

EFFECT OF ARTIFICIAL LIGHT AT NIGHT (ALAN) IN URBAN CONDITIONS ON *Taxus baccata* L. LEAF MORPHOLOGY, PHOTOSYNTHETIC CAPACITY AND ASSIMILATORY PIGMENTS CONTENT

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Abstract

Light is one of the main factors that influences the greatest phenomenon of nature, namely photosynthesis. In modern society, excessive artificial light at night (ALAN) in the conditions of the urban environment can be considered as an external polluting factor as well. The purpose of this study carried out on *Taxus baccata* L. (1-year old leaves - PYL and the current year - CYL ones) grown as a hedge in urban conditions was to explore the effects of ALAN on leaves morphology, gas exchanges and associated indicators, as well as on assimilatory pigments content. The results showed that: (1) The leaves length (LL)(mm) of CYL, as well as surface area (cm²) and leaf length to width ratio (LL/LW) were significantly lower under ALAN, than in normal (day/night cycle) light (NL). For PYL, no significant differences were noticed in the case of LL, while significantly lower values have been registered in the case of LW and leaf area to leaf fresh weight (AFWR); (2) Net photosynthesis (A) had significantly higher values during the springtime for PYL in NL as against ALAN, but opposite data have been noticed in June. The quantum yield (ϕ CO₂), water use efficiency (WUE) as well as transpiration rate (T) and stomatal conductance (g_s) proved to be affected by the light conditions. (3) The values for the content of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (TC) and carotenoids (C) were generally as that known for plants in general, with a higher amount of Chl a for PYL-ALAN, as compared to those for CYL-ALAN, or CYL-NL. Further research should be applied by the scientific community to monitor the impact of different light systems during the night and counteract the ALAN impact on plants and ecosystem.

Key words: *Taxus baccata*, artificial light at night, leaves morphology, gas exchanges, assimilatory pigments.

INTRODUCTION

Light is one of the main environmental factors which by intensity, quality, duration (photoperiod) and direction of action influences the greatest phenomenon of nature, namely photosynthesis (Taiz et al., 2015). Moreover, its beneficial effects on plant growth and development are multiple given its involvement in controlling the ontogenetic cycle, not only

due to the effects induced as an energy source, but also as a source of information (Bennie et al., 2016; see review by Singhal et al., 2019). Nevertheless, in modern society, like air pollution, light pollution can be considered as an external polluting factor as well (Argys et al., 2020; Massetti, 2020; Heinen, 2021). As for instance, an excessive artificial light at night (ALAN) has been first qualified a disturbance factor for astronomical observations, beginning

do to the alteration of natural night sky light (Cinzano et al., 2000). The negative effects of light are strongly felt especially in the conditions of the urban environment, where light emissions are associated with specific development models (roads, institutions, office buildings, commercial spaces infiltrated in urban residential areas etc.), respectively with sociocultural and institutional characteristics for lighting and light uses (Cheon & Kim, 2020). With the expansion of the urbanization process and the emergence of megacities, the need for green spaces for human health has become a sinecuanon condition and a permanent concern to reduce the potential risk of diseases burden (Stanhope et al., 2021). Industrialization and urbanization have been beneficial for the prosperity and health of people but have also introduced novel threats to wildlife and humans (Braubach, 2017; Russart & Nelson, 2018), among which is the ALAN. There has been shown that it has serious physiological consequences, especially due to the influence on the operation of the biological clock, which cause the disruption of the rhythm, not only in the case of humans, but also for domesticated animals and wildlife (see review by Russart & Nelson, 2018). This global environmental problem, which negatively affects humans, many animals and plant taxa, have also socio-cultural implications (Falcón et al., 2020; Challéat & Poméon, 2020), produces many ecological (and evolutionary) disturbances, affecting biodiversity (Gaston et al., 2017) and may reshape entire ecosystems (Holker et al., 2010). Unlike people, who can find shelter and thus manage to avoid the negative effects of ALAN, for fauna and flora the refuges are becoming more and more insufficient in the conditions of increasing in radiance and extent of ALAN in the urban environment. ALAN significantly impacts the reproduction, navigation, behavior, foraging (Shier et al., 2020; Zhang et al., 2020), habitat selection, communication, and social interactions of all living organisms (Peregrym et al., 2020), including plants live, by changes of pollination (Macgregor et al., 2015; Macgregor et al., 2019; Macgregor & Scott-Brown, 2020), photoreceptor signaling (Solano-Lamphar et al., 2018), flowering and

phenology (Ffrench-Constant et al., 2016; Škvareninová et al., 2017; Bennie et al., 2018; Zheng et al., 2021) microhabitats, as reviewed by Singhal et al. (2019) and so forth.

