

## **IN SITU AND EX SITU FOLIAR RESPONSE REACTIONS OF SPONTANEOUS AND CULTIVATED TAXA OF THE GENUS *TAXUS* L.**

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**Abstract:** Photosynthesis, the process by which green plants synthesize carbohydrates from the carbon dioxide available in the environment using light energy as a fixed energy source with the help of photoassimilate pigments, underlies all food chains in nature. The good functioning of plants (in this paper, spontaneous and cultivated taxa of the genus *Taxus* L.) in their vegetation areas is influenced, at the same time, by the level of their relationship with the environment regarding water exchange, as a vital factor for the existence of the vegetation cover on Earth. As a result, the basic physiological processes (photosynthesis, transpiration) depend to a large extent on the anatomical and micro-morphological characteristics of their leaf apparatus, on certain internal factors relating to the leaf surface, and on a series of external factors, which manifest themselves in the environment of the taxa concerned, to which they have developed specific morpho-structural and functional adaptations.

**Keywords:** *Taxus*, anatomy, micro-morphology, physiological processes, biochemical parameters

### **1. INTRODUCTION**

Plant photosynthesis is a complex biological, physical, and chemical process in which plants convert light energy into chemical energy and carry out the synthesis of organic matter, which is the premise and basis of all plant physiological activities, whereby the level of photosynthetic efficiency reflects not only the efficiency of light energy utilization in plants but also their growth potential as well as their resilience to stresses in their environment (Iszkuło et al., 2010; Adams et al., 2018; Yin et al., 2022).

Through the constant exchange of substances and energy, plants are in intimate contact with their living environment, and the intensity of the photosynthetic process is based on the interaction of external factors and internal factors determined by the very nature of the plants that carry out this process. Thus, photosynthesis, as a physiological process, is regulated simultaneously by several external factors related to the quality of the plant living environment, such as light, temperature, humidity, availability of

CO<sub>2</sub> and other gases, and quantity and quality of nutrients (Teskey et al., 1995; Wieser, 2007), but also several internal factors that determine the rate of photosynthesis that can be achieved *in situ* during a growing season at a given ambient CO<sub>2</sub> concentration and under optimal conditions of temperature, light irradiance, water vapor pressure deficit, and soil water availability (Larcher, 2001; Wieser, 2007).

At current atmospheric CO<sub>2</sub> levels, photosynthesis of all C<sub>3</sub> plants, including conifers, may be limited by CO<sub>2</sub> input (Stenberg et al., 1995). The photosynthesis in C<sub>3</sub> plants is the result of concurrent processes in which CO<sub>2</sub> is fixed by carboxylation and released in photorespiration or mitochondrial respiration processes, for which the core molecule of the entire biochemical mechanism is the enzyme ribulose diphosphate carboxylase-oxygenase (Rubisco) (Wieser, 2007; Tcherkez & Limami, 2019).

Photosynthesis of C<sub>3</sub> plants has long been thought to be limited either by CO<sub>2</sub> diffusion (regulated by stomatal opening/closing) or by CO<sub>2</sub> fixation, which depends on leaf photochemistry and, or

photosynthetic enzyme function (Gago et al., 2019). Currently, according to the literature consulted, it is known that there is a third important factor that allows the diffusion of CO<sub>2</sub> from the substomatal cavities to the carboxylation sites inside the chloroplast stroma, namely the mesophyll conductance (Pons et al., 2009; Flexas et al., 2018).

*Taxus baccata* L. - yew - is a dioecious gymnosperm, occurring throughout Europe, with scattered populations extending as far as North Africa and the Middle East (Thomas & Polwart, 2003). The species is known to be one of the most shade-tolerant tree species in the European flora (Ellenberg et al., 1992; Thomas & Polwart, 2003; Iszkuło & Boratyński, 2006; Devaney et al., 2015); its seedlings survive in the heavy shade of mature yews and frequently grow under dense crowns of *Tilia*, *Acer*, *Carpinus*, *Picea*, and *Abies*, but also under lighter *Quercus* or *Pinus* canopies and even in full sun (Thomas & Polwart, 2003). Ecological barriers limiting its distribution in Europe are low temperatures in the north, the severe continental climate in eastern Poland, prolonged droughts in Turkey, and drought and high temperatures in northern Africa (Robakowski & Wika, 2009).

For ecological reasons, *Taxus baccata* L. is generally found in areas with oceanic-mountain climates and grows well in sheltered and shady conditions (forests) with relatively high atmospheric humidity. The species is also sensitive to drought (Șofletea & Curtu, 2007; Bîrsan et al., 2017). The species is classified as vulnerable and rare in Romania (Oltean et al., 1994), being a legally protected species and declared a monument of nature, being sporadically distributed in both pristine beech forests and mixed conifer and beech forests (Sârbu et al., 2013).

This paper aims to highlight some aspects of the biology of some taxa of the genus *Taxus* L. spontaneous and cultivated in the NE of the country according to specific influencing factors (internal, leaf surface, and external factors) through anatomical, micro-morphological, physiological, and biochemical determinations carried out during three phenological phases of its ontogenetic cycle.

## 2. MATERIAL AND METHODS

### 2.1. Biological material

The biological material analyzed is represented by leaves from female yew individuals belonging to the spontaneous species *Taxus baccata* L. (T1) from the Yew Reserve - Tudora Forest, Botoșani County (lat. 47.524909° N, long. 26.691887° E, alt. 444 m) and from cultivated taxa: *Taxus baccata* (T2) and *Taxus baccata* 'Robusta' (T3), obtained from the nursery

S.C. Doropad S.R.L. Suceava, based in Dorohoi and cultivated in Vorniceni commune, Botoșani county (lat. 47.986328° N, long. 26.663299° E, alt. 185 m). Vouchers for each taxon were submitted, confirmed, and registered at the herbarium of the Faculty of Biology of the University "Al. Ioan Cuza" the University of Iasi with identification numbers 186539 - T1, 186537 - T2, 186538 - T3.

The research was carried out in two directions: field and laboratory analysis. In the specimens under study, determinations of the main physiological processes (photosynthesis, transpiration) were carried out in the ground, "in vivo" at certain defining moments of the ontogenetic cycle carried out during the 2021 calendar year, respectively in April (IV), June (VI) and September (IX). These months were chosen because they represent specific phenological phases, namely strobili production in spring, intense vegetative growth in summer, and biomass allocation to roots in autumn, according to seasons defined by Robakowski et al., 2018. We point out that no investigations were carried out during the winter resting period due to the specific weather conditions of winter 2021 in the Yew Reserve in Tudora Forest (heavy snowfall, strong wind, and consistent snow cover, which did not allow access to the field).

For further laboratory analysis, fresh material (shoots/branches) was collected and processed in the research facilities of the Faculty of Biology of "Alexandru Ioan Cuza" University of Iasi, using equipment provided by the Plant Biology Laboratory and the Integrated Centre for Environmental Science Studies for the North-East Development Region (CERNESIM), organized with funds obtained through grant No. 257/28.09.2010, SMIS/CNR 13984/901.

