medium (WPM), and Driver Kuniyuki Walnut (DKW) medium responded well during the multiplication stage. *Neolamarckia macrophylla* plantlets from WPM had the highest percentage of survival rate. The appropriate acclimatization medium for *N. macrophylla* is compost and sand (4:3). The sturdiness of stem and seedling quality index of the two-month-old seedling has already fulfilled the requirement for replanting to the field. High multiplication frequency ensures the efficiency of this protocol. This tissue culture method can be applied for the large-scale production and sustainable conservation of *N. macrophylla*, a vulnerable species for agroforestry of coastal regions for windbreak and soil reclamations.

Key words: genetic conservation, *in vitro*, jabon merah, seedling, WPM.

Introduction

Jabon merah (*Neolamarckia macrophylla* (Roxb.) Bosser) is a fast-growing tree, currently a mainstay of the timber industry, including plywood, lamina wood, and other timber industries that have favourable cultivation prospects for the community (Hal-

awane et al. 2011, Qalbi et al. 2019). However, it is still cultivated using conventional techniques; therefore, its production is unable to fulfill the needs of *N. macrophylla* seedlings (Marzuki et al. 2016). Moreover, the shortage of seedlings might also be caused by limited fruiting season, varying offspring traits, and low seed quality produced (Nursyamsi 2010, Taiyeb and Baharuddin 2017). One effort to overcome the problem of seed supply shortage can be made through tissue culture techniques. Tissue culture is a widely used technique (Yelnititis 2014) and could also be used to promote conservation purposes. Conservation actions include *ex situ* conservation in gene banks and collections stored in the form of seeds, *in vitro* plant tissue, and calluses that have not been differentiated in liquid nitrogen (cryopreservation) (Herawan and Leksono 2018a).

Tissue culture techniques start from the preparation of plant material/explants to stages in the laboratory (induction, multiplication, and rooting stages), to acclimatization and planting in the field; these processes are vital in the production of tissue culture seedlings (Herawan and Leksono 2018a). Growing media is a major factor in the propagation of tissue culture and has a profound effect on the growth and development of explants and the seeds produced (Tuhuteru et al. 2012). Rathwell et al. (2016) revealed that there are three basic media for tissue culture that are often used for woody plants: Driver Kuniyuki Walnut (DKW) medium, Murashige and Skoog (MS) medium, and Woody Plant Medium (WPM). Growth regulators, which are non-nutritional organic compounds, are other essential factors to encourage, inhibit, or qualitatively change the physiological processes of plants if added in low concentrations (Khan et al. 2011).

Research related of *N. macrophylla* tissue culture has not been conducted much. Some previous studies used basic MS media. The study by Djumat (2014) found that multiplication in shoot buds did not occur, but multiplication in axillary shoot explants occurred, with the highest number of shots at 1 $mg \cdot L^{-1}$ 6-benzylaminopurine (BAP). The best treatment to induce shoots and leaf formation was a combination of 4 $mg \cdot L^{-1}$ BAP concentration and 0.3 $mg \cdot L^{-1}$ Indole Acetic Acid (IAA) (Marzuki et al. 2016), whereas the best treatment to induce callus from red leaf leaves was a combination of 0.3 $mg \cdot L^{-1}$ Naphthaleneacetic Acid (NAA) and 4.4 $mg \cdot L^{-1}$ BAP (Gusmiaty et al. 2015), and the highest shoot and leaf induction were observed at 1.5 $mg \cdot L^{-1}$ BAP and 0.1 $mg \cdot L^{-1}$ indole-3-butyric acids (IBA) (Taiyeb and Baharuddin 2017). Since previous studies only tested specific media or growth regulators, it is, thus, necessary to conduct exploratory research to compare several tissue culture media and various concentrations of growth regulators. Therefore, this study aims to compare some basic tissue culture media (i.e. MS media, WPM, and DKW media), growth regulators (i.e. BAP and IBA), and acclimatization media to obtain a suitable method of *N. macrophylla* tissue culture techniques from multiplication to acclimatization stage.

Materials and Methods

Place and time of research

The present study was conducted at the Laboratory of Tissue Culture of 2nd Regional of Seed/Seedling Forest Tree (BPTH Wilayah II, South Sulawesi, Indonesia).

Tools and materials of research

The plant materials used were the sterile apical buds of 2-month-old plantlets of *N. macrophylla*. MS media, WPM, and DKW media were evaluated in the present study. The other materials used were agar, BAP, IBA, sterile distilled water, 70% alcohol, 96% alcohol, 0.1 N HCl, 0.1 N NaOH, sand, compost, and husk charcoal.

Methodology

This research was conducted in four stages: multiplication, root formation, acclimatization, and seedling quality observation.

Multiplication

This stage was conducted with a factorial design. The first factor was basic media consisting of three levels (MS media (A0), WPM (A1), and DKW media (A2)). The second was BAP concentration consisting of nine levels (0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, and 2 mg·L^{-1}). The experiment was repeated eight times, thus totalling in 216 experimental plants. The observation was conducted for eight weeks. The parameters observed include time of initial shoot growth, number of shoots, height of the shoot, number of leaves, and conditions of culture.