Therefore, urban green space (UGS) policies need to be improved to recover the human-nature relationship (Măcnea et al., 2021), including those connected with the application of new lighting technologies (Macgregor et al., 2019), with a view to mitigate the negative effects of ALAN on the entire ecosystems, including in order to create a bird-friendly city (Xue et al., 2020), by considering the taxonomic, functional and phylogenetic bird species communities in a large geographical range (Kosicki, 2021).

If we refer to all living organisms, the effects of night light are manifested depending on the intensity, quality, or spectral composition, as well as in terms of expansion in space and time. As such, the optimization of the processes of conservation and restoration of green space in the urban environment (Dragoş et al., 2018) to ensure health benefits cannot be achieved without considering the potentially confusing effects of ALAN (Stanhope et al., 2021). At the same time, the negative effects can derive due to the changes of the seasonal dynamics of disease-transmitting insects, as it was demonstrated by the studies of Fyie et al. (2021). It is well known that light is the primary condition for the development of photosynthesis, but in addition, it is a major environmental factor in controlling the growth and development of plants, starting from seed germination and continuing with the proper development of phases of the ontogenetic cycle (Dong et al., 2015; Cosmulescu et al., 2020). Insufficient light during the day, but also the disturbance of the biological clock activity caused by ALAN lead to damage to the photosynthetic apparatus, as highlighted by Zhang et al. (2020) following research on perennial ryegrass (*Lolium perenne* L.), a commonly used species in urban green, planting such as lawns.

Yew, *Taxus baccata* L. (Taxaceae) is a native evergreen non-resinous gymnosperm tree that growth in the temperate area of the northern hemisphere (Thomas & Polwart, 2003) and known as a good bioindicator of urban environmental pollution (Samecka-Cymerman

et al., 2011). There are numerous studies on the behavior and physiology of wild animals under ALAN, but the effects on plants grown in their specific living environment have been less studied, even if some research has been done on the behavior in laboratory or greenhouse conditions. Therefore, the objectives of this research were: (1) to examine the morphological impact; (2) to test the gas exchanges and associated indicators modifications and (3) to quantify the effects on assimilatory pigment content of artificial light at night on the *Taxus baccata* L. (female leaves) grown as a hedge in urban conditions.

MATERIALS AND METHODS

The biological material used and the study site

The biological material was represented by yew (*Taxus baccata* L.), an evergreen slow gymnosperm, grown as a hedge near to the main building of the University of Agronomic Sciences and Veterinary Medicine (USAMV) of Bucharest, Romania. This study was carried out from March 2021 to June 2021 on female leaves formed in the previous year (1-year old leaves) (PYL) and the current year (CYL), grown in normal (day/night cycle) light (NL), and artificial light at night (ALAN), also, respectively.

Parameters determined

Morphological parameters at the leaf level

For the study of yew leaves morphometric traits there were used 10 needles from one tree collected from last year's growth (2020 increment), but also leaves grown during the current year. Fresh needles were wrapped in moist paper, enclosed in Eppendorf tubes, and brought in the laboratory of the Research Center for Studies of Food Quality and Agricultural Products (USAMV of Bucharest). Leaves dimensions have been measured with a rule in terms of leaf length (LL) and leaf width (LW) (mm). Leaf area (LA) (mm²) has been established by using the millimetric graph paper. Also, the leaf area to leaf fresh weight (AFWR) (cm² g⁻¹) and the leaf length to its width ratio (LWR) have been calculated.

Leaves gas exchange parameters and associated indicators

The intensity of photosynthesis (A) (μmols CO₂ m⁻²s⁻¹), the intensity of transpiration (E) (mmol H₂O m⁻²s⁻¹), the stomatal conductance (g_s) (mols H₂O m⁻²s⁻¹), and the intercellular concentration of carbon dioxide (CO_{2i}) (μmol CO₂ L⁻¹ air) were measured with the portable system for photosynthesis LCpro-SD ADC BioScientific equipped with an infrared gas analyzer, in specific environmental conditions in 2021, in March and June. PYL and CYL grown in sun-exposed branches and a clear bright day were used for a test between hours 10:00 AM - 12:00 AM, samples randomly selected (n= 15). Leaves were enclosed in the chamber (at ambient conditions), maintained 3 minutes for acclimation, then the readings have been recorded 15 s intervals for 3 min. During measurements, the photosynthetic photon flux density (PPFD) was also recorded. Moreover, the quantum yield (φCO₂) (A/PPFD), the water use efficiency (WUE)(A/E) and the ratio of transpiration rate to stomatal conductance (E/g_s) have been calculated.