### 2.2. Research Methods and biological parameters

#### 2.2.1. Leaf morphological parameters

Morphological measurements were performed on fresh leaves. Twenty needles from one-year-old shoots of each investigated taxon were separated from the stems and scanned with the handheld compact AM 300 ADC Bioscientific determination machine, used to accurately determine leaf area (LA) (mm<sup>2</sup>) and associated parameters: length (LL) (mm<sup>2</sup>), width (LW) (mm<sup>2</sup>) and the leaf length to width ratio (LWR) were calculated.

#### 2.2.2. Micromorphological and histo-anatomical determinations

Leaf samples prepared by the *air-drying* method were examined using a scanning electron microscope (Tescan Vega II SBH) using the VegaTC Software program, taking photographs of both leaf surfaces

(adaxial and abaxial) (Hynninen et al., 2018). Histo-anatomical analysis of the plant material was processed according to the Academician Constantin Toma Laboratory of Plant Morphology and Anatomy protocol by the double staining method with iodine green and ruthenium red. Observations were carried out under an optical microscope (Euromex bScope BS.1153Pli) with photographs taken using the built-in camera, Euromex HD Ultra DC 1357.

### 2.2.3. Physiological parameters

For *in vivo* investigations to record specific functional parameters at leaf level - photosynthetic rate (A) ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and transpiration rate (E) ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), the degree of illumination available to the plant photosynthetic apparatus (Qleaf) ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ), leaf surface temperature (tleaf) ( $^{\circ}\text{C}$ ), substomatal  $\text{CO}_2$  (Ci) (vpm) and stomatal water conductance (gs) ( $\text{mol m}^{-2} \text{ s}^{-1}$ ), the portable LCi Photosynthesis System (ADC BioScientific, 2007) equipped with an infrared gas analyzer was used under specific environmental conditions in 2021. At the same time, water use efficiency (WUE) (A/E) and transpiration rate to stomatal conductance ratio (E/gs) were calculated for all investigated taxa at each time point of the proper ontogenetic cycle specified above.

The system displays the calculated values on the screen following repetitive internal calculations using newly recorded parameters and various correction factors. This produces results and intermediate values for the different photosynthetic parameters derived from the formulas established according to the LCi user guide. Registration of functional parameters was performed on clear and bright days, between 10:00 AM and 12:00 AM, on a sample of 15 one-year-old leaves randomly chosen for each individual analyzed. Leaves of the investigated taxa were enclosed in the leaf chamber under ambient conditions, held for 3 min for acclimatization, then readings were recorded at 15-second intervals for 3 min. At the time of investigations, air temperature (T) ( $^{\circ}\text{C}$ ) and atmospheric humidity (RH) (%) were measured using a thermos-hygrometer (Testo 625).

### 2.2.4. Photoassimilatory pigment assay

Leaf photoassimilate pigments were extracted in 80% acetone using 1g of fresh plant material per sample (Lichtenthaler, 1987). The absorbance of the extracts was measured by Shimadzu spectrophotometer at three wavelengths (663 nm for chlorophyll a - Chl a; 646 nm for chlorophyll b - Chl b; 470 nm for carotenoid pigments - Car). Subsequently, the content of assimilatory pigments was calculated using specific formulas reported by Boldor et al., 1983; Lichtenthaler et al., 1987; Pora et

al., 1989; Sumanta et al., 2014. Analyses were performed in replicates for each sample, and the mean values obtained were finally expressed in  $\text{mg g}^{-1} \text{ DW}$ .

## 2.3. Statistical analysis

The data reported for all parameters represent the mean value  $\pm$  standard error (SEM). Statistically significant differences between variables were assessed using one-way analysis of variance (ANOVA); Student's t-test with paired samples was also performed for the statistical interpretation of morphological parameters. Significant differences between seasons or variants were considered at  $p \leq 0.05$ . Tables were made in Microsoft Excel 2019.

## 3. RESULTS AND DISCUSSION

### 3.1. Leaf morphological parameters

According to the literature (Hageneder, 2013), the needle-shaped leaves of the tree vary in length: they are usually 16-25 mm long, but in some specimens, they can be short - 10 mm or long - 45 mm, and their usual width is 2-3 mm. The practical determinations made (Table 1) confirmed the data presented in the literature (Dempsey & Hook, 2000; Perrin & Mitchell, 2013), revealing that the spontaneous specimens from the reserve (**T1**) clearly show high plasticity in terms of leaf morphology, with shade-grown plants having almost double the leaf area of the unshaded cultivated specimens **T2** and **T3**. The higher leaf area recorded in **T1** specimens, growing spontaneously in the Tudora forest, together with various deciduous species, represents a way of adapting them to the special conditions of light exploitation, reduced in quantity and modified in quality by the richly jointed canopy of the accompanying trees, which prevents the direct penetration of light to the leaves of the yew specimens.

The literature consulted indicates that *Taxus baccata* is an unusual species among gymnosperms, being both shade and drought-tolerant (Thomas & Polwart, 2003; Niinemets & Valladares, 2006; Perrin & Mitchell, 2013). Increased levels of shading result, in specimens of this species, in the formation of a larger leaf area, with leaves that develop under intense shading being both longer and leaf widths, compared to leaves arranged in reduced shade or unshaded, which is also observed in our investigations, where leaves of **T1** specimens show the highest values of all morphological parameters investigated, in contrast to those of **T2** and **T3** individuals growing in an open area (with reduced shade).

Leaf area is an indicator of the degree of light in which leaves have grown, with increases in this

parameter being reported in plants (conifer species) generally grown under low light conditions, covering a wide range of tolerance to shade conditions (Mitchell, 1998; Reich et al., 1998; Awada et al., 2003; Qu et al., 2005; Perrin & Mitchell, 2013). Other research in conifers (Khan et al., 2000; Qu et al., 2005) also indicates that increased shading levels resulted in reduced total seedling dry weight, with biomass allocation shifting with increased shading from roots to leaves and shoot, as a characteristic survival strategy of plants acclimatized to shaded conditions (Givnish, 1988; Perrin & Mitchell, 2013).

Table 1. Morphological characteristics of *Taxus baccata* L. leaves in the intense vegetative growth phase (VI) (Each value represents the calculated mean of twenty independent measurements  $\pm$  standard error; only statistical differences have been marked with asterisks: \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ ; \*\*\*\* =  $p < 0.0001$ )

Needle morphology	Test specimens		
	T1	T2	T3
SA (mm <sup>2</sup> )	73.35 $\pm$ 3.17 ****	38.05 $\pm$ 1.51 ****	50.65 $\pm$ 2.8 ***
LL (mm)	27.28 $\pm$ 0.16 ****	16.75 $\pm$ 0.51 ****	19.84 $\pm$ 0.74 **
LW (mm)	3.36 $\pm$ 0.09 **	2.94 $\pm$ 0.08	3.26 $\pm$ 0.11 *
LWR	8.19 $\pm$ 0.24 ****	5.77 $\pm$ 0.21 ****	6.16 $\pm$ 0.25

Legend: SA = leaf area; LL = length; LW = width; LWR = length - width ratio

### 3.2. Leaf micro-morphological and histo-anatomical parameters

Leaf light-harvesting capacity for photosynthesis is determined by the interaction between the optical properties of leaves and their biological and physiological biochemical potential. Several structural features of gymnosperm leaves influence their optical property, and the interpretation of photosynthetic response to photosynthetically active radiation (PAR: 400 - 700 nm) (McCree, 1972; Clark & Lister, 1975a; 1975b; Oguchi et al., 2018) is based on an understanding of the interplay between leaf structure and function (Stenberg et al., 1995). Although much is known about the morphology and histology of conifer needles, and considerable effort has been made to characterize the photosynthetic

responses of many species in this group, few attempts have been made to relate needle leaf structure to physiological performance (photosynthesis and transpiration).

