LEAF STOMATAL TRAITS AND ASSOCIATED PHYSIOLOGICAL PARAMETERS IN DIFFERENT DECIDUOUS ORNAMENTAL TREES DURING AUTUMN SENESCENCE

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Abstract

Global warming and the climate associated changes generally influence on plant phenology throughout their entire ontogenetic cycle (including autumn phenology), also having an impact on different ecological processes and on ecosystems. Stomata are specialized cellular structures located in the plant epidermis, which have a great importance for plant physiology, evolution, and global ecology. They are known especially for their role in carrying out the gases exchange, but their contribution to the maintenance of optimal leaf temperature, water, and nutrients uptake, as well as to assuring the continuity of their transport throughout the plant cannot be neglected This paper describes: 1) characteristics of stomata in mature leaves of some deciduous ornamental trees grown in the Botanical Garden of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania; 2) water use efficiency, quantum yield, and transpiration: stomatal conductance ratio, during autumn senescence. Both indicators' categories can be promising to predict the autumn phenological shifts of the studied species driven under urban area conditions.

Key words: Botanical Garden, deciduous ornamental trees, leaf stomata, water use efficiency, leaf senescence.

INTRODUCTION

Plant ontogeny progresses by succession of specific phenological phases in relation to the species, represented as changes of traits expression due to intrinsic and extrinsic determinants, together with their interactions (Barton, 2023). Obviously, especially in recent years, in areas known to have a temperate climate, because of global warming such changes are accentuated. To address this challenge, both in the spring and autumn seasons, plants have adjusted their behavior, which has a significantly impact on different ecological processes (Xie et al., 2018; Tao et al., 2019).

It's worth mentioned that understanding the autumn phenology of deciduous trees species, but also of the climatic and meteorological factors involved can lead to the prediction of the autumn phenological shifts induced by projected climate change (Xie et al., 2018). The studies of Mariën et al. (2018) on beech, birch, and oak in forest trees in Belgium, highlighted that environmental conditions did not affect the onset of leaf senescence in mature trees, suggesting the existence of a conservative strategy. In a broad sense, autumn leaf senescence is a controlled type of programmed cell death that, unlike other stressors inducing cell death, avoids the loss of leaf nutrients (Keskitalo et al., 2005). Perennials specifically recapture leaf nutrients during autumn to relocate them to their over-wintering organs, as this is essential for their growth potential and foliage redevelopment during the subsequent year (Hagen-Thorn et al., 2006).

As previously documented, stomata are specialized cellular structures located in the plant epidermis (mainly in the stem and leaves) (Esau, 1977), having their central importance for plant physiology, evolution, and global ecology (Hetherington and Woodward, 2003; Delian, 2020; Ramakrishnan and Ray-Mukherjee, 2022). It has been extensively studied and well documented their role in carrying out the gas exchange during photosynthesis (Yin et al., 2020), and

respectively, that specific to respiration, being named the plant's "breathing" pores (Biscoe, 1872). In addition, due to the transpiration process carried out mainly at the level of the stomatal pore, but also at the level of the cuticle (Kane et al., 2020; Hazlett, 2022), these formations contribute to maintenance of optimal leaf temperature, water, and nutrient uptake, as well as the continuity of their transport throughout the plant (Chen et al., 2022). Really, due to the ability to internally regulation of the stomatal pore diameter and under the action of external factors, plants generally manage the water use efficiency (WUE), especially in drought conditions (Yoo et al., 2009; Ronzhina et al., 2023). Moreover, in-depth genetic studies are intended to increase the tolerance of plants to drought by modulating the stomatal density (SD) and their characteristics to improve WUE (Li et al., 2021; Jiao et al., 2022), also to increase the plant tolerance to drought, in parallel with climate changes (Bertolino et al., 2019). WUE is also influenced by the presence of trichomes, cuticle and cuticular waxes. A high trichome/stomata ratio improved WUE thanks to the increase in the resistance to the water vapor diffusion at the leaf level (Galdon-Armero et al., 2018, cited by Bertolino et al., 2019). Furthermore, stomata are entry gates for pathogens, such as bacteria. At this level, the fight for the plant's immune response is triggered (Wang et al., 2022) and, not least, the stomata are involved in the plant-insect interactions (Lin et al., 2022).

The stomata distribution at the leaf level, their density on a leaf unit area, as well as stomatal traits (e.g., types, size etc.) depend on the ecological conditions of plant growth (Paridari et al., 2013; Kryvoruchko and Bessonova, 2018; Yigit et al., 2019; Hurt and Doğan, 2020; Kou et al., 2023) and are permanently subject to a process of adaptation to the constantly environmental factors changing (Yin et al., 2020; Li et al., 2021; Soheili et al., 2023), in order to successfully fulfill its multiple roles and especially so that the process of photosynthesis can be negatively affected as little as possible (Ronzhina et al., 2023). For example, in *Carpinus betulus* grown at different altitudes (Hyrcanian forest, Iran) a negative correlation of stomatal sizes with altitude was determined, while for SD the correlation was positive. In addition, relative temperature and precipitation strongly influence the morphological characteristics of the leaf (Paridari et al., 2013).

The interactions between stomata's traits and the environmental conditions are also markedly highlighted in the urban environment, especially regarding the changes induced by the temperature increase (Zhu et al., 2020; Markin et al., 2023), carbon dioxide (CO_2) concentration increasing (Gardner et al., 2023), but also by different pollution sources (Petrushkevych and Korshykov, 2020), which leave their mark on the plant's morphology, anatomy, and physiology. Apart from the previously mentioned, SD, as well as the presence of trichomes influence the ability of plant species to reduce the effects of pollutants in urban environments, influencing the accumulation of air contaminants in leaves (Simon et al., 2014; Zhang et al., 2018). Therefore, Green Infrastructure (GI) is one potential passive control system for air pollution in street canyons (Tomson et al., 2021). Marek et al. (2022) highlighted the adaptation of *Pinus sylvestris* plants to the temperature increase, rather than to changes in CO_2 level. Therefore, the intraspecific relationship between SD and climate can characterize SD response to global warming (Marek et al., 2022).