Quantification of Photosynthetic Pigments

The amount of total chlorophyll (Chl T) (mg m⁻²) was estimated by the non-destructive method, using the CCM-300 Opti Sciences chlorophyll meter. The readings were taken for the adaxial needles side of 10 leaves, randomly selected from the hedge. After that, the same samples were used for leaves assimilatory pigments extraction in acetone 80%, by using 1 g needles per sample (Lichtenthaler, 1987). Then, the absorbance of the clarified extracts was measured using the spectrophotometer (Cecil CE 1021) at three wavelengths (663 nm to determine chlorophyll *a* - Chl *a*; 646 nm for chlorophyll *b* - Chl *b* and 470 nm for carotenoids). Afterwards, the content of assimilatory pigments was calculated by using the specific formulas reported by Lichtenthaler (1987). The obtained values were finally expressed as mg 100 g⁻¹ fresh weight (FW). The regression coefficient was calculated in the case of estimated Chl T and the calculated values for this parameter.

Statistical analysis

The reported data of all parameters represent the mean \pm standard error (SE). Statistically significant differences between variables were assessed using one-way analysis of variance (ANOVA). Then, the paired-samples Student T test (2-tailed) were accomplished and the significant differences among variants have been considered at $P \leq 0.05$. Pearson linear correlation analysis was also used to evaluate the relationship between some variables at 95%

confidence level. Graphs and tables were constructed using Microsoft Excel 2013.

RESULTS AND DISCUSSIONS

Morphological parameters at the leaf level

The macroscopic characteristics of leaves (length, width, and surface area) of different ages and grown in different lighting conditions (the photoperiod length for 24 hours) are shown in Table 1.

Table 1. *Taxus baccata* L. leaves morphological characteristics (current year leaves - CYL and previous year leaves - PYL) growth in normal light (day/night cycle) (NL) and in continuum light due to artificial light at night (ALAN) - June 2021

Leaf's age	Light disponible	Needle morphology				
		Leaf Length (LL)(mm)	Leaf Width (LW)(mm)	Surface area (SA) mm ² (cm ²)	Leaf area to leaf fresh weight (AFWR) (cm ² g ⁻¹)	Leaf length to width ratio (LWR)
CYL	NL	25.00\pm0.45 A**	3.12 \pm 0.08 A	60.60\pm2.34 (0.606) A***	4.35 \pm 0.17 A**	8.04\pm0.27 A***
	ALAN	21.80 \pm 0.73 B	3.10 \pm 0.04 A	47.80 \pm 0.73 (0.478) B	3.77 \pm 0.06 B	7.04 \pm 0.28 B
PYL	NL	19.80 \pm 0.37 A	3.26\pm0.19 A	46.00 \pm 1.52 (0.460) A	4.88\pm0.16 A**	6.14 \pm 0.28 B
	ALAN	21.40\pm0.68 A	3.00 \pm 0.00 A	50.20\pm3.65 (0.502) A	3.54 \pm 0.26 B	7.13\pm0.23 A***

Each value represents the calculated mean of five independent measurements \pm standard error; Means followed by different letters in the columns differ from each other by the Student T test at ***P < 0.001, **P < 0.01, *P < 0.05. T test was performed separately for CYL and PYL.

As we can see, the environmental light conditions generally had a significant effect on leaf morphology. The average leaf length was significantly higher for CYL/NL (25.00 mm), as well as SA and LWR had significantly higher values (60.60 mm²; 8.04) as compared to CYL-ALAN. On the other hand, for PYL - NL even if a higher LW (3.26 mm) was determined, non-significantly differences were noticed as against the PYL-ALAN ($P > 0.05$). The continuum light (ALAN) conditions generally determined a significantly reduction of AFWR, while for LL and LW the differences were not statistically significant as against NL conditions. It can be mentioned too, that for LWR, ALAN conditions determined significantly higher values (7.13) for PYL, as compared to PYL-NL (6.14).

Our obtained data are in a great measure consistent with those of Schirone et al. (2010) who noticed that Azorean yew leaf dimensions as means values were as followings: LL - 11.10 mm; LW - 2.30 mm, LA - 21.30 mm² and LWR - 7.7. On the other hand, after Dempsey and Hook (2000) studies, significantly smaller values were obtained for yew leaves: LL (7.7-22.4 mm); LW (1.3-2.3 mm); SA (11.2-48.4 mm²); stomatal pore length (11.1-18.2 μ m) and stomatal number (82.3-128.9). Also, the significant differences between provenances have been emphasized.