In the histo-anatomical investigations carried out on the leaves of the three yew variants examined (**T1**, **T2**, and **T3**), we found several similarities which also confirm the data already existing in the literature consulted (Baker, 1974; Günthardt & Wanner, 1982; Hunt & Baker, 1982; DeLucia & Berlyn, 1984; Stenberg et al., 1995; Spjut 2007a; Spjut 2007b; Ghimire et al., 2014; Frunzete et al., 2022), but also some differences, which particularize the variants.

The similarities relate to: - the presence of stomata only in the lower epidermis (hypostomatic lamina); - the arrangement of stomata in rows forming bands of variable widths (5-7-8-9 stomata) on either side of the midrib; - the complex organization of the stomatal apparatus and the presence of Florin's ring); - the absence of lignified sclerenchyma hypodermis; - the secretory ducts are absent; - the organization of the leaf vein; - the discrete presence of transfusion parenchyma in the lateral parts of the vascular bundle of the vein; - the cells of the lower epidermis are papilliform.

The differences, which we would prefer to call structural variations, refer to: - the unequally equifacial bifacial structure, at least at the two leaf exteriors at **T2** (Figure 1, a) versus the established hetero-facial bifacial structure at **T1** and **T3** (also consistently mentioned in the existing literature); - the mesophyll with unistratified palisadic tissue, with tall cells at **T1** (Figure 1, b) and bistratified, sometimes tristratified, palisadic tissue at **T3** with low cells (Figure 1, c); - quantitative disproportion between phloem and xylem of vascular bundle in favor of the former at **T3** (Figure 1, d).

Esau, 1977; Stenberg et al., 1995, show that the photosynthetic mesophyll of acicular leaves of some conifer species such as *Abies*, *Sequoia*, *Taxus*, and *Torreya* is differentiated into palisading and lacunose tissue, while in other species (*Pinus*, *Picea*) it remains undifferentiated, with leaves developed under high light conditions (sun leaves) also showing additional mesophyll layers (Turrel, 1936; Nobel et al., 1975; Stenberg et al., 1995), which gives them a robust, specific character.

Scanning electron microscopy (S.E.M.) investigations indicate that the micromorphology of the upper epidermis is relatively similar in all variants (**T1**, **T2**, and **T3**): the epidermal cells are long, rectangular, unequal in size, with straight lateral walls and outer walls covered by a smooth, thin cuticle on which sparse local filiform deposits of epicuticular wax are observed.

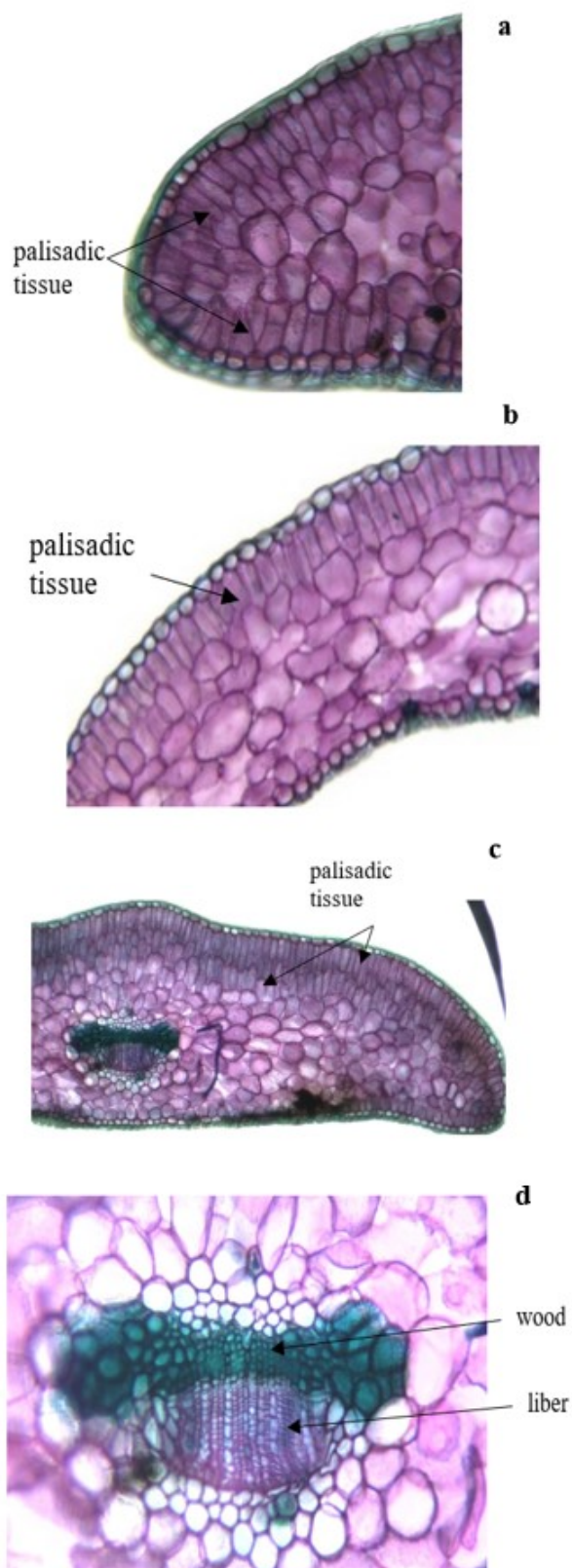


Figure 1. Cross-section through the leaf of *Taxus* sp. (wood=xylem; liber=phloem)

Because the cuticle covering the leaf is almost water-impermeable, most leaf transpiration is achieved through a diffusion process of water vapor

through the stomatal pore. This provides a low-resistance pathway for the diffusional movement of gases through the epidermis and cuticle. Stomatal resistance changes are relevant for regulating water loss by the plant and for controlling the rate of carbon dioxide uptake required for sustained carbon fixation during photosynthesis (Taiz et al., 2015).

The organization of the lower epidermis is in all three variants architecturally influenced by bands of stomata located on either side of the midrib, an arrangement otherwise characteristic of the genus *Taxus*, much different from that found in the genus *Pinus* (Goodman & Walsh, 2001; Kozłowski et al., 2019). In the **T3** cultivar (Figure 2), however, there is an asymmetry in the arrangement of the epidermal structures about the midrib: both stomatal bands are at different distances from the lateral leaf margins, which may be related to the phenomenon of fluctuating asymmetry in response to environmental stressors (De Coster et al., 2013).

