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The content of chlorophyll a and chlorophyll b in leaves of undergrowth species in hornbeam-oak forest stands of the forest-steppe zone in Western Ukraine

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Abstract. In this study, the biosynthesis of the plastid pigments chlorophyll a and b was examined for the most common shrubs in hornbeam-oak forest stands of the Western forest-steppe zone of Ukraine. The characteristics of the pigments' biosynthesis were determined in terms of plant species, vegetation period and growth conditions (under canopy cover and out in the open). The gathered data on the changes of the pigment complex with respect to the examined variables confirms the sensitivity of plastid pigment biosynthesis to environmental factors.

Keywords: shrubs, plastid pigments, chlorophyll a, chlorophyll b, hornbeam-oak forest stands, forest-steppe zone of western Ukraine

1. Introduction

As a component of the forest stand, the understory is vital in forest ecosystem functioning because of its role in providing habitats for birds and other animals, the regulation of soil microclimatic and microbiological processes (Kozlowsky 2013), as well as the improvement of physical properties of soil (Bondarenko, Marutak 2012). Yet, the effect of understory on stand production has not been adequately investigated. Our study showed that understory in hornbeam-oak forests of the Western Ukrainian forest-steppe zone is not abundant, whereas its share is contained within the range from a few specimens to 10–30% (Bondarenko 2013). This is largely the result of underestimating the importance of understory in the process of forest management.

The main species building understory under canopies of hornbeam-oak forests in the Western Ukrainian forest-steppe zone include common hawthorn (*Crataegus monogyna* Jack.), wild rose (*Rosa canina* L.), common dogwood (*Cornus sanguinea* L.), European spindle (*Euonymus europaeus* L.), common hazel (*Corylus avellana* L.), warted spindle tree *(Euonymus verrucosus* Scop.), European black elderberry (*Sambucus nigra* L.), common privet (*Ligustrum vulgare* L.), alder buckthorn (*Frangula alnus* Mill.), purging buckthorn (*Rhamnus cathartica* L.), blackthorn (*Prunus spinosa* L.), guelder rose (*Viburnum opulus* L.), common ninebark (*Physocarpus opulifolius* Maxim.) and dwarf honeysuckle (*Lonicera xylosteum* L.).

Photosynthesis is a key element of metabolic system that ensures the plant growth and development in conformity with its genetic properties. In the process of photosynthesis, the light energy is transformed into the energy of chemical bonds.

The activity of photosynthesis depends largely on plant plastids. Changes in the structure of plastids affect the intensity of photosynthesis, the level of metabolism, and the intensity of plant growth and development (Kučerjavij 2001). The content of plastids in the leaves reflects the plant condition in general.

The aim of this study was to determine the contents of chlorophyll a and b in the foliage of forest shrubs growing in the open space and under the forest canopy in the western Ukraine forest-steppe zone in different vegetation seasons. Until now, there have been no studies carried out on the content of chlorophyll a and b in the leaves of understory shrubs growing in the western Ukraine forest-steppe zone.

The biosynthesis of pigments in the plant plastids is primarily controlled by genetic structures and is also affected

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by external factors. In plants that grow in open spaces, that is, are not shaded by the forest canopy, the amount of green and yellow pigments is determined by the species genetic makeup (Margailik 1963, Nesterovič, Margailik 1969, Celniker 1982, Novikova 1985, Krynytskyi 1993). Amongst a variety of external factors that affect the formation of plastids, the most important include light intensity, environmental temperature and mineral substances (Veretennikov 1987).

Plant chloroplasts are sensitive to changes in light intensity. According to various researchers, shading of plants increases the content of chlorophylls and carotenoids in the leaves (Margailik 1963, Nesterovič, Margailik 1969). Significant reduction in the amount of chloroplasts in plant leaves indicates their deterioration and leads to disruptions of the processes of plant growth and development. The biosynthesis of pigments depends not only on light intensity but also on its spectral characteristics. Chlorophyll a absorbs more readily the far -infrared light, whilst chlorophyll b absorbs the near-infrared light (Veretennikov 1987). Under the forest canopy, where the far-infrared light prevails, the biosynthesis of chlorophyll a is intensified, whilst in the case of the near-infrared light, the biosynthesis of chlorophyll b becomes more intense.

