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Foliar traits of *Juglans regia, Aesculus hippocastanum* and *Tilia platyphyllos* in urban habitat

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Abstract

Juglans regia, Aesculus hippocastanum and Tilia platyphyllos species are widely used for planting. In the present study, two extremes of spring and autumn were chosen as sampling seasons. Leaf traits included in this analysis are: leaf relative water content (LRWC), specific leaf area (SLA), density of foliar tissue (D), leaf ash content (LAC). Those parameters are easily and rapidly screened, and relatively inexpensive. Relationships between different parameters were examined with Pearson's correlation analyses and and t-test. Results on relative water content recorded in leaves showed high variations at various stages. The results of the present study also revealed significant seasonal variability in mineral content composition. The other leaf physiological parameters differed significantly.

Keywords: leaf parameters, developmental stage, urban habitat

1. Introduction

In the leaf ontogenesis, the development starts with the initiation of a leaf primordium [1]. An expansion of the cells and subsequent differentiation gives rise to a mature leaf [2]. During the growing season, net photosynthesis rates increase and dark respiration rates decline [3]. Leaf senescence is an evolutionary selected developmental process and an oxidative process and comprises a series of biochemical and physiological events which include the final stage of development. During leaf senescence, the cell ultrastructural is changed, the photosynthetic apparatus is dismantled and the nutrients are relocated to young tissues or storage organs [4, 5]. Leaf senescence represents an important phase in the plant life [6].

Leaf characteristics play critical roles in determining rates of photosynthesis and transpiration. Leaf traits can change within a plant due to developmental age of the plant and the position of the leaf within the plant (heteroblasty) [7]. Mitton (1978) suggested that sun and shade leaves differ in developmental stability [8]. Cowart and Graham (1999) found the outside crown is a more stressful environment for leaves than the inner crown [9]. The outer leaves, because of their position, are more susceptible to the negative effects of complex mixtures of environmental variables, compared to inner leaves [10, 11].

The urbanized areas are characterised by increasing processes of xerisation, "heat islands", disruption of water relations and nutrient cycling, pollution of the air, water and soil. Foliar traits are highly sensitive to climatic conditions [12]. The objective of this study was to investigate variations in leaf traits for trees (*Juglans regia, Aesculus hippocastanum* and *Tilia platyphyllos)* growing in urban habitat located in the city of Timisoara (Romania). *Aesculus hippocastanum* (horse chestnut) belonging to the botanical family Hippocastanaceae. Is widely used for shade and ornamental purposes in streets, gardens, public parks, alleys, campuses, and other large urban spaces. The bark and leaves of *A. hippocastanum* have been employed as an astringent to treat diarrhea and hemorrhoids, venous insufficiency, and postoperative edema in order to pass kidney stones and to ease stomach aches [13]. Saponins from *A. hippocastanum* have been reported to show anti-inflammatory activity [14]. Except medicinal importance, horse chestnut was studied as a possible biomonitor of the heavy metal pollution [15]. On average, green leaves remained on trees for 130-175 days. Leaves at the top and south side of the plant are often most affected and attacks can be quite sudden. It is very sensitive to one or more pests or diseases which can affect tree health [16, 17]. Genus *Tilia* L. belongs to the family Tiliaceae. It is represented by economic and ecological important tree species [18, 19]. *Tilia platyphyllos (*large-leaf linden) is important as ornamental tree and are frequently used by urban forestry in streets and parks. *T. platyphyllos* are resistant to drought, dry winds and low temperatures; they are suitable for commercial and protective planting [20]. Infusions are widely used in traditional medicine for the treatment of enterocolitis and gastroenteritis. It is considered to be a diuretic and antispasmodic. Traditionally, plant was used as a diaphoretic to promote perspiration, but mainly due to its sedative activity in cases of anxiety and heart palpitations. Leaves can be used to improve perspiration and reduce fever [21]. The quantification of foliar bioaccumulation shows *Tilia* trees have the biggest accumulation capacity for zinc, meaning that all can be used as biomonitoring agents for this element [22]. Enrichment factors of toxic elements were considerably high in the dust deposited on *Tilia* leaf surface [23]. Data suggest that *Tilia* leaves may represent a reliable indicator for developmental stability evaluation studies [11]. Comparing acclimation strategies of *Tilia platyphyllos* leaves, Majer et al (2013) found that sun leaves were better protected against stress than shade leaves [24]. No significant effect on photochemistry of photosystem II was evident in leaves of *Tilia platyphyllos* infested with eriophyid mite *Eriophyes tiliae* [25]. *Juglans regia (walnut)* belongs to family Juglandaceae and has been used in traditional medicines from ancient times. Its leaves has been widely used in traditional medicine for the treatment of skin inflammations, venous insufficiency, fungal or microbial infections, hyperhidrosis and ulcers and for its antidiarriec, antihelmintic, antiseptic and astringent properties. Antiradicalar and antibacterial activities have also described [26]. Walnut has been used in the treatment of diabetes and prostate and vascular disturbance [27]. In addition, *J. regia* have other components that may be beneficial for health including plant protein, dietary fiber, melatonin, plant sterols, folate, tannins, α-tocopherol and polyphenols [28; 29; 30; 31]. *J. regia* leaves have antibacterial effects and may be a suitable alternative remedy for protection and treatment of dental plaque [32]. Several extracts of the leaves and fruits were assessed for their neuroprotective effects through antioxidant and anti-cholinesterase methods [33]. The leaves extracts could be considered as a promising analgesic and anti-inflammatory agents against diseases such as rheumatoid arthritis [34]. The juglone is one of the strongest naturally occurring phytotoxic and allelopathic chemical compound [35; 36, 37]. Juglone and some other phenolics from leaves may be involved into the defense mechanism [38]. Sun et al. (2007) showed insecticidal activities [39]. The seasonal changes of juglone in leaves showed a linear decrease over growing season. Leafs contains approximately 10% tannins of the ellagitannins type and 3.4% flavonoids [40]. The phenolic acids are connected with plant resistance to insects and antimicrobial properties. *Juglans regia* leaves are useful for biomonitoring of the atmospheric deposition of Zn [41].