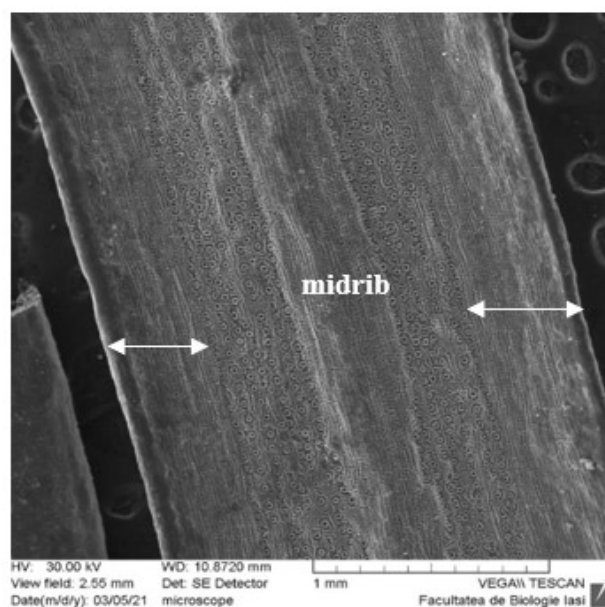


Figure 2. Organization of stomata on the lower epidermis

The stomatic apparatus in yew is located in the lower epidermis, minimizing air movement and water vaporization, except that the epicuticular wax, which helps regulate water evaporation in other conifers, is absent. The presence of elevated subsidiary cells around the stomata in yews controls, by enlarging and shrinking them, stomatal opening and hence gas exchange, as well as the release of water by evaporation, as a functionally important adaptation during periods of drought, as the stomatal apparatus is capable of extremely rapid responses to changing weather conditions (Dempsey & Hook, 2000; Thomas & Polwart 2003; Hageneder, 2013).

### 3.3. Physiological parameters related to internal and external factors

The process of photoassimilate is regulated simultaneously by several factors: factors that intervene at the level of the photosynthetic apparatus (amount of light incident on the leaf –  $Q_{leaf}$  and leaf temperature - leaf) as well as environmental factors available to plants during their ontogenetic cycle: quantity, quality, and duration of light, water quantity, ambient heat, carbon dioxide content, and other atmospheric gases; these factors, together with carbohydrate demands from absorption reservoirs, can induce direct effects on biochemical processes in the photosynthetic pathway. These, in turn, are caused indirectly by changes in specific non-photosynthetic processes, which in turn change the rate of photosynthesis, (Teskey et al., 1995).

From the analysis of the practically obtained data (Table 2), it can be deduced that the **photosynthetic rate (A)** shows differences in values as a response to the leaves of specimens belonging to the investigated taxa in the three analysis times, namely: spring (IV), summer (VI) and autumn (IX). It is observed that the leaves of specimens of cultivated taxa **T2** and **T3** show higher values compared to the leaves of spontaneous taxon **T1** in all three research stages, with the highest values being observed in spring, when the strobili are formed (Zarek, 2016). According to the literature, net photosynthetic performance in *Taxus* is extreme under optimal growing conditions, with trees unable to utilize high-light exposure effectively. Hence, in summer periods when temperatures are high, a tree loses a lot of energy due to an intense respiratory process and its inability to balance this loss with more efficient photosynthesis.

However, yew compensates by photosynthesis during winter, when there is more moisture in the soil and lighter in the environment because deciduous trees in the upper story are leafless, so it creates additional energy stores, which it conserves until the middle of the warm season of the following year (Pietzarka, 2005). Another factor that may limit the photosynthetic rate of this species is the age of the leaves: literature data show that yew leaves live between four and eight years, but their photosynthetic capacity decreases with age, reaching only 50% for seven-year-old needles compared to younger ones (Hageneder, 2013).

In turn, the stomata represent an essential pathway for gas and water exchange in plants as a powerful influence on leaf characteristics associated with photosynthesis and transpiration, as they control leaf apparatus temperature and water use efficiency

(WUE) and are vital to the plant's existence. The characteristics of the stomata are influenced to a large extent by environmental conditions; thus, research (Beerling & Chaloner, 1993; Woodward & Kelly, 1995; Li et al., 2017) demonstrated that an increase in  $CO_2$  concentration and atmospheric air temperature causes a decrease in stomatal density. Under conditions of short-term water stress, plants increase their WUE by reducing stomatal aperture and hence transpiration rate as an adaptation to drought stress and adjust the relationship between water, transpiration, photosynthesis, and WUE through stomatal changes to maximize  $CO_2$  assimilation (Li et al., 2017).

The removal of water from the plant body in the form of vapor (the transpiration process), although dependent on atmospheric humidity, is closely linked to the structure and functions of the plant. In woody plants, it occurs through all tree organs except the root system via the stomata (about 81% of the process), the cuticle (in conifers with an intensity of 12-15 mg  $H_2O/dm^2/hour$ , a value representing about 3% of total transpiration, according to Burzo & Dobrescu, 2005) and the lenticels.

In the biological material analyzed, the **transpiration rate (E)** (Table 2) shows significant differences in spring (IV), when strobili formation takes place, and in autumn (IX), when biomass allocation to roots takes place. The lowest values of transpiration rate were recorded in the leaves of taxon **T1** (0.78 mmol  $H_2O\ m^{-2}s^{-1}$ ; 0.86 mmol  $H_2O\ m^{-2}s^{-1}$ ), and the highest in the leaves of taxon **T2** (2.15 mmol  $H_2O\ m^{-2}s^{-1}$ ); at the same time, a general trend of reduced water loss at leaf level was observed in the leaves of taxon **T3**, in which during the autumn, when biomass allocation to the roots takes place, the water content decreases in favor of a gain of dry matter, accumulated through the process of photosynthesis.

The physiological processes of photosynthesis and transpiration are influenced to a greater or lesser extent by several factors acting on the leaf surface or in the environment (environmental factors). Thus, from the investigations carried out on the taxa studied, we can state that leaf-level factors influence the intensity of photosynthesis and transpiration processes; among these, the main factor influencing the photosynthesis process intensity is **the amount of light on the leaf ( $Q_{leaf}$ )**. The research carried out practically deduced that the lowest values of leaf light quantity occur in yew specimens belonging to taxon **T1**, which vegetates spontaneously in the Tudora Forest Yew Reserve at all times of analysis, because the other deciduous individuals in the forest limit the direct penetration of sunlight to the leaves of yew



Table 2. Physiological parameters correlated with leaf factors in *Taxus baccata* L.