Root formation

In this stage, the factors' basic media consisted of three levels (MS media (A0), WPM (A1), and DKW media (A2)), and IBA concentrations consisted of nine levels (0 (b0), 0.25 (b1), 0.5 (b2), 0.75 (b3), 1 (b4), 1.25 (b5), 1.5 (b6), 1.75 (b7), and 2 (b8) mg·L^{-1} BAP), were also considered. The experiment was repeated five times at each media level, resulting in 135 experimental units.

There are five plants in the observation conducted for six weeks with parameters: time of root growth, number and length of roots.

Acclimatization

In the acclimatization stage, the materials used were compost, rice husk, compost + sand (4:3), compost + sand + rice husk (1:1:1), and compost + rice husk (2:1). For each basic media (MS media, WPM, and DKW media), each combination of acclimatization media and basic media was repeated five times at each medium acclimatization, resulting in 75 experimental units. The observation was conducted for four weeks. The observation parameter was the percentage of survival.

Physical quality of seeds

To measure the quality of seedlings, height, diameter, dry root weight, dry leave weight, and dry stem weight at the age of 0 months or right after seedling removal to polybag, 1 month, 2 months, and 3 months with a sample of five seeds each were measured. Formula (1) was used to calculate the index (Dickson et al. 1960).

$$SQI = \frac{TW}{\frac{H}{RCD} + \frac{SW}{RW}}, \quad (1)$$

where: *SQI* is seedling quality index, *TW* is total weight (g per plant), *H* is height (cm), *RCD* is root collar diameter (mm), *SW* is shoot weight (g per plant), *RW* is root weight (g per plant).

Data analysis

The data obtained from observations in the analysis of variance and comparative

tests between treatments were analysed using Statistical Product and Service Solution 22.0 program, and if the treatment had a significant effect, Duncan's multiple range test (DMRT) with a 95% confidence level was conducted.

Results and Discussion

Multiplication

Multiplication is the process of multiplying prospective plants by planting explants in the media. The results of the analysis of variance at the multiplication stage are as follows.

Day after planting (DAP)

The analysis of results on the various basic media showed a very significant effect (p-value = 0.000), BAP concentration was significantly affected (p-value = 0.050), and the interaction between the basic media and BAP concentration had no significant effect (p-value = 0.149) on DAP.

The results of further tests on basic media using DMRT with 95% confidence level showed that WPM and MS media were not significantly different, whereas DKW media was significantly different. WPM responds the fastest, with a mean value of 7.46 DAP, and DKW media responds the slowest, with a mean value of 10.19 DAP (Fig. 1).

The results of further tests of BAP concentration using DMRT with a confidence level of 95% showed that 1 mg·L^{-1} BAP concentration was significantly different from other BAP concentrations and responded most quickly at 6.5 DAP. It is

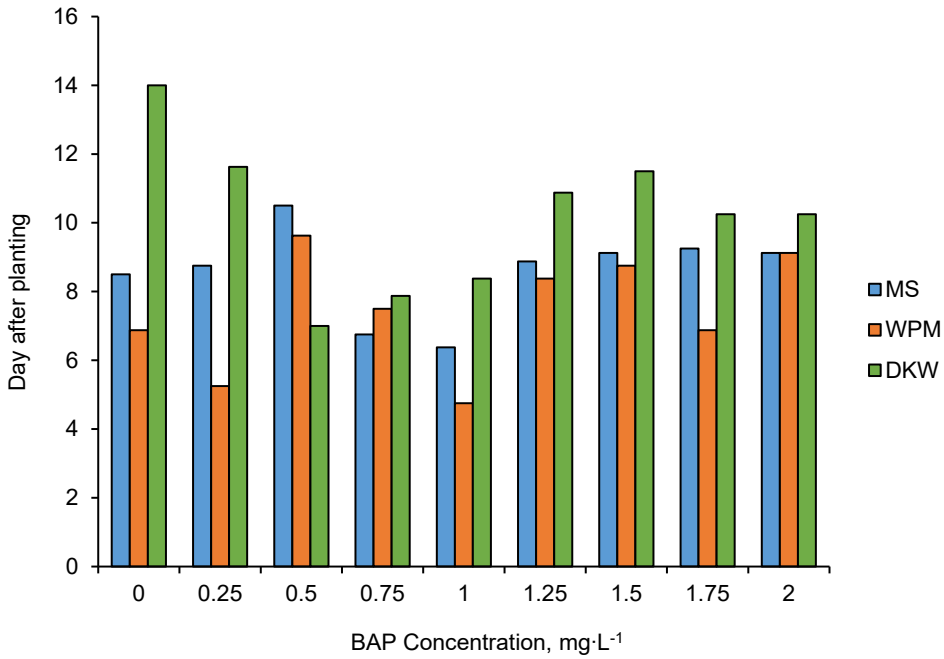

Fig. 1. Days needed for the explant to grow a new shoot after planting in different BAP concentration and planting media.