Previous studies carried out by Devaney et al. (2014) on the influence of light provided at different intensities (low light, medium light and high light) demonstrated that the morphology of yew leaves was influenced by

lighting conditions. Besides the specific dimensions of the leaves in relation to the lighting conditions, the studies conducted by Kwak et al. (2017) on the effects of providing ALAN led to certain conclusions regarding the presence of waxes, respectively at the level of the abaxial surface of the leaf, where a dense epicuticular layer of wax crystals has been produced compared to the control. Associated with this observation, a reduction in the degree of opening of the ostioles of the stomata was also estimated. At the same time, the negative impact of ALAN was manifested at the level of the chloroplast's ultrastructure, by increasing the number of thylakoid membranes per grana, as well as the number, respectively the dimensions of plastoglobules.

Studies on yew species conservation and restoration carried out by Zhao et al. (2017) have shown that prolonging the photoperiod (the extension of the photoperiod to 18 hours / day, compared to a natural photoperiod of 10.5 hours/day - Northeast China) for one year old *Taxus cuspidata* seedlings leads to accelerated growth in slow-growing species, as compared to those grown under the natural ones. However, there was a decrease in nutrient content in all organs of the plant, because for new growths the translocation of nitrogen (N) was insufficient, and annual organs of old shoot and root showed phosphorus (P) depletion. As a result, that promotion of growth in conditions of prolonged photoperiod and accumulation of dry mass in seedlings was not associated with an improvement in quality, given the dilution of nutrients and reduction of their use. So, the authors suggest the need for studies focused on the alleviate the nutrient dilution and to improve N and P utilizations in transplant of *T. cuspidata* seedlings.

The ability to adapt the leaves (formed in the previous year and in the current year) (on plants aged 3 years) to various conditions of light intensity was studied by Wyka et al. (2008). The authors did not find any anatomical changes in leaves formed in the previous year, but those in the current year kept in full light (HL) were thicker, with thicker palisade and lacunous tissue, and respectively with a higher

leaf mass relative to their surface, compared to providing 18% illumination, or 5% illumination.

Leaves gas exchange parameters and associated indicators

The obtained results as regard as of the leaf level gas exchanges and other associated parameters are shown in Table 2.

It can be observed that related to net photosynthesis (A) there are differences in the response of leaves to different light conditions, as well as depending on leaves ages (see values for March and June). Considerably lower photosynthesis values have been registered for PYL-NL ($1.03 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in June and the highest ones ($4.94 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) at NL in the springtime (CYL-NL), followed by those of CYL-NL (June - $3.93 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and $3.26 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for PYL-ALAN (June), respectively.

Transpiration rate (E) ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) presented similar values as evolution, with those determined in the case of photosynthesis, being registered differences in relation to the age of the leaves and the lighting conditions, respectively. The highest value ($2.74 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) was noticed for PYL-ALAN and there was a higher significantly difference as against the PYL-NL ($P < 0.001$). As it was expected, stomata conductance (g_s) ($\text{mols H}_2\text{O m}^{-2} \text{ s}^{-1}$) had also the same trend.

As regard as the intercellular carbon dioxide (CO_2i) the June determined values (around $320 \mu\text{mol L}^{-1}$) were not different from statistical viewpoint, but, in March significantly small value ($259 \mu\text{mol L}^{-1}$) was registered for CYL-NL, as compared to $284 \mu\text{mol L}^{-1}$ (CYL-ALAN).

In general, quantum yield (ϕCO_2) follows the same trend as photosynthesis intensity.

Also, the same trend can be observed for WUE, during March, with the significantly highest value for CYL-NL (3.93) as compared with those for CYL-ALAN (2.04) ($P < 0.01$), while in June values were small, without significantly differences between leaves growth in PYL-ALAN (1.20) or PYL-NL (1.66) ($P > 0.05$).

Table 2. *Taxus baccata* L. female previous year leaves (PYL) and current year leaves (CYL) gas exchanges and related indicators, growth under normal light (day/night cycle) (NL) and in continuum light due to artificial light at night (ALAN), in urban environment conditions - 2021