Indicators	SEASONS								
	IV - SPRING (strobili production)			VI – SUMMER (intensive vegetative growth)			IX – AUTUMN (biomass allocation to roots)		
	(means $\pm$ SE; n = 15)			(means $\pm$ SE; n = 15)			(means $\pm$ SE; n = 15)		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
<b>A</b> ( $\mu\text{moli CO}_2$ $\text{m}^{-2}\text{s}^{-1}$ )	<b>3.68</b>	<b>8.81</b>	<b>8.97</b>	<b>2.54</b>	<b>3.85</b>	<b>6.16</b>	<b>2.94</b>	<b>4.29</b>	<b>6.39</b>
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.66	0.68	1.95	0.34	0.63	1.15	0.54	0.54	0.96
	**			**			**		
<b>E</b> (mmol H <sub>2</sub> O $\text{m}^{-2}\text{s}^{-1}$ )	<b>0.78</b>	<b>1.96</b>	<b>1.65</b>	<b>1.12</b>	<b>1.32</b>	<b>1.23</b>	<b>0.86</b>	<b>2.15</b>	<b>1.41</b>
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.08	0.12	0.16	0.16	0.10	0.14	0.11	0.17	0.14
	****			****			****		
<b>Qleaf</b> ( $\mu\text{moli m}^{-2}\text{s}^{-1}$ )	<b>81.67</b>	<b>445.13</b>	<b>814.20</b>	<b>55.53</b>	<b>583.6</b>	<b>1580.40</b>	<b>19.20</b>	<b>155.73</b>	<b>525.20</b>
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	16.36	58.82	123.01	7.44	132.69	129.24	2.47	13.42	112.31
	****			****			****		
<b>tleaf</b> (°C)	<b>25.01</b>	<b>28.16</b>	<b>28.15</b>	<b>23.79</b>	<b>32.03</b>	<b>29.33</b>	<b>24.86</b>	<b>29.99</b>	<b>28.15</b>
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.20	0.27	0.37	0.15	0.16	0.71	0.15	0.06	0.42
	****			****			****		
<b>Ci</b> (vpm)	<b>304</b>	<b>220.40</b>	<b>271.80</b>	<b>382.53</b>	<b>270.27</b>	<b>251.73</b>	<b>375.40</b>	<b>303.80</b>	<b>278.93</b>
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	38.09	13.70	26.36	26.95	27.11	39.23	32.41	21.28	22.02
				*			*		
<b>gs</b> (mol $\text{m}^{-2}\text{s}^{-1}$ )	<b>0.04</b>	<b>0.08</b>	<b>0.07</b>	<b>0.08</b>	<b>0.04</b>	<b>0.04</b>	<b>0.05</b>	<b>0.10</b>	<b>0.06</b>
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.005	0.006	0.008	0.014	0.004	0.005	0.006	0.009	0.006
	***			*			***		
<b>WUE</b>	<b>5.20</b>	<b>4.51</b>	<b>5.10</b>	<b>3.77</b>	<b>3.05</b>	<b>5.15</b>	<b>3.74</b>	<b>2.39</b>	<b>5.45</b>
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.97	0.29	0.89	0.82	0.53	1.05	0.60	0.25	1.18
							*		
<b>E/gs</b>	<b>20.86</b>	<b>24.61</b>	<b>25.09</b>	<b>16.87</b>	<b>34.19</b>	<b>29.49</b>	<b>19.14</b>	<b>22.97</b>	<b>24.36</b>
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.97	0.86	2.52	3.23	2.19	1.80	1.39	0.68	3.52
				****					

Legend: **A** = Photosynthesis rate; **E** = Transpiration rate; **Qleaf** = P.A.R. incident on leaf surface; **tleaf** = Leaf surface temperature; **Ci** = Sub-stomatal CO<sub>2</sub>; **gs** = Stomatal conductance of H<sub>2</sub>O; **WUE** = Water use efficiency; **E/gs** = Ratio of transpiration rate to stomatal conductance; only statistical differences have been marked with asterisks: \* = p<0.05; \*\* = p<0.01; \*\*\* = p<0.001; \*\*\*\* = p<0.0001.

trees, and the highest values are recorded in the leaves of specimens of taxon **T3**, which vegetates in full sun. Although **Qleaf** in the latter case is the highest, namely 1580.93  $\mu\text{moli m}^{-2} \text{s}^{-1}$  of the values recorded for all taxa analyzed in summer (VI), the intensity of the photosynthesis process is lower compared to that achieved by the same individuals in spring (IV), when the mean value of the **Qleaf** parameter was 814.20  $\mu\text{moli m}^{-2} \text{s}^{-1}$ .

Incident light at the leaf level (**Qleaf**) and ambient light (penetrating through the canopy) directly influence the photosynthesis process; it acts as a regulatory factor through intensity, quality (spectral composition), and duration (Bennie et al., 2016; Delian

et al., 2022). Shaded leaves typically capture ambient light more efficiently (Kull & Koppel, 1987; Teskey et al., 1995), yet such leaves have a lower capacity to carry out subsequent light energy conversion reactions compared to light-exposed leaves because shaded leaves have proportionally more nitrogen in thylakoids than in ribulose diphosphate carboxylase compared to sunny leaves (Yushkov & Zav'yalova, 1989; Teskey et al., 1995).

The transpiration process is affected by several internal factors due to which, under the same environmental conditions, the intensity of water removal is not the same in all woody plants. Among the internal factors involved in the regulation of this

process are the following: leaf surface, which intensifies transpiration in the case of resinous plants, even if their leaves have structures adapted to reduce it; leaf orientation - leaves placed parallel to the direction of the sun's rays transpire less, compared to those in a perpendicular position; the number of stomata - according to Grudnicki, (2006) resinous species have between 90-130 stomata/mm<sup>2</sup>; the arrangement of stomata on the leaf epidermis and their degree of openness; plants regulate the intensity of leaf transpiration by mechanisms of closure and opening of the ostioles, controlled by internal factors such as the presence of chloroplasts and external factors such as light and water.

**Leaf temperature (tleaf)** is, together with leaf light intensity, a relevant factor that determines the change in the photosynthesis and transpiration intensity directly proportional to its variation in value. In the practical determinations (Table 2), the lowest values of this parameter are recorded in the leaves of spontaneously growing yew specimens (**T1**), in which the intensity of photosynthesis and transpiration is also lower than in the leaves of cultivated taxa **T2** and **T3**. It is worth noting that in the leaves of specimens of taxon **T2**, even though this factor registers the highest value (32.02°C), it is observed that the intensity of photosynthesis is inversely proportional to the leaf temperature, presenting higher values in spring. The same cannot be said for the transpiration rate, which increases directly proportional to leaf temperature, except for the value recorded in the leaves of **T2** taxon specimens during autumn, when the transpiration rate is higher (2.15 mmol H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup>), even though the leaf temperature is lower than in summer.

Analyzing, at the same time, the quantitative variation of **sub stomatic CO<sub>2</sub> (Ci)**, it is observed that in the leaves of the spontaneous taxon **T1**, the intensity of the photosynthesis process decreases in value with the increase of the sub stomatic CO<sub>2</sub> concentration, while a reduction of this parameter leads to an intensification of the photosynthetic process in the leaves of the cultivated taxa **T2** and **T3**, which leads us to the interpretation that, in addition to factors acting on the leaf surface, environmental factors such as atmospheric CO<sub>2</sub> concentration are also involved in the photosynthesis process.