A lot of attention has been devoted to the role of photosynthetic pigments in biomass production by various tree species (Zaïka et al., 2010, Kenzor et al., 2010, Terelâ et al., 2014), and a clear trend has been established, illustrating the accumulation of photosynthetic pigments in pine, oak, beech, maple, larch, and spruce growing in plantation forests in the Ukrainian part of Roztocze. The above authors also determined the relationship between the production of tree biomass and the mass of photosynthetic pigments. Details concerning the accumulation of photosynthetic pigments in pine stands infected by pathogens were described by Derevânčuk and Zaïka (2011), whilst those for pine stands were classified into various selection categories – by Dan'kevič et al. (2014) and Zaïka et al. (2010) – and for young trees under the stand canopy – by Zaïka and Dereh (2014).

In light of the fact that the questions of understory status and its resilience as well as its response to changes in environmental conditions under the forest canopy, and especially, chloroplast formation and responses to shading, have been insufficiently investigated, the latter direction of studies remains valid. The present study provided opportunity for determining the amplitude of ecological responses of understory species based on the content of chlorophyll a and b, and for possible application of the results obtained in the forest management (2014).

2. Study object and methods

The fresh hornbeam-oak forests in the western Ukraine forest-steppe zone have been typically developed as a complex multi-layered stands, comprising two or three strata of woody vegetation. The upper layer is usually built by pedunculate oak (*Quercus robur* L.), European beech (*Fagus sylvatica* L.) and common ash (*Fraxinus excelsior* L.), whilst the lower one is usually built by European hornbeam (*Carpinus betulus* L.), small-leaved linden (*Tilia cordata* Mill.), sycamore (*Acer pseudoplatanus* L.) and Norway maple (*Acer platanoides* L.). Such a dense crown cover transmits not more than 1% of solar light, which is the main factor affecting the occurrence and development of understory species.

The study plot was located in the maturing 75-year-old stand with moderate canopy density (Table 1). In the stand species composition, there prevailed common ash, showing a high growth intensity, similar to European beech and Norway maple. The latter species attained the height of 26.8–29.1 m and diameter at breast height (DBH) of 26.2–36.4 cm, whilst pedunculate oak was lower than the aforementioned species. The understory was composed of common hazel, spindle, warted spindle tree, European black elderberry, alder buckthorn and common dogwood. The main stand metrics were taken from the description of forest taxation made in the Medobory Reserve (Materìali 2007), whilst canopy density was evaluated at the study plot.

Table 1. Main forest stand metrics at the study plot

Study plot	Stand composition	Species	Age [years]	Average		Canopy density	Volume [m³/ha]
				DBH [cm]	H [m]		
1	4Jw1Ds2 Kp2Gz 1Bz	Jw4 Ds1 Kp2Gz2 Bz1	75 75 75 75 70	26.2 22.8 36.4 30.5 20.7	27.2 23.8 29.1 26.8 20.8	Moderate	276

Gz, European hornbeam (*Carpinus betulus*); Jw, common ash (*Fraxinus excelsior*); Ds, pedunculate oak (*Quercus robur*); Bz, European beech (*Fagus sylvatica*); Kp, Norway maple (*Acer platanoides*).

The plants were growing in the open area, on the slopes and at the foothills of the Gostra mountain, where the dominating shrub species included common hawthorn, guelder rose, common hazel, blackthorn, European spindle, common dogwood, European black elderberry, warted spindle tree, alder buckthorn, guelder rose, common ninebark and purging buckthorn.

The study on the chlorophyll content in the leaves of understory shrubs was made in the region of Ternopol, in the Medobory Reserve (Viknianskie Forestry) and in hornbeam -oak forests of the forest-steppe zone in Western Ukraine. The following species were examined: common hawthorn, wild rose, common dogwood, European spindle, common hazel, warted spindle tree, European black elderberry, common privet, alder buckthorn, purging buckthorn, blackthorn, guelder rose, common ninebark and dwarf honeysuckle. The study was conducted in leaves both under the stand canopy and in the open area in various stages of the growing season (from May to September) in the years 2010–2012.