Considering the previously presented, the aim of this study was to describe: 1) stomata characteristics in mature leaves of 19 deciduous ornamental trees species grown in the Botanical Garden of the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV of Bucharest), Romania, using light microscopy; 2) leaves water use efficiency and quantum yield during autumn senescence. Both indicators' categories can be promising to predict the autumn phenological shifts of the studied species driven under urban area conditions.

MATERIALS AND METHODS

Study sites and species
This research was carried out during autumn senescence, on leaves of 19 deciduous ornamental trees species grown in the Botanical

Garden of the Faculty of Horticulture, USAMV of Bucharest, Romania, North latitude of 44^{0}24' N and 26^{0}05' East longitude, and an altitude which varies between 60 m and 90 m above sea level, with a temperate-continental climate.

The species belong to 19 different families, as follows: (A) Sapindaceae (Aceraceae) - 1. *Acer platanoides* L.; 2. *Acer pseudoplatanus* L.; (B) Anacardiaceae - 3. *Cotinus coggygria* Scop.; (C) Betulaceae: 4. *Betula pendula* Roth (*B. verrucosa*); 5. *Corylus avellana* L.; 6. *Corylus colurna* L.; 7. *Carpinus betulus* L.; (D) Caesalpiniaceae - 8. *Cercis siliquastrum* L.; (E) Calycanthaceae - 9. *Calycanthus floridus* L.; (E) Cornaceae - 10. *Cornus mas* L.; 11. *Cornus sanguinea* L.; (F) Ebenaceae - 12. *Diospyros virginiana* L.; (G) Fagaceae - 13. *Quercus rubra* L. (*Q. borealis* Michx.); (H) Ginkgoaceae: 14. *Ginkgo biloba* L.; (I) Magnoliaceae: 15. *Liriodendron tulipifera* L.; (J) Moraceae - 16. *Maclura pomifera* (Rafin.) C.K. Schneid.]; (K) Oleaceae: 17. *Forsythia x intermedia* Zabel; (L) Tiliaceae: 18. *Tilia tomentosa* Moench (*T. argentea* DC.); (M) Ulmaceae: 19. *Celtis occidentalis* L.

Leaf traits
The stomata density and their characteristics features
Stomatal distribution, stomata density (SD) in leaves and their traits have been analysed on mature healthy still green leaves, in October 2023, by the method of stomatal impression described everywhere in specialized works. A thin layer of nail polish was applied on both sides of the leaves, on an area of about 2 cm^2. After solvent evaporation (about 20 minutes later), the transparent stomatal impression of the leaf epidermis was taken with the help of a sheet of transparent shells and placed on a microscopic slide, which was labelled with the name of the sample.

The observations, images and measurements of the anatomical structures were made with the optical microscope Leica DM1000 LED, Camera video Leica DFC295 the Laboratory of Microscopy and Plant Anatomy of the USAMV of Bucharest. For stomatal density (SD), ob. 40 x. was used, and the photos and measurements of the stomata were made at ob. 20 x.

SD was counted and expressed as number per mm^2. The guard cells length (GcL) (µm), guard cells width (GcW) (µm), stomatal pore length (SPL) (µm) and stomatal pore width (SPW) (µm) were measured. Then, the stoma area (STArea) as well as the stoma pore area (STPArea) were calculated, too (µm^2). Also, photos were taken for the stomatal impressions of the lower epidermis of each sample.

Physiological associated indicators
Based on the net photosynthesis rate (A - µmol CO_2 m^{-2} s^{-1}) and transpiration rate (E - mmol H_2O m^{-2}s^{-1}) (data are not shown) measured *in-situ*, for still green leaves, using the portable infrared gas analyser (LCPro-SD-ADC BioScientific Ltd, Hoddesdon, UK), there were calculated two associated indicators, namely: water use efficiency (WUE) (A/E) (µmol CO_2 m^{-2} s^{-1}/ mmol H_2O m^{-2}s^{-1}) and quantum yield (φCO_2) (A/ Photosynthetic Photon Flux Density (µmol CO_2 m^{-2} s^{-1}/ µmol photons m^{-2}s^{-1}).

Statistical analysis
Data were processed using Microsoft Excel (version 2010) and are shown as average values ± Standard Error (SE). The analysis of variance (ANOVA) was performed. Then, the post hoc Duncan Multiple Range Test (DMRT) by using IBM SPSS Statistics software was carried out to determine where there were statistically significant differences between different species. Statistically significant differences have been considered at the value of $p \leq 0.05$.
Pearson correlation coefficients were also calculated to evaluate the possible relationship between stomatal traits and physiological indicators at 95% confidence level. Graphs were constructed using Microsoft Excel 2010.

RESULTS AND DISCUSSIONS

An overview of the leaves micromorphological characteristics is shown in Figure 1. The variability of the epidermal cells shapes and size of the for the 19 species studied, the type of stomata, as well as the presence of trichomes in some cases can be observed. The stomata are present only at the level of the lower epidermis (abaxial) and in most of the analyzed species, they are of the paracytic and anomocytic type. In the case of 9 *Acer* studied species, most of

the stomata were of anomocytic type, but on *A. platanoides* there were observed some that resemble the paracytic type (Toma et al., 2015). As regards the presence of trichomes, the types present and their respective sizes, the species *C. occidentalis* stood out, which at the level of the upper epidermis presented multicellular hairs, with a diameter of 8.43 μm and a length of 106.7 μm. In *C. sanguinea*, they were located on both epidermises, but the length varied (155 μm adaxially, respectively 217 μm abaxially). For *T. tomentosa*, stellate hairs were observed, and in the case of *F. x intermedia* the secretory hairs consisted of 8 cells, with a mean diameter of 22.33 μm. Our results agree with those obtained by Gülz et al. (1991) who observed in the leaves of *T. tomentosa* just emerging from the bud, dense trichomes (consisting of eight stellate cellular hairs) located on the abaxial side, while in mature leaves, because of the growth of differentiated cells, the density of the hairs was reduced. On the adaxial surface there were only solitary stellate hairs, to which solitary glandular trichomes were added. Simon et al. (2014) also found that in *C. occidentalis* leaves, in addition to the stomata size and their distribution as having a major importance in the accumulation of contaminants from the air, the presence of numerous trichomes leaves its mark on the dust deposition. Also, unicellular glandular hairs have been observed by Toma et al. (2015) on the *A. platanoides* petiole epidermis. On *A. platanoides* and *A. campestre* lamina there were observed unicellular (or tricellular on the latter species) eglandular hairs.