suspected that the endogenous cytokinins found in the explants have been able to encourage the formation of shoots and leaves; it requires 1 mg·L^{-1} BAP cytokinins to form buds and leaves of *N. macrophylla* explants. The results of this study were different from those of Putriana et al. (2019), in which MS basic media without addition of growth regulators was used and responded more quickly in comparison with the addition of growth regulators. The interaction between the basic media and BAP concentration had no significant effect, which meant that the basic media of MS, WPM, and DKW with addition of 0–2 mg·L^{-1} BAP were not significantly different even though the addition of 1 mg·L^{-1} BAP produced better result. In addition, the previous study demonstrated that WPM media provided better multiplication for *Syzygium densiflorum* Wall. ex Wight & Arn. due to its potential in organogenesis process.

Number of leaves

The analysis of results on the various basic media showed no significant effect (*p*-value = 0.195), BAP concentration had a very significant effect (*p*-value = 0.000), and the interaction between the basic media and BAP concentration had a very significant effect (*p*-value = 0.000) on the number of leaves.

The results of further tests of BAP concentration of DMRT with a confidence level of 95 % showed that 1 mg·L^{-1} BAP concentration produced the highest number of leaves with a mean value of 10.42 leaves, which was not significantly different from the addition of 1.5 mg·L^{-1} BAP, with a mean value of 10.17 leaves. The interaction between DKW basic media and 0.75 mg·L^{-1} BAP concentration produced a mean value of 13 leaves; however, the result was not significantly different from the interaction between MS basic media and 1 mg·L^{-1} BAP concentration, with a mean value 10.75 leaves. WPM basic media and 2 mg·L^{-1} BAP concentration produced a mean value of 12 leaves. It is assumed that DKW basic media, which already contains 52.1 µM·L^{-1} nitrogen (N) and 198.0 µM·L^{-1} manganese (Mn) was sufficient to increase leaf growth; thus, the addition of 0.75 mg·L^{-1} BAP was able to produce more leaves compared with MS basic media and 1 mg·L^{-1} BAP and WPM basic media and 2 mg·L^{-1} BAP (Fig. 2).

Number of shoots

The analysis of results of various basic media showed no significant effect (*p*-value = 0.260), BAP concentration had a very significant effect (*p*-value = 0.000), and the interaction between basic media and BAP concentration also had a very significant effect on the number of shoots (*p*-value = 0.000).

The results of further tests of BAP concentration using DMRT with a confidence level of 95 % showed that the addition of 1.5 mg·L^{-1} BAP produced the highest number of shoots with a mean value of 2.46 shoots. The average value of shoots produced on the media with the addition of BAP was higher by 2 times than in the media without addition of BAP.

This is in line with the previous study conducted by Widiastoety et al. (1997) which concluded that BAP administration in the media can stimulate the formation and multiplication of shoots from explants (tissue pieces). BAP was predicted to stimulate RNA synthesis and protein in various tissues which can further encourage cell division (Yu et al. 2019). In this case, BAP played a role as exogenous factor which supported the shoot produc-

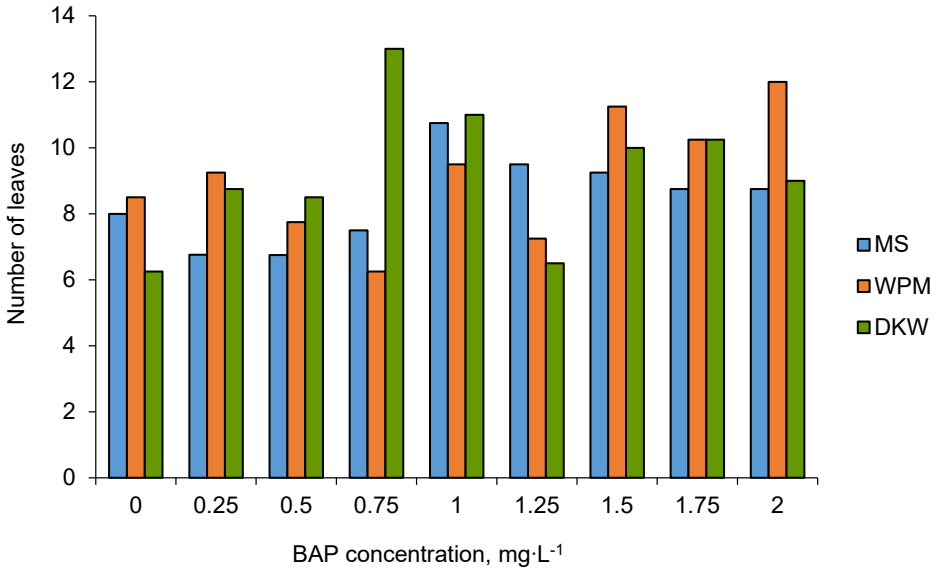

Fig. 2. Number of leaves appeared after 8 weeks of planting with various BAP concentrations and different media.

tion as the endogenous. It is strengthened by Gunawan (1992) that stated the interaction between exogenous and endogenous growth regulators determines the direction of culture development.