At the current levels of CO<sub>2</sub> in the atmosphere, photosynthesis of all C<sub>3</sub> plants, including conifers, may be limited by its contribution because the carboxylation enzyme Rubisco also functions as an oxygenase - competition between CO<sub>2</sub> and atmospheric O<sub>2</sub> for its active sites. Although enzyme data on photosynthesis in conifers are sparse, it appears that the kinetic characteristics of Rubisco, including its affinity for CO<sub>2</sub>, are similar to those of spinach

(Weidner & Kraus, 1987; Gezelius, 1975; Beadle et al., 1983; Teskey et al., 1995).

Another parameter analyzed was **stomatal conductance (gs)**, usually measured in mmol m<sup>-2</sup> s<sup>-1</sup>. According to the definition, this parameter conditions the net molar flux of CO<sub>2</sub> entering or water vapor exiting through the stomata of a leaf, calculated for a given CO<sub>2</sub> or water vapor concentration difference between the atmosphere and the stomatal cavity; as a result, molar fluxes are conditioned by both CO<sub>2</sub> on net assimilation rate and water vapor in transpiration intensity.

Stomatal conductance is often strongly correlated with the photosynthetic rate (Wong et al., 1979; Lawson et al., 2018), ensuring an appropriate balance between CO<sub>2</sub> uptake for photosynthesis and water loss through transpiration (water that is essential for translocation as well as evaporative cooling). However, such observations often refer to long-term or steady-state measurements, as short-term environmental perturbations (irradiance, for example) often lead to a spatial and temporal mismatch between stomatal behavior and photosynthesis (Kirschbaum et al., 1988; Tinoco-Ojanguren & Pearcy 1993; Lawson & Weyers 1999; Lawson et al., 2010; McAusland et al., 2015; Lawson et al., 2018).

For example, rates of photosynthesis respond to changes in light within seconds of a change in intensity, whereas changes in stomatal conductance take from a few seconds to tens of minutes (Kirschbaum et al., 1988; Lawson et al., 2010), and when light intensity increases, lags and the slow nature of the stomatal response can result in low gs, which restricts CO<sub>2</sub> diffusion into the leaf, thereby limiting photosynthesis (McAusland et al., 2016; Lawson et al., 2018; Xiong & Flexas, 2020).

Discussing this specific functional parameter, the literature states that a fundamental challenge for terrestrial plants is to strike a balance between the benefits of CO<sub>2</sub> uptake and the risk of dehydration resulting from concomitant water loss through transpiration (de Boer et al., 2016; Leakey et al., 2019; Xiong & Flexas, 2020). In theory, stomatal conductance (gs) is determined by the stomatal density and the diffusion efficiency of individual stomatal pores. In mature leaves, anatomical stomatal features are nearly fixed and leaves adjust the stomatal aperture to achieve real-time stomatal conductance within a range between near zero and anatomically determined maximum stomatal conductance. Maximum stomatal conductance is calculated in practice using stomatal density and anatomical stomatal pore parameters, suggesting that high maximum stomatal conductance would necessarily be related to small stomatal pore size and high stomatal density (Franks & Farquhar, 2001; Franks & Beerling,



2009; Sack & Buckley, 2016; Xiong & Flexas, 2020). But regardless of stomatal characteristics and their distribution on the leaf surface, the literature clearly states that the stomatal opening state during photosynthesis depends on the plant's ability to replace water evaporated into the atmosphere to prevent leaf dehydration (Xiong & Flexas, 2020).

Recent studies have analyzed the stomatal conductance of fast-growing tree species to identify the water consumption of different species. This research concluded that leaf water potential before sunrise remained constant over the months, while leaf water potential at midday showed seasonal variation. For example, the stomatal conductance of the canopy had a higher water potential in July compared to October. Research in that experiment suggested that stomatal conductance allowed constant water use per unit leaf area (Zhu et al., 2015).

Other studies have looked at the relationship between drought stress and stomatal conductance. Through these experiments, researchers concluded that a drought-tolerant plant regulates its transpiration rate through stomatal conductance to minimize water loss and allow the plant to survive under low environmental water levels (Li et al., 2017).

At the leaf level, the measurement of stomatal CO<sub>2</sub> conductance and CO<sub>2</sub> flux in the leaf can be used to assess the rate limitation of biochemical reactions of photosynthesis, for example, the rate of CO<sub>2</sub> diffusion through stomata versus the biochemical limitations of photosynthesis. Recent relative research has concluded that in conifers grown under conditions of efficient water and nutrient supply, the photosynthesis process is enhanced, and stomatal conductance is reduced under long-term CO<sub>2</sub> enrichment (Eamus & Jarvis, 1989; Teskey et al., 1995). However, according to the results of research on *Pinus ponderosa*, (Surano et al., 1986; Teskey et al., 1995) is likely that higher leaf temperature and better heat resistance of plant individuals lead to progressive stomatal closure, which in turn is induced by the amount of CO<sub>2</sub> in the environment and leads to progressive reductions in transpiration. During the field research on the yew

specimens under study, in parallel with *in vivo* determinations of the fundamental physiological processes (photosynthesis and foliar transpiration), environmental factors with direct effects on them were also recorded: temperature (°C) and relative air humidity RH (%) (Table 3).

According to the literature, data consulted, the optimal air temperature range for photosynthesis in *Taxus baccata* specimens is between 14 and 25°C, the highest of all the literature ranges for gymnosperms, with a maximum in summer of 38 - 41°C (Piesek et al., 1969; Thomas & Garcia-Martí, 2015; Hageneder, 2013). In the present determinations, the mean air temperature recorded during *in vivo* investigations for the three seasons showed values between 19 - 26°C, within the range presented in the literature (Thomas & Garcia-Martí, 2015). The temperature range for photosynthesis in yews is the same as for all other European tree species and is particularly wide. As a result, yew can photosynthesize throughout mild winters, which is particularly useful for this taxon, which occupies the lower vegetation floor under deciduous trees such as ash or beech, which are not photosynthetically productive in winter. This characteristic enables it to compensate for its relatively low photosynthetic efficiency in summer (Hageneder, 2013).

Since most conifers retain their leaves for several years, their foliage is constantly subjected to a wide range of environmental temperatures as the seasons change, with rates of photosynthesis varying according to this factor: they fall both above and below the optimum growing temperature (Wood & Brittain, 1973; Teskey et al., 1995), which is generally in the range of 15 - 30°C (Belous, 1986; Teskey et al., 1995). As well as the ambient temperature, the intensity of the photosynthesis process is also influenced by the humidity of the atmospheric air, which regulates the degree to which the stomata open. Low humidity causes dehydration of guard and attached cells, leading to stomatal closure, while near saturation point humidity maintains the turgor of guard and attached cells, favoring stomatal opening (Burzo & Dobrescu, 2005).

Table 3. Environmental factors recorded at the time of investigation of the physiological parameters of *Taxus baccata* L.