Table 1 shows the average values ± SE of the determined indicators, as well as the significance of the differences at the level of $p < 0.05$. Stomata number, as well as their sizes varied widely among evaluated species, being strong significantly different from a statistical point of view. According to ANOVA test, p values were < 0.001 for each studied parameter. Stomatal density per mm^2 leaf area ranged between the lower one – 70.33 mm^{-2} (*A. pseudoplatanus*) to the highest registered for *C. occidentalis* (467 mm^{-2}), with a mean value of 172 mm^{-2}.

The length and width of the guard cells also showed a wide variability. The length was higher than the width, ranging between 8.70 μm (*A. platanoides*) and 22.80 μm (*B. pendula*), with an average value of 15.86 μm. The lowest value for guard cell width was 2.86 μm, (*C. occidentalis*), while the maximum one was 7.80 μm (*G. biloba*). It can be observed that for *A. platanoides* the lowest values were noticed also for stoma pore length (5.26 μm), stoma pore width (1.50 μm), stoma area (74.98 μm^2) and stoma pore area (7.95 μm^2). At the opposite pole was *B. pendula*, with STArea of 418.91 μm^2 and *C. mas* with a SPArea of 63.84 μm^2, species in which SD had a low value of only 85 stomata mm^{-2}.

The low values of the water use efficiency and quantum yield (Table 1) can be explained by the fact that the determinations regarding net photosynthesis and transpiration rate were carried out during autumn, when naturally, physiologically, the rate of net photosynthesis decreases in relation to the onset of leaf senescence, with all the consequences that arise on the physiological processes in plants. However, if the degree of senescence is variable depending on the species, the mentioned indicators also show very different values.

The Pearson correlations between stomatal traits and associated physiological indicators are shown in Figure 2. Green represents a negative correlation, while red represents a positive correlation. Overall, we notice a wide variability in relation to the studied species. SD showed rather a negative correlation with different indicators (more evident in *A. platanoides*), except *C. siliquatrum* and to a certain extent *Q. rubra*. Also, the stomata traits, water use efficiency and quantum yield were strongly positive correlated (r > 0.7) in *A. pseudoplatanus*, and to a lesser extent in descending order of the species *C. betulus*, *C. siliquastrum* and *F. intermedia*. Negative correlations were recorded especially in *C. coggygria*, *B. pendula*, *C. floridus* and to some extent in *C. occidentalis*.
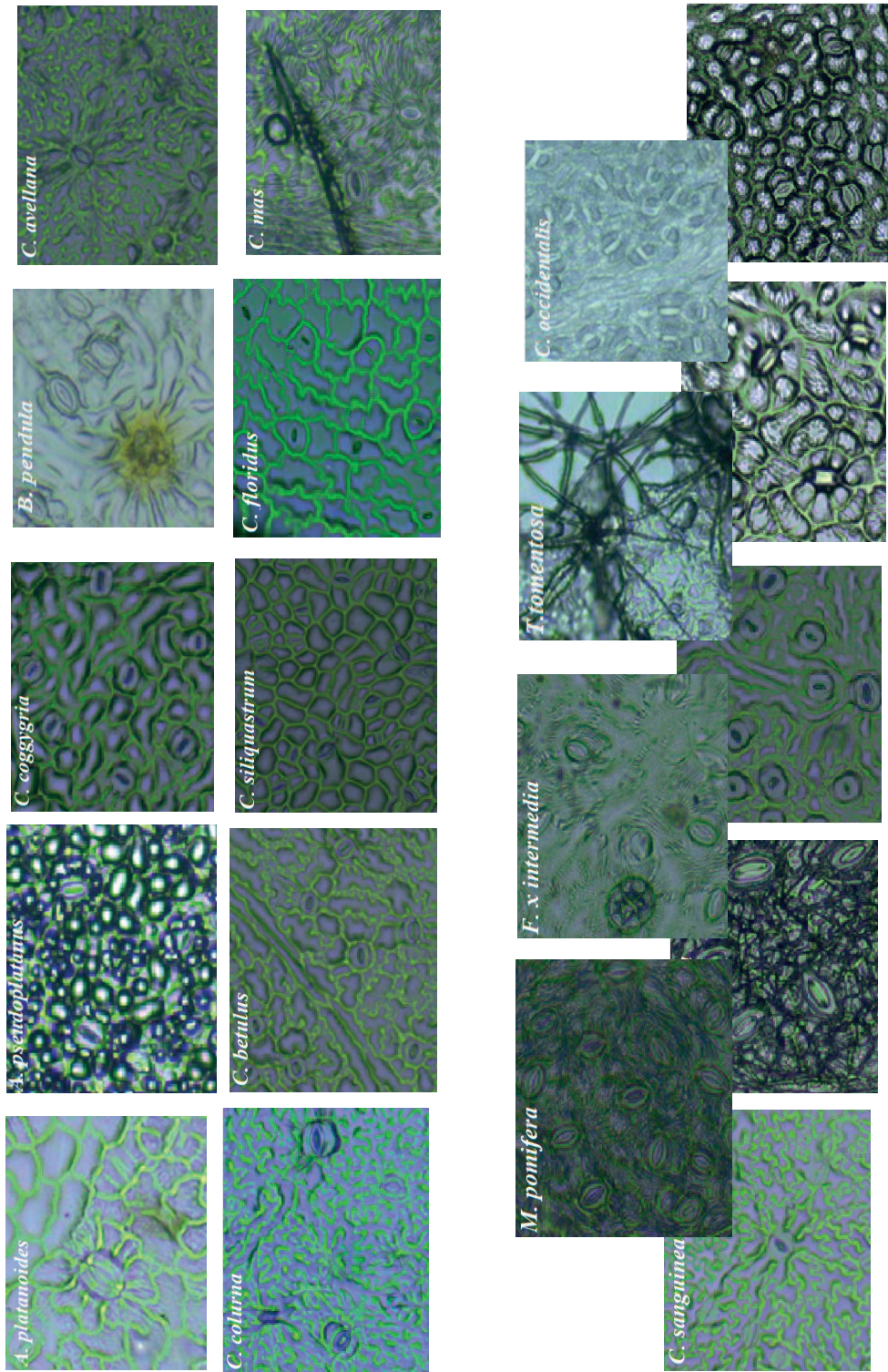
Figure 1. Stomatal photographs (20 x) of the leaves abaxial impressions for different deciduous ornamental trees during autumn senescence