The leaves for determining the content of chlorophyll a and b were taken from 5 to 10 trees of each species studied, from the upper part of the crown. The contents of chlorophyll a and b were measured by the method of Brayon et al. (1995) and Gusejeva (1982). In conformity with this method, 100 mg of leaves was ground to homogeneous mass and extracted in 96% alcohol using the Wintermans and DeMots method. The extract was filtered through a Schotti filter.

The optical density of the extract obtained was determined at 440.5, 649 and 665 nM with the FEK KFK-3 apparatus. The concentration of chlorophyll (C) was calculated using the Vintermans formula: $Ca = 13.70 \times D_{665} - 5.76 \times D_{649}$ (mg/L) and $Cb = 25.80 \times D_{649}\text{-}7.60 \times D_{665}$ (mg/L). The content of chlorophyll (A) was calculated according to the formula:

$$A = \frac{CV}{P \cdot 1000}$$

where A is the content of chlorophyll a and b in plant material (mg/g wet mass),
V is the capacity of chloroplast extract (mg/L),
P is the weight of plant material (g),
C is the concentration of chlorophyll (mg/L).

The quantitative measurement of the content of plastids, depending on the environmental conditions, yielded characteristics of the photosynthetic apparatus of understory shrubs. The average number of repetitions was three.

3. Results

The results of measurements of the content of chlorophylls a and b in shrub leaves in various stages of the growing season are given in Figure 1.

The results of the measurements indicate a high interspecific differentiation in the chlorophyll content and in the response to changes in light intensity between the understory shrubs in various stages of the growing season. Wild rose, warted spindle tree and alder buckthorn were found to display the highest genetically determined level of chlorophyll biosynthesis: 1.197–1.626, 2.513 and 1.700 mg/g wet weight, respectively, in the period from the end of May to the beginning of June. The values of indicators for some species vary considerably between the respective years of observations. The content of chlorophyll varies from 0.631 to 2.142 mg/g in the leaves of common hawthorn, from 0.652 to 1.783 mg/g in common dogwood, from 787 to 1.388 mg/g in European black elderberry and from 0.796 to 1.201 mg/g in blackthorn. The above differences in the values of indicators between the examined species testify to a varying rate of leaf development in response to the impact of climatic factors. A relatively low chlorophyll concentration was found at the beginning of growing season in species such as common hazel (0.656–0.758), purging buckthorn (0.845) and common ninebark (0.792–1.072) encountered in the open area.

At the initial phase of growth and formation, the leaves are particularly sensitive to light availability; however, this response is species specific and depends on the species light demand. The results of the authors' own study provide evidence that the process of chlorophyll biosynthesis shows interspecific variation in response to changes in light intensity and depends on the shrub species. In some species, there occurs an increase in the chlorophyll content in the assimilatory apparatus and, conversely, a decrease in other species. A significant increase in the chlorophyll content was found in common hazel (2.5–3.8 times) and common ninebark (by 21.3–24.1%) growing under the forest canopy. In the leaves of wild rose growing under the canopy, the content of chlorophyll turned out to be 8.2–37.9% lower than that in the open area. Other understory species did not display any clear response of chlorophyll biosynthesis to light intensity in the first half of growing season. At the end of May 2011, a slight decrease in the chlorophyll content (by 5.4–6.3%) was observed in the leaves of European spindle and common dogwood growing under the forest canopy, whilst at the beginning of June 2012, this content was 2.1–2, 4 times greater than that in the open area. We are of the opinion that this was due to the differences in the leaf development rate in species building the forest canopy, because the transmission of light was decreasing with the increasing canopy closure. In June 2012, light conditions under the forest canopy were not favorable for understory species. The observed reduction in the chlorophyll concentration in the leaves of wild rose and European black elderberry could be due to destructive processes, which varied between the species examined.