Environmental factors	SEASON								
	SPRING (IV)			SUMMER (VI)			AUTUMN (IX)		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
<b>Temperature</b> <b>T (°C)</b>	<b>21.03</b> ± 0.13	<b>22.45</b> ± 0.95	<b>22.85</b> ± 1.25	<b>19.15</b> ± 0.13	<b>24.55</b> ± 0.95	<b>26.45</b> ± 1.25	<b>20.32</b> ± 0.18	<b>26.55</b> ± 0.25	<b>25.7</b> ± 0.6
<b>Relative humidity</b> <b>RH (%)</b>	<b>51.80</b> ± 2.87	<b>44.70</b> ± 1.60	<b>47.75</b> ± 3.25	<b>63.12</b> ± 0.42	<b>46.65</b> ± 2.65	<b>49.15</b> ± 3.45	<b>57.55</b> ± 0.52	<b>49.7</b> ± 0.1	<b>49.45</b> ± 0.15

As a result of the recordings made of the RH parameter, it can be observed that the intensity of the photosynthesis process shows a decrease in the leaves of the spontaneous taxon **T1**, where the relative air humidity recorded an average value of 63.12 %. Air relative humidity, in turn, depends on the environmental temperature, which modifies the atmosphere's capacity to retain water vapor. Increasing the ambient temperature raises this capacity of the atmospheric air, which leads to a decrease in its relative humidity and, consequently, a reduction in the degree of stomatal opening. In this way, the air humidity and temperature continuously change the intensity of the photosynthesis process (Burzo & Dobrescu, 2005).

The intensity of transpiration is also influenced by environmental factors, among which light, temperature, atmospheric pressure, air currents, and air and soil humidity all have a significant influence (Burzo & Dobrescu, 2005).

### 3.4. Photoassimilatory pigments

The rate of photosynthesis is correlated with the level of chlorophyll pigments in leaf tissues, the main factors in the process equation, alongside water and dry matter content (Trifu & Bărbat, 1997), data presented in Table 4. In the determinations carried out in practice, leaves belonging to the spontaneous taxon **T1** show the highest quantity of photoassimilate pigments in all the seasons analyzed, followed by leaves belonging to the taxa **T3** and **T2**. In the case of the yew, the life span of the leaves depends on the environmental conditions, being on average 4 to 8 years (Bartkowiak et al., 1978; Zarek, 2016). Their

progressive aging eventually leads to an imbalance between chlorophyll biosynthesis and degradation and can be observed, among others, by changes in pigment concentrations and their degradation products (Kutík, 1998; La Porta et al., 2006). The amount of photoassimilatory pigments in tree leaves are subject to changes during their growth and development; this depends, among other things, on the age and position occupied by the leaves in the tree crown, light conditions, and access to water and minerals in the environment (Merzlyak et al., 1999; Silkina & Vinokurova, 2009; Sarala & Saravana, 2011; Zarek, 2016). Since the amount of light absorbed and used by the plant in the photosynthetic process is based on the number of photosynthetic pigments present in its photosynthetic tissues, the direct influence of chlorophyll content on the photosynthetic potential of the plant and its primary production can be understood.

On the biological material available, under its specific growing conditions, it can be observed that in the spring season of strobili formation, chlorophyll *a* and *b* fractions and the number of carotenoid pigments are closely related to variations in environmental factors (temperature and humidity). At the same time, from the analysis of the data obtained practically, we observe that the leaves of the specimens of the spontaneous taxon **T1** showed the highest values of photoassimilate pigment content at all times of analysis, compared to the leaves of the cultivated taxon specimens, **T2** and **T3**, which showed lower values, but not significantly, the main factor that determined these results being, we believe, the different degree of illumination that their leaves had in the growing environment.

**Table 4.** Photosynthetic pigments *Taxus baccata* L.

Photosynthetic pigments	SEASON								
	SPRING (IV)			SUMMER (VI)			AUTUMN (IX)		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
<b>Chl a</b> mg g <sup>-1</sup> DW	<b>1.87</b> ± 0.03	<b>1.15</b> ± 0.04	<b>1.35</b> ± 0.03	<b>2.21</b> ± 0.15	<b>1.25</b> ± 0.02	<b>2.17</b> ± 0.04	<b>3.74</b> ± 0.09	<b>2.39</b> ± 0.04	<b>1.96</b> ± 0.07
	****						****		
<b>Chl b</b> mg g <sup>-1</sup> DW	<b>0.60</b> ± 0.02	<b>0.31</b> ± 0.02	<b>0.31</b> ± 0.01	<b>0.73</b> ± 0.12	<b>0.39</b> ± 0.01	<b>0.60</b> ± 0.03	<b>1.26</b> ± 0.02	<b>0.67</b> ± 0.01	<b>0.58</b> ± 0.01
	****			*			****		
<b>Car</b> mg g <sup>-1</sup> DW	<b>0.92</b> ± 0.04	<b>0.53</b> ± 0.02	<b>0.58</b> ± 0.01	<b>0.94</b> ± 0.04	<b>0.53</b> ± 0.01	<b>0.88</b> ± 0.02	<b>1.69</b> ± 0.07	<b>1.00</b> ± 0.02	<b>0.84</b> ± 0.02
	***			****			****		
<b>Chl a/b</b>	3.11	3.77	4.29	3.04	3.21	3.61	2.96	3.59	3.40
<b>Chl/Car</b>	2.70	2.76	2.88	3.13	3.07	3.14	2.96	3.04	3.03

Legend: Chl *a* = chlorophyll *a*; Chl *b* = chlorophyll *b*; Car = carotenoid pigments; only statistical differences have been marked with asterisks: \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ ; \*\*\*\* =  $p < 0.0001$

Analyzing the recorded chlorophyll values in leaf tissues of yew individuals in different specific phenological phases, we note that this fraction was biosynthesized and accumulated in the highest amount in the leaves of spontaneous *Taxus baccata* (T1) specimens both in the strobili formation in April and during the following stages investigated. The obtained results are practically in agreement with the literature, according to which chlorophyll *a*, shows a slight increase in value from March (1.95 mg g<sup>-1</sup> DW) to April (1.99 mg g<sup>-1</sup> DW) (Zarek, 2016).

Following the dynamics of the second chlorophyll fraction - chlorophyll *b*, it was observed that its level gradually increased in the leaves of spontaneous taxon specimens (T1) from April (0.60 mg g<sup>-1</sup> DW) to June (0.73 mg g<sup>-1</sup> DW), the data obtained practically being close to those obtained by Zarek, 2016 (0.61 mg g<sup>-1</sup> DW in March and 0.75 mg g<sup>-1</sup> DW in May 2015). In turn, carotenoid pigments, as component fractions of foliar assimilatory pigments with specific contributions to the proper course of the photo assimilation process, but also with a protective role of chlorophyll fractions against the destructive action of ultraviolet radiation in the light spectrum (Gonçalves et al., 2001; Robakowski & Wika, 2009) have recorded specific quantitative variations in the leaves of the taxa analyzed, induced by the intensity and quality of light, as an essential environmental factor for the photo assimilation process available to the leaf apparatus of wild and cultivated yew trees. Thus, in the leaf of spontaneous specimens (T1), the content of carotenoid pigments shows approximately the same concentrations for April and June months, recording 0.92 mg g<sup>-1</sup> DW, respectively, 0.94 mg g<sup>-1</sup> DW, a result that is close to the values presented in the literature, Zarek (2016) reporting in June 2015 a concentration of 0.84 mg g<sup>-1</sup> DW of carotenoid pigments.