Table 1. Leaf epidermis stomatal traits and synthetic physiological indicators for leaves of 19 woody ornamental trees, during autumn season

Species	Stomata density: No mm⁻²	Guard cell length (GcL)(μm)	Guard cell width (GcW)(μm)	Stoma pore length (SPL)(μm)	Stoma pore width (SPW)(μm)	Stoma area (StArea)(μm²)	Stoma pore area (SPArea)(μm²)	Water use efficiency (WUE)	Quantum yield (qpCO₂)
A. platanoides	197±7.02 cde	8.70±1.41 h	3.43±0.43 gh	5.26±0.24 k	1.50±010 h	74.96±17.98 i	7.95±0.86 i	0.14±0.13 e	8.39E-05±7.61E-05 fg
A. pseudoplatanus	70.33±6.14 i	18.33±0.13 bcd	6.60±0.15 cd	10.70±0.82 def	3.30±0.20 cdef	302.59±11.03 bcd	35.65±4.80 cde	1.01±0.25 cde	58E-04±15E-04 cd
C. coggygria	227.67±16.50 c	11.27±0.34 gh	4.53±0.12 f	7.93±0.18 ji	2.60±0.11 gf	131.52±5.38 jik	20.65±1.25 fg	0.10±0.03 e	11E-04±2.8E-05 efg
B. pendula	102.67±6.74 ghi	22.80±2.25 a	7.53±0.18 b	14.03±0.73 a	3.33±0.23 cdef	418.91±38.37 a	46.53±2.28 b	0.64±0.09 cde	9E-04±1E-04 abc
C. avellana	135.33±11.57 fg	13.53±1.05 fg	5.78±0.30 e	7.27±0.24 j	1.50±0.11 h	177.77±22.22 ghi	10.95±1.18 hi	4.24±0.61 a	6E-04±9.01E-05 cd
C. columa	92.67±11.05 hi	17.13±0.93 bcde	4.53±0.32 f	9.87±0.69 fg	3.33±0.31 cdef	213.99±26.49 fgh	33.32±5.50 e	-2.11±0.18 fg	-4.9E-04±3.1E-05 h
C. betulus	130.33±10.74 fgh	16.50±1.23 cdef	5.50±0.26 e	10.00±0.64 efg	4.47±0.12 ab	256.21±25.88 def	44.73±3.45 bc	-2.94±0.30 g	-18E-03±14E-04 i
C. siliquastrum	160.33±6.74 ef	16.03±1.32 cdef	4.37±0.18 f	13.33±0.41 ab	3.23±0.22 cdef	193.06±22.99 fghi	43.07±2.95 bcd	0.98±0.21 cde	0.001±2E-04 ab
C. floridus	105±8.66 ghi	15.93±1.38 cdef	5.47±0.18 e	8.40±0.12 hij	2.27±0.12 g	209.89±16.00 fgh	19.03±0.98 fgh	0.91±0.16 cde	0.001±2E-04 a
C. mas	85±5 i	17.27±1.22 bcde	6.00±0.12 cde	12.83±0.32 ab	4.97±0.23 a	296.41±29.25 abc	63.84±4.28 a	1.37±0.35 cd	0.0003±8.5E-05 defg
C. sanguinea	185.33±2.67 de	13.30±0.40 fg	5.90±0.15 de	7.73±0.38 i	2.73±0.23 efg	193.41±9.45 fghi	21.17±2.32 fg	0.74±042 cde	7.7E-05±4.37E-05 fg
D. virginiana	165.33±11.35 ef	18.30±0.26 bcd	3.93±0.18 fg	12.43±0.23 bc	4.50±0.36 ab	226.47±13.08 fgh	55.78±3.60 cde	0.26±0.07 de	0.0005±0.00015 cde
Q. rubra	303.33±9.28 b	15.30±0.91 efg	9.27±0.38 a	9.60±0.31 fgh	4.50±0.26 ab	358.35±29.57 b	46.55±3.88 b	-1.60±0.07 f	-0.0055±0.00022 j
G. biloba	85.33±14.07 i	19.87±0.97 b	7.80±0.29 b	9.17±0.34 ghi	4.83±0.26 a	342.88±13.53 bc	15.47±2.10 ghi	1.75±0.22 bc	0.0003±4.72E-05 defg
L. tulipifera	179.67±10.91 de	15.13±0.64 efg	3.85±0.22 fg	7.73±0.29 j	3.40±0.06 cde	168.39±12.40 hij	26.28±0.90 ef	1.02±0.16 cde	0.00086±2.78E-05 bc
M. pomifera	287.67±22.18 b	17.17±0.93 bcde	4.03±0.14 fg	11.20±0.43 cde	3.97±0.12 bc	206.51±11.15 fgh	44.33±0.68 bcd	2.24±0.16 b	0.00066±2.35E-05 bcd
F. x intermedia	210.33±4.33 cd	19.00±0.17 bc	4.40±0.21 f	12.30±0.15 bc	3.57±0.30 cd	235.04±14.38 efg	43.87±3.70 bcd	2.48±1.09 b	0.0003±0.0001 defg
T. tomentosa	77.67±11.05 i	14.83±0.32 fg	6.73±0.24 c	11.40±0.26 cd	2.90±0.21 gfed	243.05±14.08 defg	33.07±2.61 e	0.04±0.40 e	-1.9E-05±0.0001 e
C. occidentalis	467±27.22 a	10.93±0.56 gh	2.87±0.09 h	7.83±0.22 j	4.47±0.29 ab	111.39±5.23 jk	34.99±2.51 de	0.98±0.22 e	0.0005±0.0002 cdef