Indicators	March (means \pm SE; n = 10)		June (means \pm SE; n = 5)			
	1-year-old leaves (CYL)		1-year-old leaves (PYL)		current year leaves (CYL)	
	NL	ALAN	NL	ALAN	NL	ALAN
(A) $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	4.94 \pm 0.23 A ^(xxx)	2.29 \pm 0.24 B	1.03 \pm 0.34 B	3.26 \pm 0.20 a ^(xxx)	3.93 \pm 0.51 A (^{xx})	1.94 \pm 0.13 B
(E) (mmol H ₂ O m ⁻² s ⁻¹)	1.29 \pm 0.05 A ^(xx)	1.14 \pm 0.03 B	0.60 \pm 0.08 b	2.74 \pm 0.12 a ^(xxx)	1.62 \pm 0.05 A ^(x)	1.47 \pm 0.06 B
(g _s) (mol H ₂ O m ⁻² s ⁻¹)	0.05 \pm 0.003 A ^(xxx)	0.03 \pm 0.002 B	0.02 \pm 0.003 b	0.1 \pm 0.007 A ^(xxx)	0.05 \pm 0.003 A	0.04 \pm 0.003 A
(CO _{2i}) ($\mu\text{mol L}^{-1}$)	259 \pm 8.66 B	284 \pm 8.20 A ^(x)	325 \pm 12.73 a	326 \pm 4.51 a	317 \pm 12.06 A	325 \pm 12.73 A
(ϕCO_2) (A/PPFD)	0.0032 \pm 0.000141567 A ^(xxx)	0.0015 \pm 0.0001482 07 B	0.0006 \pm 0.0002 b	0.002 \pm 0.0001 a ^(xxx)	0.002 \pm 0.0003 A ^(xx)	0.001 \pm 7.88343E-05 B
(WUE) (A/E)	3.93 \pm 0.32 A ^(xx)	2.04 \pm 0.24 B	1.66 \pm 0.56 a	1.20 \pm 0.08 a	2.45 \pm 0.32 A ^(xxx)	1.32 \pm 0.06 B
(E/g _s)	27.89 \pm 1.03 B	42.88 \pm 3.52 A ^(xxx)	30.73 \pm 1.69 a	27.63 \pm 0.77 a	32.66 \pm 1.52 A	30.98 \pm 1.15 A

Legend: A = Net photosynthesis; E=Transpiration rate; g_s = Stomatal conductance; CO_{2i} =Intercellular CO₂ concentration; ϕCO_2 = Quantum yield; PPFD = Photosynthetic photon flux density; WUE = Water use efficiency; E/g_s = Ratio of transpiration rate to stomatal conductance

The data are shown as means \pm SE. The significant differences (P<0.05) between variants are indicated by different letters in the row: for June - in capital letters, the differences between the current year leaves; in small letters, differences between one-year leaves. In June light intensity was around 1532 $\mu\text{mol m}^{-2} \text{ s}^{-1}$.

NS - non-significant; *significance at $P \leq 0.05$; **significance at $P \leq 0.01$; ***significance at $P \leq 0.001$

On the other hand, very significantly higher values have been noticed at CYL-NL (2.45), as compared to CYL-ALAN conditions in June (1.32) (P<0.001).

Ratio of transpiration rate to stomatal conductance (E/g_s) had significantly higher value for CYL-ALAN in March (42.88) as compared to CYL-NL (27.89) (P<0.001), while in June no significant differences were registered for the leaves of the current year, but neither for those of one year old grown in different lighting conditions.

Leaves assimilatory pigments content

As can be seen in Figure 1a, the maximum values were recorded for chl *a*, followed by chl *b* and carotenoids, respectively, a characteristic situation known for plants in general. We notice that for the leaves from the previous year, the amount of total chlorophyll was significantly higher, with values of 113.22 mg 100 g⁻¹FW (PYL-ALAN) and, respectively, 108.70 mg 100 g⁻¹FW (PYL -NL), compared to the one determined in the leaves of the current year (83.16 mg 100g⁻¹ FW for CYL-ALAN, respectively, 79.89 mg 100g⁻¹ FW for CYL-NL). It is also worth noting that the total

chlorophyll content can be estimated by the non-destructive method, given the strong positive correlation ($R^2 = 0.8819$) between the results obtained by the spectrophotometric method (destructive) and those estimated using the chlorophyll meter CCM-300 (Figure 1b).

For chl *b*, significantly higher values were recorded for the leaves of the previous year, in lighting conditions assured also during the night (84.65 mg 100 g⁻¹FW- ALAN-PYL), compared to those specific to the leaves exposed to the normal lighting cycle (71.24 mg 100 g⁻¹FW- NL-PYL). Significantly lower values were determined for the leaves of the current year (27.12 mg 100 g⁻¹ FW-ALAN-CYL; 25.81 mg 100 g⁻¹FW- NL-CYL), without finding statistically significant differences between the latter values.

In the case of carotenoids, the trend was somewhat different, in the sense that significantly higher values were determined in the leaves of the previous year, in normal lighting conditions (25.38 mg 100 g⁻¹FW), and the lowest values characterized the leaves that were also exposed to lighting during the night (9.28 mg 100 g⁻¹FW). In contrast, for the current year's leaves, there were no significant

differences in relation to the lighting conditions to which the leaves were exposed.