As the literature indicates, the process of chlorophyll biosynthesis and degradation is controlled by light, and at high levels of light intensity, the degradation processes outweigh the synthesis processes, so plants growing in full sunlight have lower chlorophyll content than plants growing in the shade (García-Plazaola & Becerril, 2001; Swanberg & Verhoeven, 2002). In the case of the cultivated yew taxa, it was observed in the determinations carried out that the leaves of the T3 taxon showed a value close to those of the T1 taxon for chlorophyll *a* in June, namely 2.17 mg g<sup>-1</sup> DW, compared to 2.21 mg g<sup>-1</sup> DW in T1.

**Chlorophyll *a*/chlorophyll *b* ratio** - the research carried out aimed to contribute with the results obtained to complete the specific data presented in the literature on this parameter, considered to vary widely, for C<sub>3</sub> photosynthetic type plants in the range of about 3:1 (Hoffman, 1987; Sebanek, 1992; Delian,

2010). Its value gives us information on the degree of illumination supported and exploited by the foliar apparatus of the taxa analyzed, with specific references to its photosynthetic efficiency, respectively to the biosynthetic possibilities and the rate of accumulation of the main photo assimilation products in plants (Burzo et al., 1999). The determinations carried out in this context revealed that for the taxa investigated, this ratio varies between 3.04:1 and 4.29:1, its high value observed in the leaves of *T. baccata* specimens mainly due to their relatively low chlorophyll *b* content.

In turn, **the ratio of chlorophyll pigments to carotenoid pigments** increases slightly with leaf/needle age (2.7:1 in April; 3.14:1 in June; 3.04:1 in September), which is due to a faster increase in chlorophyll content, while the carotenoid pigment content increases slightly less. The increase in the chlorophyll pigment/carotenoid pigment ratio with leaf aging was also noted, for example, in *Pinus sylvestris* (Miazeck & Ledakowicz, 2013), with the authors reporting that its value was lower in younger pine needle tissues, with this value increasing with advancing age. The authors point out that for the sensitive and young tissues, a higher content of carotenoid pigments relative to chlorophyll content may serve as a protective function against oxidative stress or excessive light radiation during the spring season, and more intense decomposition of chlorophyll pigments compared to the carotenoid content during the growing season will lead to a decrease in the discussed ratio.

### 3.5. The Importance of *Taxus* sp. in Ecology and environmental protection

The ecological and environmental value of *Taxus baccata* is supported primarily by its longevity (some specimens can reach an age of about 1000 years) and tolerance of abiotic stress (Thomas & Polwart, 2003). The application potential of the use of the organs of yew specimens, still under research attention, may offer new opportunities to exploit the taxon in different practical directions of interest (Sharma et al., 2022).

Key elements supporting its ecological and environmental importance include the following: the species *T. baccata* is very suitable for dendrochronological research, with taxa of the genus *Taxus* representing integral components with significant roles in the plant associations in which they grow, for example, the *Taxo - Fagetum* association (Sedmáková et al., 2020). They can protect vulnerable habitats from erosion due to their ability to grow on a wide range of soils and produce decay-resistant wood (Thomas & Polwart, 2003).

Species of the genus *Taxus* play a significant role in biodiversity, providing a valuable habitat for a variety of organisms, including endophytes, which contribute to

the adaptation of plants to environmental changes; thus, a total of about 70 species of various endophytes were observed (*Aspergillus* sp., *Penicillium* sp., *Alternaria* sp., *Cladosporium* sp., *Fusarium* sp.) in different yew taxa, and their isolation and characterization is crucial for understanding the ecology associated with these conifers (Adhikari et al., 2023).

The yew trees are populations well adapted to the specific living conditions of the habitats in which they grow and play a vital role in the production of oxygen in these areas through the process of photosynthesis throughout the calendar year, including the winter season, when they have local exclusivity in this regard.

At present, the species *Taxus baccata* is heavily affected due to illegal logging for various +/- objective reasons (source of raw material for taxol extraction and the production of furniture and high-quality wooden objects, the tendency to be removed from certain areas as a "potential source of poisoning for animals and humans"), due to failure of the natural regeneration process, loss of genetic diversity, unfavorable conditions offered by the soil/substrate on which it grows, pressure from herbivores and climate change (Alavi et al., 2020; Sedmáková et al., 2020).

According to Hematzadeh et al., (2023) under these conditions, maintaining the genetic diversity and the size of natural yew populations is essential for their adaptation, sustainability, and evolution. Consequently, various practical actions have been recommended for their protection, such as fencing the natural populations to preserve their integrity and the number of individuals that make them up and thinning the forest canopy in a mixture with other species in which the yew grows, to favour a photo-assimilation process that will ensure their robustness and longevity (Iszkuło, 2011).

#### 4. CONCLUSIONS

From an anatomical and micromorphological point of view, the leaves of the studied taxa show similarities that confirm the data already existing in the literature: absence of epicuticular wax, complex organization of the stomatic apparatus on the lower face, arrangement of the stomata in two bands located on either side of the vein, absence of hypodermis and secretory ducts, uniqueness of the leading fascicle of the vein, papillary cells of the lower epidermis. The differences that do occur fall more into the category of structural variations and consist of the appearance of unequally equifacial bifacial structures, bi-tristratified palisade tissue, quantitative disproportions between the phloem and the wood of the midrib vascular bundle, the occurrence of fluctuating asymmetries concerning leaf width, all of which are correlated with ecological conditions and are adaptive response reactions of the

leaves.

Physiological and biochemical determinations carried out in the field - "in vivo" and in the laboratory allow us to state that the leaf area in spontaneous and cultivated representatives of the genus *Taxus*, under vegetation conditions specific to the N-E area of Romania varies in value in correlation with the degree of illumination / increased levels of leaf shading, conditions efficiently exploited by the foliar apparatus, resulting in the formation of a larger leaf area and an increased content of photoassimilate pigments in yew specimens growing spontaneously in the forest of the Tudora Reserve; the development of fundamental physiological processes (photosynthesis, transpiration) varies according to the specific phenological phases, leaf factors and climatic conditions; the content of foliar photo assimilatory pigments shows quantitative variations depending on the peculiarities of the biological cycle of the taxa investigated and on the variations of internal (Qleaf, tleaf, Ci, gs) and external factors available to them (temperature, atmospheric humidity).

Species of the genus *Taxus*, through the biotic and abiotic interactions they maintain in the habitats in which they grow, are essential for biodiversity maintenance, ecological balance, and environmental protection; they represent important ecosystem components, and their protection as species at high risk of survival in the face of climate change is essential for their adaptation, sustainability, and evolution.

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