Data are shown as mean value ± SE (n = 3). The comparison was done on columns between different species, by the post hoc Duncan Multiple Range Test (DMRT) by using IBM SPSS Statistics software. Statistically significant differences have been considered at the value of $p \leq 0.05$ and are represented by different letters.

Figure 2. Pearson correlation analysis of stomata traits and associated physiological indicators

We recorded that for WUE there is a very strongly positive correlation with the quantum yield, as logically expected. The exception was *L. tulipifera*, in which case a strong negative correlation was noticed (r = - 0.95).

Although the stomata ostioles occupy a small area of the total leaf surface, through them significant amounts of water pass in the form of vapors (during transpiration), as well as CO_2 (raw material in photosynthesis) (Chen et al., 2022). Regarding their characteristics, Yin's et al. (2020) studies on 45 species of woody plants highlighted the relationship between the maximum rate of photosynthesis and stomatal characteristics (SD, stomatal length, maximum stomatal conductance) and revealed some contradictory results compared to those obtained through previous studies. The authors' conclusion was that plants that possess a smaller number of stomata (but with larger sizes) can have a maximum stomatal conductance with lower values. As a result, under conditions of increased CO_2 concentration and reduced water availability, as is predicted to happen in the future, the rate of photosynthesis can still be maintained at adequate values.

Yigit et al. (2019) highlighted the dependence of the micromorphological characteristics of the leaves on the growing conditions. Thus, in *T. tomentosa*, apart from the length and width of the stomatal pore, where the differences were statistically insignificant (the average values varied from 9.76 - to 10.73 μm; respectively 3.38 – 4.35 μm), for stoma length (14.36 to 18.89 μm), stoma width (9.36 to 15.07 μm) and stomata density (20 to 176) the differences depending on the area were significantly different. In our study we found that SD was 77.67 mm^{-2}, so close to the one recorded in the city of Sivas (Turkey) (72), and the stoma length of 14.83 μm (close to the one measured in Izmir - 14.36 μm). The changes in stomatal characteristics are specific to the species, the cultivar and depend on the climate specific to the plant growth conditions, otherwise confirming the results previously obtained by other authors (Hurt and Doğan, 2020; Sevic et al., 2020). Although a negative relationship was usually found between SD and the concentration of carbon dioxide in the atmosphere, the research carried out by Marek et al. (2022) in *Pinus sylvestris* highlighted the link between SD variation and temperature changes, respectively a response to climate warming.

Naturally, plants can regulate the degree of stomata opening to maximize the CO_2 assimilation, simultaneously with the reduction of water losses. But the studies have also demonstrated that in the case of extreme heat, a decoupling of stomatal conductance - net photosynthesis rate takes place, which allows leaves survival in such conditions, as well as a quickly depletes available water (Marchin et al., 2023). Maximum daytime operating stomatal conductance have been characterized by having lower instantaneous water use efficiency (iWUE), while water potential gradients were highly varied. As a result, the faster stomatal response can be useful for such leaves. Thus, smaller stomata with a faster dynamic feature can be integrated to plants selection for stomata conductance as an adding trait for enhancing photosynthesis performances, as well as to improve agricultural qualities (Drake et al., 2012).

Moreover, genetic manipulation of stomata, with the aim of making their role more efficient is a current concern of researchers. In this context, the proof is in-depth studies carried out by Li et al. (2021) on poplar. It has been demonstrated the role of *PdERECTA* in stomatal modeling with the aim of increasing the efficiency of water use, respectively reducing SD, and increasing their size in the case of overexpression of the mentioned gene. It was thus emphasized that stomatal conductance decreased and implicitly the intensity of transpiration done the same, which increased WUE (without significantly affecting CO_2 absorption). It was appreciated that the *PdERECTA* gene is of interest for the genetic modification of poplar to obtain drought tolerance trees. Jiao et al. (2022) isolated an EPIDERMAL PATTERNING FACTOR (EPF) secreted Cys-rich small peptide *PdEEPFL6* from NE19 [*Populus nigra* × (*P. deltoides* × *P. nigra*)] that was highly induced by dehydration treatment in poplar. Overexpression of *PdEPFL6* determined a significantly decrease of the *PdSPCH* and *PdMUTE* transcription factors expression, which are implicated in stomata development. Thus, stomata density

was reduced and respectively, drought resistance was improved.

Eensalu et al. (2008) highlighted variations in *B. pendula* stomata traits depending on the leaf position in the crown, respectively in relation to the intensity of the light incident on the leaf. In the upper part of the crown, a higher SD and a smaller leaf surface were determined, while no significant changes were noted regarding the epidermal cell density. Also, the length and width of the guard cells at the abaxial leaf level had lower values in the case of reduced light availability. To detect some bioindicators of the state of the urban environment, Petrushkevych and Korshykov (2020) studied different measurements of *B. pendula* exposed to different levels of aerotechnogenic loading. Among them, there was a decrease in the length, width, and stomata area, while the thickness of the leaf and the SD increased. In addition, in *B. pubescens*, in the case of high temperatures and water deficit, the size and number of stomata decreased, while the number of mesophyll cells and chloroplasts per surface unit increased, as measures to counteract the effects of the decrease in leaf conductance (Ronzhina et al., 2023).