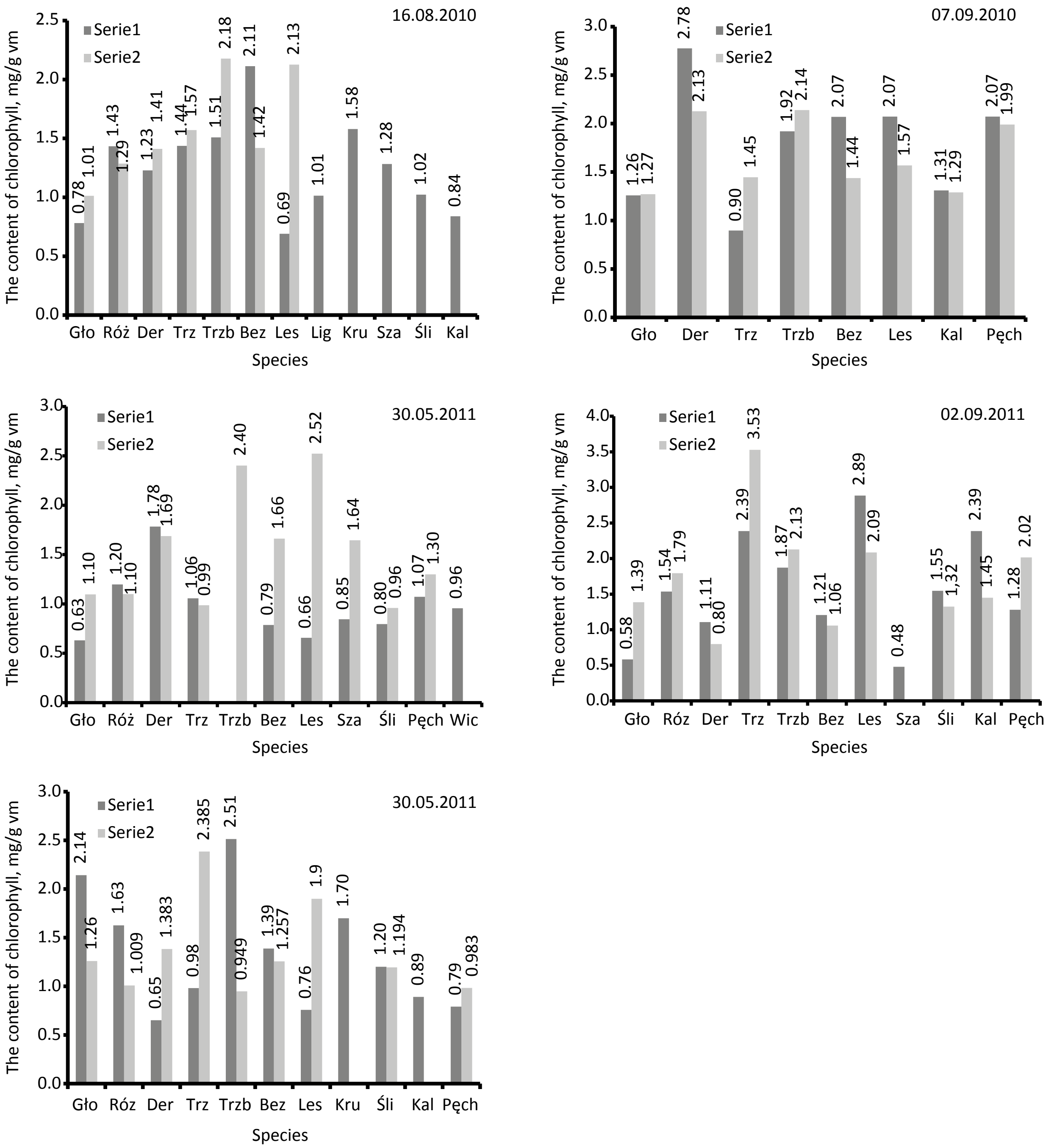

Figure 1. Content of chlorophylls a and b in leaves of understory species in various stages of the growing season [mg/g]