The interaction between WPM basic media and 2 mg·L^{-1} BAP produced the highest number of shoots, which were three buds. However, it was not significantly different from the interactions between WPM basic media and 1.5 mg·L^{-1} BAP, MS basic media and 1 mg·L^{-1} BAP, MS basic media and 1.5 mg·L^{-1} BAP, DKW basic media and 0.75 mg·L^{-1} BAP, and DKW basic media and 1 mg·L^{-1} BAP. This is due to the role of N and phosphorus (P) contained in MS media, WPM, and DKW media. WPM had 14.8 µM·L^{-1} N and 1.3 µM·L^{-1} P contents, which were lower than in MS and DKW media. Thus, it requires BAP greater than 2 mg·L^{-1} in comparison with MS and DKW media (with around 0.75–1.5 mg·L^{-1}) to produce shoots on *N. macrophylla* explants (Fig. 3).

Shoot height

The analysis of results of various basic media had no significant effect (p-value = 0.173), BAP concentration had a very significant effect (p-value = 0.000), and the interaction between basic media and BAP concentration also had a very significant effect (p-value = 0.000) on shoot height.

The results of further tests of BAP concentration using DMRT with a 95% confidence level showed that the shoot height was 3.9 cm higher without BAP in comparison with the addition of any BAP concentration. MS basic media without addition of BAP gave the best results, with a median value of 5.56 cm, and was not significantly different from DKW basic media with 0.75 mg·L^{-1} BAP. It is estimated that the planted explants produce endogenous auxin with a high enough concentration without BAP; thus, the cell elongation process occurs. On the other hand, with the addition of BAP, the activity of endogenous

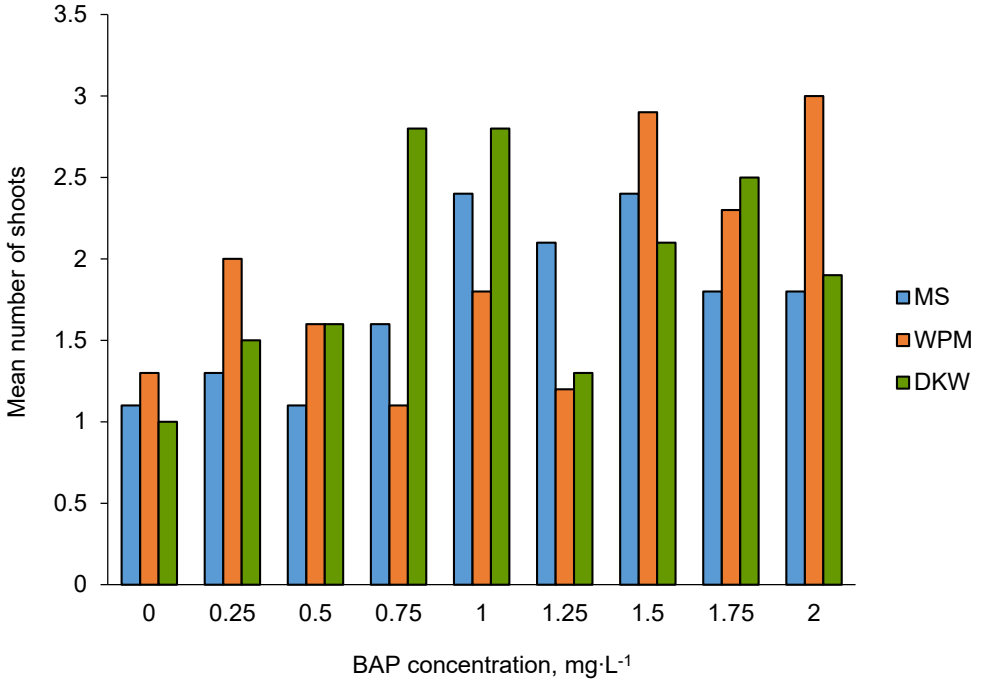

Fig. 3. Number of shoots after 8 weeks of planting with various BAP concentrations and different media.