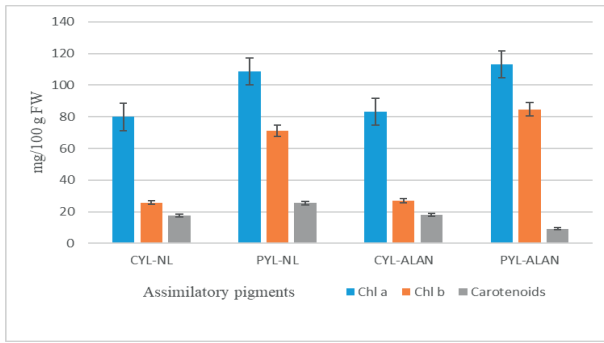


Figure 1a. Assimilatory pigments contents of *Taxus baccata* L. (current year leaves - CYL and previous year leaves - PYL) growth in normal light (NL) and in artificial night light (ALAN) - June 2021

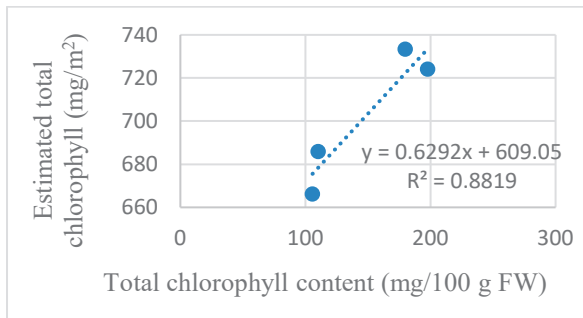


Figure 1b. Correlation between determined and estimated of total chlorophyll

As regard as the Chl *a*/Chl *b* ratio, the values were significantly higher in the case of CYL (3.09 and 3.06) as against to PYL (1.52 and 1.33) for NL, respectively ALAN conditions. With a view to estimate the linear relationship between two parameters, the Pearson correlation coefficients (*r*) were calculated, and values ranges between -1 and 1. As we can see in Table 3, there were generally strong significantly (positive or negative) correlations (values close to 1) between some indicators and ($P < 0.001$), while in some of them a moderately ($P < 0.01$) or slightly correlation ($P < 0.05$) has been registered. Also, nonsignificant correlations ($P > 0.05$) have been calculated in the case of (E) CYL-ALAN vs. (E) CYL-NL; (gs) CYL-ALAN vs. (gs) CYL-NL; (CO_2i) CYL-NL vs. (CO_2i) CYL-ALAN. Each of these last are related to the stomata behavior. When a negative number was calculated the indication was that an inversely proportional relationship between variables was noticed. Light influence on photosynthesis is manifested by light quantity (intensity), its quality (spectral

compositions), as well as its duration of action (Bennie et al., 2016). Despite the enhancing of photosynthesis rate in normal light conditions, the supplementary lighting practices are used, especially in greenhouses and plants factories, based on light emitted diodes (LED), with a view to enhance plants productivity and crop yield quality, too. Robakowski and Wyka (2009) studies showed that ensuring a high light intensity causes a decrease in the value of chlorophyll fluorescence (F_v/F_m below 0.8) (from November to May - study period), reaching even 0.37 in January and February, which means the manifestation of a process of photoinhibition during the winter at the level of *T. baccata* seedlings leaves subjected to acclimatization at different light intensities.

Nevertheless, in the case of urban lighting, which is necessary in the conditions of today's civilization and known to have many beneficial effects, from the biological point of view, artificial light during the night (ALAN) can be considered as an external polluting factor as well (Argys et al., 2020), which can have adverse effects not only on mammals behavior (Russart & Nelson, 2018; Cheon & Kim, 2020), but also on plant morphology and physiology (Singhal et al., 2018).

Studies performed for different plants by Meravi and Prajapati (2020) in relation to certain photosynthetic parameters determined using the Chlorophyll Fluorometer have highlighted that the normal physiological processes are disturbed in plants grown near the street lighting sources. If under normal living conditions, in general, for C3 type plants the quantum productivity has similar values (around 0.093) (Long et al., 1993 cited by Bauerle et al., 2020), the stressful lighting conditions can induce the process of photoinhibition, with a decrease of the photosynthesis efficiency, also with a decrease in dark respiration intensity.

As regard as the leaves age, as Bauerle et al. (2020) mentioned, in the new leaves that must build their own structure and ability to carry out physiological processes, there is often an intensification of photosynthesis, while with the achievement of leaves specific dimensions, for leaves of many plant species, a linear decline in the rate of this process is noticed, even if the growing conditions are favourable.