Regarding the contribution of the stomatal transpiration has in total transpiration, Kane et al. (2020) underline the existing differences in *Q. rubra* depending on the age of the leaves, respectively the presence or absence of stomata and the presence of the cuticle (and its thickness). The authors obtained different data as compared to those previously reported. It was considered that intense transpiration in young growing leaves is due to keeping the stomatal ostiole open. The recent explanation was that the ability to close would develop as stomata are exposed to low humidity and high concentrations of abscisic acid.

Akinshina et al. (2020) described *L. tulipifera* stomata, regarding the position on the leaf, the specific type, as well as the influence of light on SD. Thus, in the case of leaves exposed to the sun, the number of stomata was by 82% higher compared to the number of stomata in the shade leaf (182 vs. 100 mm^{-2}; length - 39 µm vs 32 µm; width 21 µm vs. 13 µm). The ecological plasticity of this species relative to light and temperature is highlighted, which improves its acclimatization to arid climate

conditions. The ability to quickly close the stomata, as the water potential of the leaves decreases (respectively the isohydric response) denotes an ability of *L. tulipifera* to avoid the stress caused by drought, compared to other species such as *Pyrus* and *Quercus* characterized by an anisohydric response (Cregg et al., 2023). In the same vein, the research carried out by Kryvoruchko and Bessonova (2018), in *Q. robur* and *Q. rubra* species grown alone, highlighted an increase in SD by 30.6% and 25.3%, respectively, compared to those grown in groups. At the same time, there were changes in the anatomy of the leaf, which for solitary plants leads to xeromorphism type behavior, as adaptation reagents to the greater shortage of air and soil moisture.

Miller-Rushing et al. (2009) investigated whether the tree species grown individually in the Arnold Arboretum in Boston, Massachusetts underwent changes for 100 years (1893 to 2006), regarding SD, guard cell length, and intrinsic water use efficiency (iWUE), examining leaves from 74 herbarium specimens collected from three genera: *Acer* (maples), *Quercus* (oaks), and *Carpinus* (hornbeams). In oak and hornbeam, a negative correlation was found between SD and the length of the guard cells, respectively, the SD decreased, while the length of the guard cells increased. The WUE values did not undergo significant changes over time. The authors appreciate that iWUE does not respond to changes in CO_2 concentration, probably due to changes in stomatal characteristics, such as their density and the guard cells sizes.

In the species *C. coggygria*, in China, Li et al. (2022) studied the effects of unusual continuous rainy weather and determined a significant positive correlation with the values of some environmental indicators such as sunshine duration, temperature, photosynthetically active radiation (PAR) and daily precipitation (DPD) greater than or equal to 0.1 mm. Also, the transpiration rate was significantly correlated with SD and temperature, PAR, DPD, and the atmospheric CO_2 concentration.

Following the meta-analysis carried out by Gardner et al. (2023), it was suggested that the intensification of the photosynthesis process,

rather than the reduction of stomatal conductance, leads to the improvement of iWUE, under conditions of increased CO_2 concentration in all analyzed species. In addition, leaf–air vapor pressure difference (D). factor was also important to compare the different species and the obtained results emphasized that the most responsive to D were angiosperms, as against gymnosperms. The studies carried out by Avci and Aygün (2014) on 18 Turkish hazelnut (*Corylus avellana* L.) revealed that the leaves are hypostomatic and there are statistically significant differences regarding the number of stomata and respectively the size of the stomata, appreciating that these indicators could be used to identify the varieties.

The drought applied to *B. pendula* seedlings significantly affected the anatomy of the leaf (Kou et al. 2023), regarding the length of the stomata ($p<0.05$) and had a highly significant effect ($p<0.01$) on the change in stomatal structure. At the control, SD was 56.33 mm^{-2}, while the higher drought stress level (of 25 % polyethylene glycol) induced an increase of SD to 73 mm^{-2}. At the same time, stomata length, stomata width and stomatal aperture decreased from 44.33 µm to 39.36 µm; 36.53 µm to 32.67 µm, respectively from 5.71 µm to 1.75 µm in the case of the last parameter. Authors appreciate that these data can serve as a theoretical basis for the selection and breeding of new drought-tolerant European birch species and the promotion of new drought-tolerant species in China. According to the previously data, our obtained results of SD – 102.67 mm^{-2} or guard cells length (22.80 µm – evident below 39.36 um as noted before) possible signify an adaptation of this specie to the drought stress.

CONCLUSIONS

Global warming and the climate associated changes generally influence on plant phenology throughout their entire ontogenetic cycle (including autumn phenology), also having an impact on different ecological processes and on ecosystems.

The micromorphological characteristics of the mature leaves of the studied ornamental deciduous trees are very closely related to the species and the specific growing conditions. The striking variability regarding the stomata density, but also their dimensions, as well as the marked differences compared to the existing data in the specialized literature, are the expression of the fact that the biological material was represented by *in situ* mature, old individual trees (not grouped), grown, and adapted along the time to the environmental conditions of an urban botanical garden, located in a temperate climate.

Overall, the results regarding the interrelationships between micromorphological and physiological associated characters, such as water use efficiency and quantum yield, suggest that trees belonging to different botanical families have a specific behavior during leaf senescence, in the autumn season. Some species are less photosynthetically efficient than others, which may mean that a negative photosynthesis can be associated with a higher rate of respiration. In autumn conditions, the intensification of the catabolic processes at the leaves level before they fall should not be considered as a minus for the plant, but a benefit for what means an efficient compounds re-translocation in perennial plant organs before the leaves fall and not ultimately a proper preparation of the plant to get through the winter and start vegetation the following year.

To our knowledge, these are the first results on this topic in Romania. These are added to those previous obtained by other researchers, contributing to expanding our understanding and support the prediction of the autumn micromorphological and physiological shifts driven under urban area conditions, helping also, to understand plants adaptation to the future changing environmental climate.