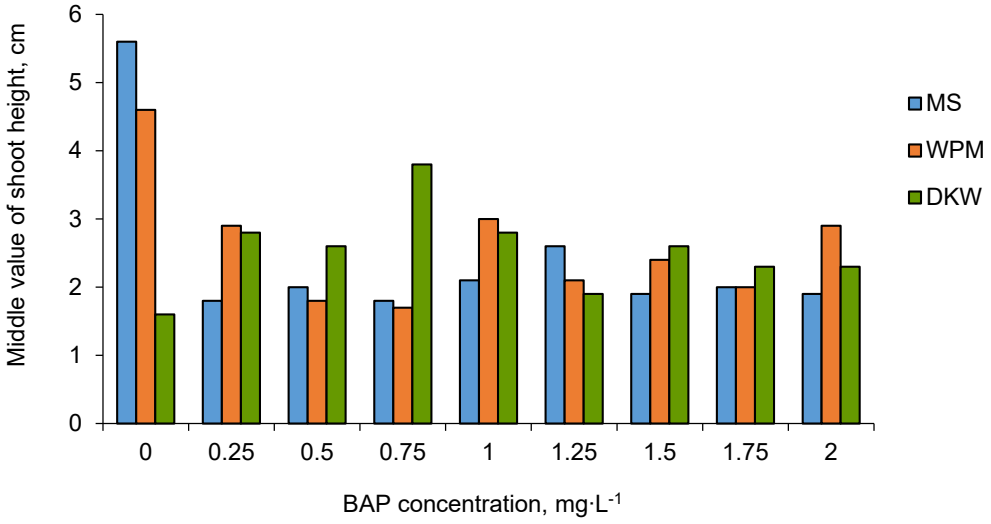

Fig. 4. Shoot height of explants after 8 weeks of planting with various BAP concentrations and different media.

auxin is inhibited because of the presence of exogenous cytokinins (Fig. 4).

Colour and callus of the shoot

The leaves and callus were observed to be formed at the bottom of the plantlet stem. The basic media of MS and WPM were similar in colour, which was dark green, whereas the basic media of DKW was light green. On the basic media of MS and DKW, callus was not found in the lower part of the plantlet stem, but in the basic media of WPM, solid callus was found at the bottom. The callus formed in this research was green and dense. It is called a proliferative callus, it can grow but is unable to develop toward organogenesis or embryogenesis (Fig. 5).

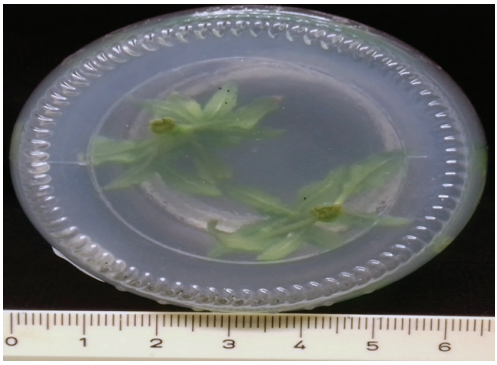

Fig. 5. Solid callus found at the bottom of the plantlet stem on WPM media.

Rooting

The rooting stage happens when the buds are transferred to the root induction medium, which contains hormones for root formation.

Day after rooting induction

The analysis of results of various basic media had a very significant effect (p-value = 0.000), BAP concentration had a very significant effect (p-value = 0.001), and the interaction between the basic media and BAP concentration had a very significant effect (p-value = 0.000) on DAP.

The results of further tests of basic media of MS, WPM, and DKW using DMRT with a 95% confidence level showed significant difference in DAP. WPM basic media responds faster at 7.16 DAP compared with MS and DKW basic media, because the concentration of macronutrients in WPM basic media was lower than that in MS and DKW basic media. The high N content in MS (30 $\mu M \cdot L^{-1}$) and DKW (26.05 $\mu M \cdot L^{-1}$) media compared with N content in WPM (7.4 $\mu M \cdot L^{-1}$) media caused the inhibition of root induction (Fig. 6).

The analysis of results of further BAP concentration using DMRT with a 95% confidence level showed that 2 $mg \cdot L^{-1}$ IBA concentration had a faster root formation, which was 7.13 DAP, whereas 0 and 1.25 $mg \cdot L^{-1}$ IBA showed a significant result and had the slowest root formation, which was 10 DAP.

The interactions between WPM basic media and 0.75 $mg \cdot L^{-1}$ IBA and between WPM basic media and 1.5 $mg \cdot L^{-1}$ IBA resulted in a faster root formation at 5.6 DAP, and DKW basic media and without the addition of IBA resulted in the slowest root formation at 13.2 DAP. The result of this study is in line with that of Yunita and Nugraha (2021), who showed that IAA, IBA, and NAA treatments were to obtain the appropriate type and concentration of auxin for *in vitro* induction of *Alternanthera reineckii* Griseb. roots.

Number of roots

The analysis of results of various basic media had a very significant effect (p-value = 0.003), BAP concentration had