Explanation:
Total content of chlorophyll a and b in leaves of undergrowth species:
1. Species growing in the open air
2. Species growing under the canopy
Gło, common hawthorn (*Crataegus monogyna* Jack.); Róż, wild rose (*Rosa canina* L.); Der, common dogwood (*Cornus sanguinea* L.); Trz, European spindle (*Euonymus europaeus* L.); Les, common hazel (*Corylus avellana* L.); Trzb, warted spindle tree (*Euonymus verrucosus* Scop.); Bez, European black elderberry (*Sambucus nigra* L.); Lig, common privet (*Ligustrum vulgare* L.); Kru, alder buckthorn (*Frangula alnus* Mill.); Sza, purging buckthorn (*Rhamnus cathartica* L.); Śli, blackthorn (*Prunus spinosa* L.); Kal, guelder rose (*Viburnum opulus* L.); Pęch, common ninebark (*Physocarpus opulifolius* Maxim.); Wic, dwarf honeysuckle (*Lonicera xylosteum* L.).

The effect of forest canopy on the chlorophyll biosynthesis was also observed in other stages of the growing season. In the second half (August) of the season, there was a marked or significant increase in the chlorophyll concentration in the leaves of the majority of understory species encountered under the forest canopy. During this period, the largest differences in chlorophyll concentrations between the shrubs growing in the open area and under the stand canopy were observed in the case of common hazel (3.1 times) and warted spindle tree (2.1 times). In European spindle and common hawthorn, the differences ranged from 9% to 29.7%. In the first half of the growing season, there was a drop in the chlorophyll content in the leaves of wild rose and European black elderberry growing under the forest canopy by 10.3–32.8% compared to the open space.

At the end of the growing season, the most visible were the effects of microclimate factors on the processes of chlorophyll accumulation. The leaves in most of the shrubs examined lost their functional properties faster under the canopy than in the open area. The decrease in the chlorophyll content by 1.6–39.3% was observed under the forest canopy in common dogwood, common hazel, blackthorn, European black elderberry and guelder rose. Only hawthorn, European spindle, warted spindle tree and common ninebark preserved a higher content of chlorophyll under the forest canopy compared to the open area.

According to Gûbbenet (1951), the ratio of both chlorophyll types is an important factor that characterises the effect of light conditions on biosynthesis of photosynthetic pigments. This author suggests that with insufficient light intensity, there is increase in the synthesis of chlorophyll a and carotenoids (plastids of shade). On the basis of our results, we can infer that some phenomena result from the nature of the biosynthesis of chlorophyll a and b. The ratio of chlorophyll a to chlorophyll b in the first half of the growing season increases from several percent to 2–3 times in most understory species growing under the canopy. At the beginning of leaf formation, the biosynthesis of chlorophyll a is more intense under the canopy than at the forest edge. However, in the second half of the vegetation season, when the leaves are fully formed, the role of chlorophyll b under the forest canopy increases. In the majority of species, the ratio of chlorophyll a to chlorophyll b under the canopy decreases to 66.5%, whilst in some shrub species, it increases or remains at the same level as at the forest edge.

4. Conclusions

The differences in the biosynthesis of chlorophyll a and b observed in understory shrubs in various stages of the growing season reveal changes in the absorption of sunlight, which ensures survival under conditions of low light availability.

The best adaptation capabilities to shading show species such as common hazel, European black elderberry and common ninebark, in which the concentration of chlorophyll under the stand canopy increases significantly compared to the concentration under the canopy of the forest. Shrubs under the forest canopy show a significant increase in the chlorophyll b biosynthesis, which suggests changes in the absorption of light and the increase in the absorption of infrared radiation by understory species.

Changes in the complex of chloroplasts in the examined shrub species reflect the adaptation capabilities of plants to the lighting conditions in the environment and are the main cause of the photosynthesis inactivation. They also indicate the sensitivity of this system to the impact of environmental factors. The study results show that plant assimilation organs are adapted to the intensity of light and thus to the environmental conditions.

Conflict of interests

The authors declare no conflict of interests.

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Author's contributions

V.Z. – conceptualization, methodology, statistical analysis; T.B. – measurements, literature review, writing, text revision.

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