Table. 3. Pearson's linear correlation coefficients between different studied indicators and paired T-test, for *Taxus baccata* female leaves - June 2021

A. Current year leaves (CYL) (June)								
	(A) CYL-NL	(A) CYL-ALAN	(E) CYL-NL	(E) CYL-ALAN	(gs) CYL-NL	(gs) CYL-ALAN	(CO ₂ i) CYL-NL	(CO ₂ i) CYL-ALAN
(A) CYL-NL	1							
(A) CYL-ALAN	0.81**	1						
(E) CYL-NL	-0.15*	-0.40*	1					
(E) CYL-ALAN	0.72**	0.76**	-0.78 ^{ns}	1				
(gs) CYL-NL	-0.39***	-0.19***	0.73***	-0.77***	1			
(gs) CYL-ALAN	0.63***	0.62***	-0.78***	0.97***	-0.84 ^{ns}	1		
(CO ₂ i) CYL-NL	-0.50***	-0.52***	0.82***	-0.92***	0.85***	-0.98***	1	
(CO ₂ i) CYL-ALAN	-0.35***	-0.83***	0.38***	-0.45***	-0.18***	-0.30***	0.26 ^{ns}	1
B. Previous year leaves (PYL) (June)								
	(A) PYL-NL	(A) PYL-ALAN	(E) PYL-NL	(E) PYL-ALAN	(gs) PYL-NL	(gs) PYL-ALAN	(CO ₂ i) PYL-NL	(CO ₂ i) PYL-ALAN
(A) PYL-NL	1							
(A) PYL-ALAN	0.79***	1						
(E) PYL-NL	0.42 ^{ns}	0.45***	1					
(E) PYL-ALAN	-0.5**	0.12*	-0.1***	1				
(gs) PYL-NL	0.37**	0.33***	0.97***	-0.23***	1			
(gs) PYL-ALAN	-0.44*	0.17***	-0.06***	0.99***	-0.22***	1		
(CO ₂ i) PYL-NL	-0.64***	-0.43***	0.2***	0.27***	0.34***	0.18***	1	
(CO ₂ i) PYL-ALAN	-0.81***	-0.41***	-0.31***	0.82***	-0.42***	0.81***	0.28 ^{ns}	1

Values close to 1 mean a strong correlation, instead the value zero means the independence of variables. Values between 0-0.2 mean a weak relationship (yellow); those over 0.2 and lower or equal with 0.7 there is a moderately strong relationship (orange), and those that surpass 0.7 signify a very strong relationship (red) (in a positive direction, if the value is positive, or in a negative one if the value is negative) (Illowsky and Dean, 2017, cited by Baranyai, 2021)

***Correlation is significant at P < 0.001 level (2-tailed)

**Correlation is significant at P < 0.01 level (2-tailed)

*Correlation is significant at P < 0.05 level (2-tailed)

^{ns}Correlation is not significant at P > 0.05 level (2-tailed)

Similar results were obtained by Robakowski & Bielinis (2017) on different ages of *Abies alba* leaves, along a light gradient. The lowest values of assimilation (A) were recorded in the oldest leaves (2 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), while in the current year needles, A had the highest values. A similar trend was recorded in terms of transpiration rate, stomatal conductance, and water use efficiency. Devaney et al. (2015) pointed out that the photosynthetic capacity of yew needles depends primarily on lighting conditions, while the stage of development is a factor that has a lower impact on this physiological indicator, although the

morphological characteristics of the fruits are larger influenced by the size of the plant. Dong et al. (2015) following studies performed in wheat showed that in terms of biomass there were no significant differences between the variant that was provided with continuous light and that treated with intermittent light. In contrast, some photosynthetic characteristics in the case of intermittent light were superior to those of the control and the maximum sensitivity was recorded at flowering, when the effects of the light/dark cycle proved beneficial on the intensity of photosynthesis, transpiration rate, and implicitly on water use efficiency.

Our data shows that stomatal conductance (g_s) under ALAN conditions was highly significantly higher ($0.1 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) as against the normal light cycle ($0.02 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) in the case of one year old leaves (in June) demonstrating that ALAN mediated an increase of the leaf's stomatal conductance. As pointed out by Zhang et al. (2018) with the increase of the concentration of carbon dioxide in the air, it can be felt beneficial effects on plants, because of increasing its inflow into leaves, a process associated with decreased g_s , and not least with the increase of the values regarding the water use efficiency.

Regarding the total chlorophyll content, according to the data obtained by Robakowski et al. (2018), it changed over time, registering minimum values in March (4.3 mg/g), respectively maximum values in September (10.8 mg/g). Also, for carotenoids the seasonal trend was similar. Zarek (2016) studies performed on quantitative and qualitative analysis of assimilatory pigments (Chl *a*, Chl *b*, Chl *a/b* ratio) in female and male plants, in the dynamics led to obtaining significant differences between the two types, but larger during autumn and winter, while for protoporphyrin IX, magnesium protoporphyrin IX, protochlorophyllide, chlorophyllide *a*, and chlorophyllide *b*, there were no significant differences by sex and date of sampling.