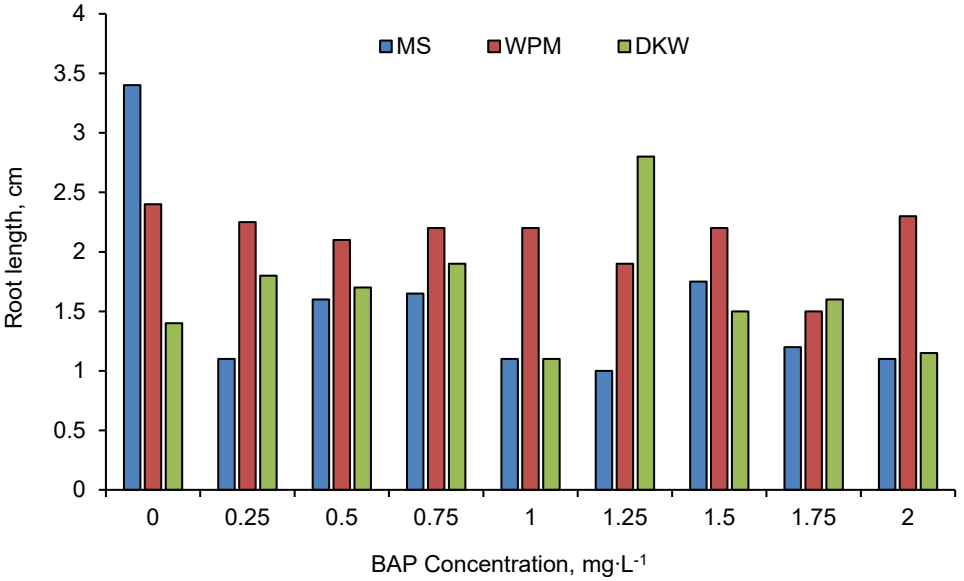

Fig. 6. DAP for explants producing roots by various IBA concentrations and different media.

a very significant effect (*p*-value = 0.000), and the interaction between basic media and BAP concentration had no significant effect (*p*-value = 0.054) on the number of roots.

The results of further tests of the basic media using DMRT with a 95% confidence level showed that the number of roots formed in WPM was 50.7, which was more than MS and DKW media. This is due to the sufficient and balanced nutrients in WPM needed by plantlets to grow.

The results of the further analysis of IBA concentration using DMRT with a 95% confidence level showed that the number of roots produced at 0.75 mg·L^{-1} IBA concentration was 61, which is significantly different from other concentrations. These results were presumed by the ratio of auxin which was higher than that of organogenesis cytokinin. Hence, it tended to lead the root formation. This finding was in line with the study conducted by Kristina and Syahid (2012).

Length of the root

The results of the analysis of various basic media had a very significant effect (*p*-value = 0.000), IBA concentration had a very significant effect (*p*-value = 0.000), and the interaction between basic media and IBA concentration had a very significant effect (*p*-value = 0.000) on root length.

The results of further basic test analysis using DMRT with a 95% confidence level of root length in WPM significantly differed from MS and DKW basic media. Further test results of IBA concentrations using DMRT with a 95% confidence level on addition of IBA with a concentration of 0.25–2 mg·L^{-1} showed a significant result. The treatment without IBA showed the longest root length, which was 2.43 cm, whereas the shortest root length with IBA concentration treatment (1.75 mg·L^{-1}) was 1.43 cm. The treatment without IBA produced the longest root length, but not the highest number of roots; this is due to the unavail-

ability of optimum auxin in *N. macrophylla* plantlets. It requires exogenous auxin to produce a greater number of roots. When the higher concentrations of IBA level were given, the lower the average length value produced. These results are in line with that of Ak et al. (2021); giving high concentrations of IBA will inhibit root lengthening, whereas lower concentrations produce longer roots in 'Garnem' culture (Fig. 7).

Shape and callus of the root

The root media without addition of IBA formed fiber roots, whereas the addition of IBA initially formed callus at the bottom of the stem. Besides, the leaves touched the root media, and this happened to MS and DKW rooting media. Rooting in WPM is not followed by callus formation. The appearance of roots turned out to have a different pattern on MS and DKW media: they felt soft, and there was callus. On the other hand, on WPM, the root extraction looked larger and felt rough, and there was no callus because IBA played a vital role in the process of cell division and enlargement, especially at the beginning of root formation. The concentration of IBA absorbed by the plant will affect the cell division.

Callus formed on MS and DKW media had crumb texture (friable) with white colour. Although the callus formed in this study had been able to form roots that need to be developed further with various combinations of auxin and cytokinin growth regulators; thus, callus becomes embryogenic. This is in line with the study of Gusmiaty et al. (2015), in which it was found that the use of MS media with addition of 0.3 mg·L^{-1} NAA and 4.4 mg·L^{-1} BAP formed callus with friable texture and varying colours on the explants of *N. macrophylla* leaves (Fig. 8).

Acclimatization

The analysis of results on acclimatization variety of media treatments had a significant effect (p-value = 0.021); basic me-