REFERENCES

Akinshina, N., Duschanova, G.M., Azizov, Azamat, A.A., Khalmurzaeva, A.I., & Toderich, K.N. (2020). Xeromorphic features of the leaves of Liriodendron tulipifera (L.) Magnoliaceae in the arid climate of Central Asia. *Moscow University Biological Sciences Bulletin*, *75*, 212-217.

Avci, N., & Aygün, A. (2014). Determination of stomatal density and distribution on leaves of Turkish hazelnut (*Corylus avellana* L.) cultivars. *Journal of Agricultural Sciences – Tarim Bilimleri Dergisi*, *20* (4):454-459.

Barton, K.E. (2023). The ontogenetic dimension of plant functional ecology. *Functional Ecology*. 00:1–16.

Bertolino, L.T., Caine, R.S., & Gray, J.E. (2019). Impact of stomatal density and morphology on water-use efficiency in a changing world. *Frontiers in Plant Science, 10*:225. doi: 10.3389/fpls.2019.00225.

Biscoe, T.D. (1872). The Breathing Pores of Leaves. *The American Naturalist, 6* (3), 129-133.

Chen. Z., Li, S., Wan, X., & Liu, S. (2022.) Strategies of tree species to adapt to drought from leaf stomatal regulation and stem embolism resistance to root properties. *Frontiers in Plant Science, 13*:926535. doi: 10.3389/fpls.2022.926535.

Cregg, B., Rouse, R., & Ellison-Smith, D. (2023). Genotypic variation in water relations and gas exchange of urban trees in Detroit, Michigan, USA. *Urban Forestry & Urban Greening, 81*, 127858.

Delian, E. (2020). Stomata-a key factor with multiple functions in the conditions of global climate change. A brief overview. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development, 20*(1), 151-160.

Drake, P.L., Froend, R.H., & Franks, P.J. (2012). Smaller, faster stomata: scaling of stomatal size, rate of response, and stomatal conductance. *Journal of Experimental Botany, 64*(2), 495–505.

Eensalu, E., Kupper, P., Sellin, A., Rahi, M., Sõber, A., & Kull, O. (2008). Do stomata operate at the same relative opening range along a canopy profile of Betula pendula? *Functional Plant Biology, 35*(2) 103-110.

Esau, K. (1977). Anatomy of Seed Plants. Wiley, New York.

Galdon-Armero, J., Fullana-Pericas, M., Mulet, P. A., Conesa, M. A., Martin, C., & Galmes, J. (2018). The ratio of trichomes to stomata is associated with water use efficiency in tomato. *Plant Journal,* doi: 10.1111/tpj.14055.

Gardner, A., Jiang, M., Ellsworth, D S., MacKenzie, A R., Pritchard, J. et al. (2023). Optimal stomatal theory predicts CO_2 responses of stomatal conductance in both gymnosperm and angiosperm trees. *New Phytologist, 237*(4): 1229-1241.

Gülz, P.G., Prasadb, R.B.N., & Müller, E. (1991). Surface Structure and Chemical Composition of Epicuticular W axes during Leaf Development of Tilia tomentosa Moench. *Zeitschrift für Naturforschung, 46c*, 743-749.

Hagen-Thorn, A., Varnagiryte-Kabasinskiene, I., Nihlgård, B., & Armolaitis, K. (2006). Autumn nutrient resorption and losses in four deciduous forest tree species. *Forest Ecology and Management, 229*, 33-39.

Hazlett, D. (2022). Importance of transpiration in plants. *Global Journal of Agricultural Research and Reviews, 10* (1), 7-8.

Hetherington, A., & Woodward, F. (2003). The role of stomata in sensing and driving environmental change. *Nature, 424*, 901–908.

Hurt, H., & Doğan, A. (2020).Determination of stoma density and distribution of hazelnut (*Corylus avellana* L.) cultivars and genotypes grown in different ecologies. *Yüzüncü Yil Üniversitesi Journal of Agricultural Sciences, 3*, 544-552.

Jiao, Z.Y., Han, S., Li, Z., Huang, M.B., Niu, M-X., Yu, X., Liu, C., Wang, H-L., Yin, W., & Xia, X. (2022). PdEPFL6 reduces stomatal density to improve drought tolerance in poplar. *Industrial Crops and Products, 182*, 114873.

Kane, C.N., Jordan, G.J., Jansen, S., & McAdam, S.A.M. (2020). A permeable cuticle, not open stomata, is the primary source of water loss from expanding leaves. *Frontiers in Plant Science, 11*:774. doi: 10.3389/fpls.2020.00774.

Keskitalo, J., Bergquist, G., Gardeström, P., & Jansson, S. (2005). A cellular timetable of autumn senescence. *Plant Physiology, 139*(4):1635-1648.

Kou, J., Yan, D., Qin, B., Zhou, Q., Liu, C., & Zhang, L. (2023) Physiological response mechanism of European birch (Betula pendula Roth) to PEG-induced drought stress and hydration. *Frontiers in Plant Science, 14*:1226456.

Kryvoruchko, A. P., & Bessonova, V. P. (2018). Anatomical leaves characteristics of Quercus rubra L. and Quercus robur L. and stand density. *Ukrainian Journal of Ecology, 8*(1), 64-71.

Li, X., Wu, T., Cheng, Y., Tan, N-D., Jiang, F., Liu, S-Z., Chu, G-W., Meng, Z., & Liu, J-X. (2021). Ecophysiological adaptability of four tree species in the southern subtropical evergreen broad-leaved forest to warming. *Chinese Journal of Plant Ecology, 44* (12):1203-1214.

Li, H., Yang, Y., Wang, H., Liu, S., Jia, F., Su, Y., Li, S., He, F., Feng, C., Niu, M., et al. (2021). The receptor-like kinase ERECTA confers improved water use efficiency and drought tolerance to poplar via modulating stomatal density. *International Journal of Molecular Sciences, 22*, 7245.