As Wyka et al. (2008) noticed, there were changes in the content of assimilating pigments in the case of lower lighting, respectively an increase in their quantity, only that for those of the current year the changes were weaker. In addition, the Chl *a*/Chl *b* ratio was not affected. No significant effects on photosynthesis were determined, but the increased light intensity favored photochemical yield, photochemical quenching, apparent electron transport rate and inducible non-photochemical quenching (NPQ) in needles formed in the current season. For those formed in the previous year, the increased light intensity increased only NPQ. At the end of the experiment, the sensitivity to photoinhibition was tested by transferring the plants to intense light and determined a reduction of photoinhibition in those formed in the previous year and therefore almost a lack of their plasticity, compared to the leaves formed in the current year.

Studies carried out by Ffrench-Constant et al. (2016) have also shown ALAN influence on the timing of budburst in deciduous trees, with no significant effect of Defense Meteorological Satellite Program's Operational Linescan System (DMSP OLS) value on the species with earliest budburst (such as *Acer pseudoplatanus*), but significant negative influence in the other species, in order of budburst, *Fagus sylvatica*, *Quercus robur* and *Fraxinus excelsior*. In the brightest areas compared to the darkest areas, the date of budburst will be advanced by up to 7.5 days, so the phenology of woodland tree species is affected by light street pollution, with more pronounced negative effects on the smaller plants growing below the height of streetlights. The authors emphasize the need to study the impact of ALAN on phenology and interactions between species, including the effects of light quality generated by different light sources.

In the case of *Acer pseudoplatanus* and *Rhus typhina* species, proven to be very sensitive to light pollution, the delay of the phenological phases in autumn were 6 to 9 days in terms of leaf colouring and 6 to 7 days in terms of their fall, data that should be taken in consideration by the planning authorities (Škvareninová et al., 2017).

It is known that the pollination process can be affected in the case of ALAN. But, studies on wildflower (*Silene latifolia*) carried out by Macgregor et al. (2019) as regard as effects of two street lighting technology (in lamp type: from high-pressure sodium lamps to light-emitting diodes) and in lighting regime: from full-night (FN) to part-night (PN) lighting demonstrated that the pollination has been done both diurnally and nocturnally. The lamp type, the lighting regime, and the distance from the light all significantly affected aspects of pollination quality. Consequently, the authors confirmed that street lighting could affect plant reproduction through indirect effects mediated by nocturnal insects and recommend that novel lighting technologies should mitigate the effects of ALAN on ecosystems. In addition, as Macgregor and Scott-Brown (2020) pointed out future research directions are needed concerning on nocturnal pollination services (based plant interactions with insect pollen-vectors and the direct and indirect

vulnerabilities caused by anthropogenic activities) with a view to be fully understood and ultimately conserved.

The issue of the effects of ALAN on plants in general and of the changes regarding their phenology is in the attention of researchers, especially considering the climate changes and the urbanization process, so that analysing satellite data intensity across the United States are performed with great interest (Zheng et al., 2021).

CONCLUSIONS

Even if light is one of the main factors that influences photosynthesis, in modern society, excessive artificial light at night (ALAN) in the conditions of the urban environment can affect leaves' morphological characteristics, the gases exchanges (and associated physiological indicators), as well as the assimilatory pigments content

For the *Taxus baccata* L. (1-year old leaves -PYL and the current year -CYL ones) grown as a hedge:

(1) The leaves length of current year leaves, as well as their surface area and leaf length to width ratio were significantly lower under artificial light at night (ALAN), than in normal (day/night cycle) light (NL). For PYL, no significant differences were noticed in the case of LL, while significantly lower values have been registered in the case of LW and leaf area to leaf fresh weight (AFWR);

(2) Net photosynthesis (A) had significantly higher values during the springtime for PYL in NL as against ALAN, but opposite data have been noticed in June. The quantum yield (ϕCO_2), water use efficiency (WUE) as well as transpiration rate (T) and stomatal conductance (g_s) proved to be affected by the light conditions.

(3) The values for the content of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids were generally as those known for plants in general, with a higher amount of Chl *a* for PYL-ALAN, as compared to those for CYL-ALAN, or CYL-NL.

Further research should be applied by the scientific community on redesigning the exterior lighting as part of the urban landscape (Ardavani et al., 2020). Also, the researchers

must join the decision-makers and their joint efforts must be channeled towards intensifying the actions of monitoring and mitigate the effects of ALAN on plants and ecosystems (Marcantonio et al., 2015).

Lighting professionals, energy manager and governance workers need to make outdoor lighting more beneficial for Sustainable Development Goals (SDGs) (Tavares et al., 2021).

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