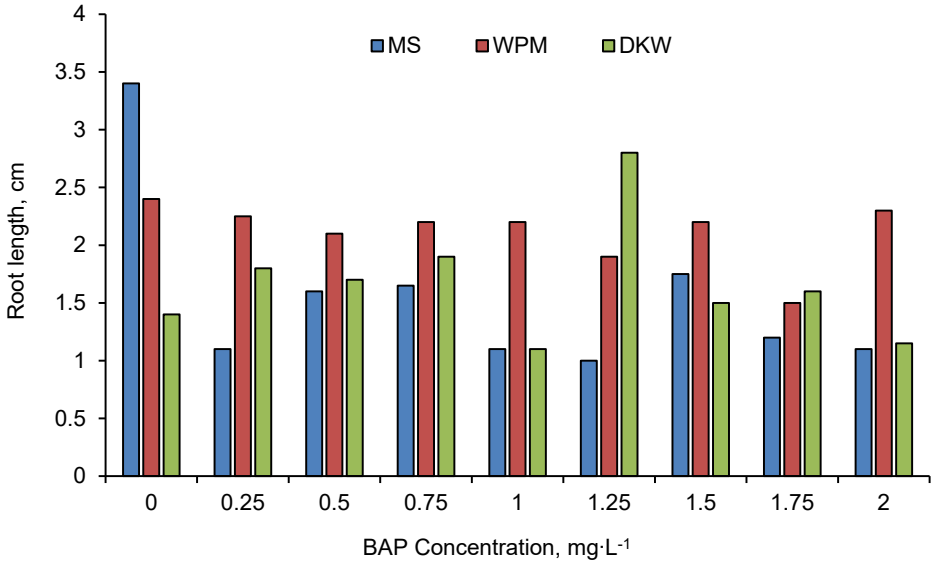

Fig. 7. Root length after 6 weeks in different rooting media with various BAP concentrations.

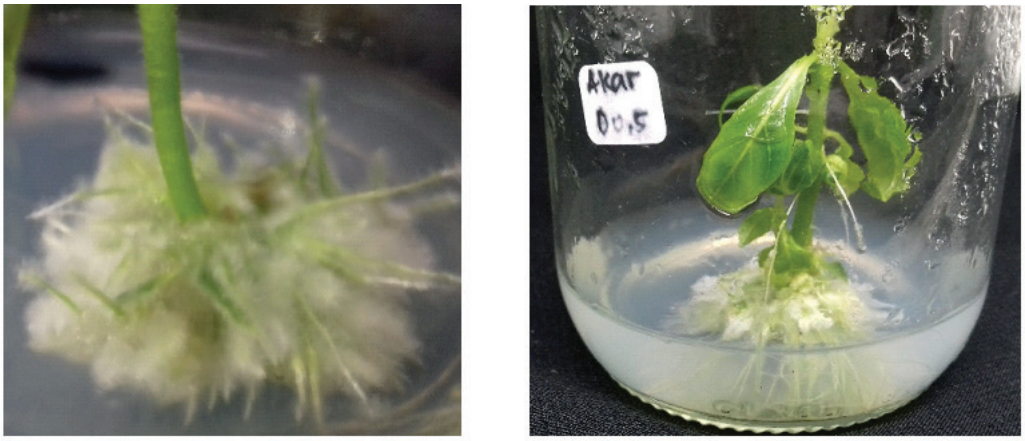

Fig. 8. Callus formed in the MS medium (left) and DKW medium (right).

dia treatment of tissue culture had a very significant effect on the percentage of living plantlet in the acclimatization media (*p*-value = 0.000) (Table 1). A further test showed that acclimatisation in compost + sand media produced 93.3 % survival rate of the explant, which was not significantly different from only compost media and compost + rice husk + sand media. It was due to the addition of sand to the loose structure of the media (Fig. 9). The aeration and drainage of the planting media were also good, and plant roots can grow circulation optimally, and the availability of adequate air is needed by root cells to breathe.

The use of single husk charcoal acclimatization media resulted in the lowest percentage survival, which was 13.3 %, and it was not significantly different from the compost and husk charcoal media. The low percentage survival can be seen from the high mortality of plantlets during acclimatization. The root system produced in *in vitro* culture contributes to a high percentage of viability at the acclimatization stage to the green house or field. Therefore, the selection of substrate materials needs to be done carefully. This can be caused by an adaptive process that is not carried out gradually, as in *Thalictrum foliolosum* (Mishra et al. 2020).

The results of further test analysis of tissue culture basic media treatment using DMRT with a confidence level of 95 % showed that the survival rate of WPM basic media was 74 %, which was not significantly different from DKW media. Meanwhile, Thengane et al. (2006) reported that the successful acclimatization

Table 1. Average survival percentage of seedlings in various acclimatization media.

Acclimatization medium	Average survival, %	DMRT (95% confidence level)
Compost	86.7	a
Rice husk	13.3	b
Compost + sand (4:3)	93.3	a
Compost + rice husk + sand (1:1:1)	80.0	a
Compost + rice husk (2:1)	36.7	b