Li, L., Wang, Y., Xue, X., Zhang, W., Wu, J., Gao, L., Tan, X., Rong, X., Duan, R., & Liu, Y. (2022). Response of Cotinus coggygria photosynthesis and coloration to weather change in Chongqing. *Journal of Nanjing Forestry University (Natural Sciences Edition). 46*(5), 95- 103.

Lin, P.A., Chen, Y.T., Ponce, G., Acevedo, F. E., Lynch, J. P., Anderson, C. T., Ali, J. G., & Felton, G. W. (2022). Stomata-mediated interactions between plants, herbivores, and the environment. *Trends in Plant Science, 27*(3), 287-300.

Marek, S., Tomaszewski, D., Zytkowiak, R., Jasinska, A., Zadworny, M., Boratynska, K., Dering, M., Danusevicius, D., Oleksyn, J., & Wyka, T. P. (2022).Stomatal density in Pinus sylvestris as an indicator of temperature rather than CO_2: evidence from a pan-european transect. *Plant, Cell & Environment, 45* (1):121-132.

Marchin, E.M., Medlyn, B.E., Tjoelker, M.G., & Ellswor, D.S. (2023). Decoupling between stomatal conductance and photosynthesis occurs under extreme heat in broadleaf tree species regardless of water access. *Glob Change Biology, 29*, 6319–6335.

Mariën, B., Dox, I., De Boeck, H.J., Willems, P., Leys, S., Papadimitrou, D., & Campioli, M. (2018). Does drought advance the onset of autumn leaf senescence

in temperate deciduous forest trees? *Agricultural and Forest Meteorology*, *250–251*, 127-137.

Miller-Rushing, A.J., Primack, R.B., Templer, P.H., Rathbone, S., & Mukunda, S. (2009). Long-term relationships among atmospheric CO_2, stomata, and intrinsic water use efficiency in individual trees. *American Journal of Botany*, *96*(10), 1779-1786.

Paridari, I.C., Jalali, S.G., Sonboli, A., Zarafshar, M., & Bruschi, P. (2013). Leaf macro- and micro-morphological altitudinal variability of Carpinus betulus in the Hyrcanian forest (Iran). *Journal of Forestry Research*, *24*(2): 301−307.

Petrushkevych, Y.M., & Korshykov, I.I. (2020). Ecological and biological characteristics of Betula pendula in the conditions of urban environment. *Trees Structure and Function*, *15* (8): 483-491.

Ramakrishnan, S., & Ray-Mukherjee, J. (2022). Contextualizing Stomata. *Reson 27*, 579–597.

Ronzhina, D.A., Migalina, S.V., & Yusupov, I.A. (2023). Gas flaring cause shifts in mesophyll and stomatal functional traits of Betula pubescens Ehrh. *Journal of Forestry Research, 34*, 2079- 2087.

Sevik, H., Cetin, M., Ozel, H. B., Erbek, A., & Cetin, I. Z. (2020).The effect of climate on leaf micromorphological characteristics in some broad-leaved species. *Environment, Development and Sustainability*, *23* (4):6395-6407.

Simon, E., Baranyai, E., Braun, M., Cserháti, C., Fábián, I., & Tóthmérész, B. (2014). Elemental concentrations in deposited dust on leaves along an urbanization gradient. *Science of the Total Environment*, *490*, 514-520.

Soheili, F., Heydari, M., Woodward, S., & Naji, H.R. (2023). Adaptive mechanism in Quercus brantii Lindl. leaves under climatic differentiation: morphological and anatomical traits. *Scientific Report*, *13*, 3580.

Tao, Z., Xu, Y., Dai, J., Wang, H. (2019). Divergent Response of leaf coloring seasons to temperature change in Northern China over the past 50 years. *Advances in Meteorology*, Article ID 2706803.

Toma, C., Ifrim, C., & Gațu, I. (2015). Some aspects concerning the anatomy of the offshoot of *Acer* L. species from the collection of the botanical garden Iassy. *Analele Ştiinţifice ale Universităţii „Al. I. Cuza" Iaşi.s. II a. Biologie vegetală*, *61*, 1-2, 27-37.

Tomson, M., Kumar, P., Barwise, Y., Perez, P., Forehead, H., French, K., Morawska, L., & Watts, J. F. (2021). Green infrastructure for air quality improvement in street canyons. *Environment International*, *146*, 106288.

Wang, M., Ji, Q., Liu, P., & Liu, Y. (2022). Guarding and hijacking: stomata on the move. *Trends in Plant Science*, *27*(8), 736-738.

Xie, Y., Wang, X., Wilson, A.M., & Silander Jr, J.A. (2018). Predicting autumn phenology: How deciduous tree species respond to weather stressors. *Agricultural and Forest Meteorology*, *250–251*, 127-137.

Yigit, N., Cetin, M., Ozturk, A., Sevik, H., & Cetin, S. (2019). Variation of stomatal characteristics in broad leaved species based on habitat. *Applied Ecology and Environmental Research*, *17*(6), 12859-12868.

Yin, Q., Tian, T., Kou, M., Liu, P., Wang, L., Hao, Z., & Yue, M. (2020). The relationships between photosynthesis and stomatal traits on the Loess Plateau. *Global Ecology and Conservation*, 23 ref.44. Elsevier, Amsterdam, Netherlands.

Yoo, C.Y., Pence, H.E., Hasegawa, P.M., & Mickelbart, M.V. (2009). 'Regulation of transpiration to improve crop water use'. *Critical Reviews in Plant Sciences*, *28* (6), 410 - 431.

Zhang, W., Zhang, Z., Meng, H., & Zhang, T. (2018). How does leaf surface micromorphology of different trees impact their ability to capture particulate matter? *Forests*, *9*(11), 681.

Zhu, J., Zhu, H., Cao, Y., Li, J., Zhu, Q., Yao, J., & Xu, C. (2020). Effect of simulated warming on leaf functional traits of urban greening plants. *BMC Plant Biol*, *20*, 139.