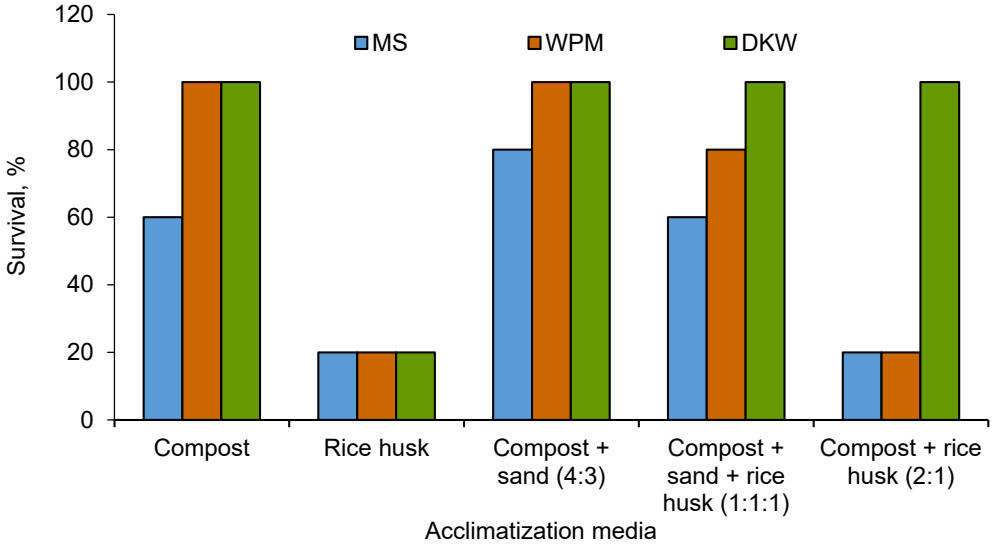

Fig. 9. Survival percentage of seedlings in various acclimatization media taken from various basic media from the rooting stage.

(up to 80 %) of *Calophyllum inophyllum* L. was due to WPM. Also, Kruczek et al. (2021) showed that acclimatization of two cultivars of goji regenerated on the medium with chitosan were successfully acclimatized and established in the mixture of 90 % peat and 10 % perlite under field conditions. Hence, these findings could be a further alternative as acclimatization media.

Physical quality of seeds

The analysis of results on the variety of sturdiness of seeds showed a significant effect (*p*-value = 0.029), the root:shoot ratio (RPA) (*p*-value = 0.030) was significantly affected, and the seed quality index (IMB) had a very significant effect (*p*-value = 0.008). The results of further sturdiness tests on RPA and IMB using DMRT with a 95% confidence level showed that seed sturdiness increases with seedling age. The sturdiness of seedlings at 2 (4.698) and 3 (4.886) months were not significantly different but was very significantly different from seedlings at 1 month (3.522). An optimum sturdiness value is 4–5 (Febrianti et al. 2013). Furthermore, the results of this study had met the optimum sturdiness criteria. Root:shoot ratio (RSR) is a valuable selection criterion that can be used for phytoremediation and associated phytotechnologies (Rogers et al. 2019).

The results of RPA follow-up test at 2 (2.232) and 3 (3.980) months were not significantly different. However, it showed a very significant difference at 1 month (3.0480). The lower RSR generally results in higher plant survival and adaptability (Komala et al. 2008). Mindawati et al. (2005) stated that seeds with RPA value of 2–5 would be ready to be moved to the field. A high total dry weight will affect the seed quality index (SQI); this means that the higher the total dry weight, the higher the BMI in a treatment (Bayau 2018). Those criteria could be used as the quality information for seeds. Plants with SQI greater than 0.09 will have high survival in

the field. Furthermore, the analysis of result of the seed quality index in this study showed that all age level treatments would be able to grow in the field because BMI value was greater than 0.09. The tissue culture method can be used in the *ex situ* conservation of *N. macrophylla* in the form of plantlets and can be further developed for breeding activities and as conservation efforts.

The use of MS, WPM, and DKW basic media responded well to the multiplication stage. WPM and 1 mg·L^{-1} BAP responded faster to the multiplication stage, DKW and 0.75 mg·L^{-1} BAP produced the greatest number of leaves, WPM and 2 mg·L^{-1} BAP produced the highest number of shoots, and MS without addition of BAP produced the highest shoot height.

The use of MS, WPM, and DKW rooting media was able to induce root formation. Several previous studies (Batti et al. 2020, Pandey et al. 2022) got the similar results to this present study. WPM rooting media was better than MS and DKW media (Fadladeen et al. 2021). WPM and 0.75 mg·L^{-1} IBA as a rooting media and WPM and 1.5 mg·L^{-1} IBA responded faster to root formation, WPM and 0.75 mg·L^{-1} IBA produced the highest number of roots, and MS without addition of IBA as a rooting media produced the longest roots. The appropriate acclimatization media for *N. macrophylla* were compost and sand (4:3) and red jabon plantlets originating from WPM, which produced the highest percentage of survival. By the age of 2 months, the physical quality of *N. macrophylla* seedlings (height, diameter, sturdiness, RSR, and SQI) meets the requirements to be planted in the field. In this research, the effects of media on plant growth showed a good result and a protocol is necessary to optimize the efficient micropropagation of *N. macrophylla*.

Conclusions

In this study, an effective micropropagation system for *N. macrophylla* has been developed by using three media, including DKW, WPM, and MS. Those media produced equally effective results. At the age of two months, physical characteristics of *N. macrophylla* seedlings (height, diameter, sturdiness, shoot ratio, and seed quality index) met the requirements for field planting. Hence, these findings are promising to be applied in the conservation fields.

Acknowledgements

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