2. Materials and Methods

Field and laboratory measurements

The study was performed in 2013 in the city of Timisoara, western Romania. The leaves were collected from the external belt of the crown, around its entire circumference, at heights from 1,5 m to 1,75 m. Leaf samples were collected for phisiological determination separately from each tree during april and september. We collected the samples during the later morning for all species. Ten leaves were collected from each species. The selection was aimed at finding areas where trees adjacent to streets are exposed to the influence of characteristic of urban areas, and in particular occurring in the vicinity of busy roads [42]. This paper presents the results concerning infestation by pests with visual method. The tree condition was evaluated with a scale to express the level of leaf infestation (degree 0 was no symptoms, healthy leaf; degree 1 meant traces of infestation – up to 5% of leaf blade area; degree 2 was slight infestation – up to 25% of leaf blade area; degree 3 – medium infestation – up to 50% of leaf blade area; degree 4 – heavy infestation – up to 75% of leaf blade area; level 5 – very heavy infestation – over 75% of leaf blade area) [43]. The trees were divided into two categories: trees with no visible damage to the leaf blade (juvenile and healthy" in april) and trees with emerging damage to the leaves ("old, senescence and sick" in september). The visual damages affected over 75% of the leaf surface in *Aesculus*. On the same days, leaf relative water content (LRWC) was determined [44]. Leaf fresh weight (FW) was determined using Kern analytical balance within 2 h after excision. Turgid weight (TW) was obtained after soaking the leaves for 2 h in distilled water and weighed. Leaves were placed in labeled envelopes and put in the oven at 80°C for 24 hours for dry weight purpose. All dry leaves were re-weigh separately to estimate leaf dry weight (DW). Leaf relative water content (or percentage moisture content) was calculated from the following equation: RWC = (FW – DW)/(TW – DW) x 100. Several leaf physiological parameters were calculated: density of foliar tissue (D= DW/FW X 1000: in g/kg), specific leaf area (SLA), leaf ash content (LAC). 15 randomly selected leaf discs (with calculated area) were sampled and dried in an oven at 80°C for 24 hours. Dry weight (DW) of the samples was measured and used for calculation of specific leaf area (SLA in cm^2/g), where S is the area of leaf discs [45, 46]:

$$\text{SLA}\ [\text{cm}^{-2}\ \text{g}^{-1}] = \frac{S[cm^2]}{DW[g]}$$

For leaf ash content (LAC), the porcelain crucibles with the dry samples were transferred into a furnace, which was set at 500ºC for 2 h to ensure proper ashing. The ash was then cooled in a dessiccator and weighed. Ash content is expressed as mass percentage of dry biomass [44, 47].

Species descriptions

The *Aesculus* leaves are opposite and palmately divided into 5-7 leaflets. They are oval, serrated and have 10-25 cm [48]. In urban areas *Aesculus* plants were infected by different disease agents [49]. A powdery mildew, caused by the fungus, *Erysiphe flexuosa* was sometimes found covering the underside of leaves with a white mould. A small brown circular leaf spot caused by *Septoria hippocastani* was an occasional problem on horse chestnut. Bleeding canker symptoms (the causal agent responsible has been identified as *Pseudomonas syringae* pathovar *aesculi*) include foliar discoloration [50]. The fungus *Guignardia aesculi* causes drown and irregular blotches with a yellow halo [51]. These trees suffered heavily from attacks by a leaf mining insect known as *Cameraria ohridella* [52]. *Cameraria ohridella* has spread very rapidly and it is now present across most of the country. The diseases of *Aesculus hippocastanum* reduces their aesthetic value [53]. The *Juglans* leaf is imparipinnate. The leaflets are oblong ovate and up to 12 cm. The leaf margin is smooth [54]. *Aceria erinea* and *Aceria tristriata* appeared to be the most important and detrimental eriophyoids on *Juglans regia* [53, 55]. The most common walnut diseases are fungal anthracnose caused by *Gnomonia leptostyla* [56]. The *Tilia* leaves are cordate, sharply serrated [57]. The most widespread linden-tree leaf disease agents were *Discula umbrinella* and *Cercospora microsora* [58]. The subspecies of the species *Eriophyes (=Phytoptus) tiliae* are feeding specialists on *Tilia platyphyllos* where they create distinctive galls [53, 59]. Statistical significance was determined by the two-sample t-test. Differences at P<0.05 were considered to be significant. Patterns of trait correlations were examined by calculating the pairwise Pearson correlation coefficients.

3. Results and discussion

Laboratory measurements are summarized in Fig 1-3. The values of fresh leaf weights are significantly elevated from one season to another for *Tilia* and *Juglans.* The values of dry leaf weights are significantly higher for old, senescence and sick leaves towards juvenile and healthy leaves, for all species. The same significant increase in autumn we recorded for the amount of ash in leaves incinerated. The mean values of physiological parameters calculated for each species are shown in Figures 4-7. Analysis of significant differences were also examined (Table 1). In this survey, significant seasonal difference was found in SLA at *Tilia* leaves. Variability of moisture content (LRWC) was relatively high, leaves sampled in spring contained significantly more moisture than those sampled in autumn. Date of this investigation revealed that ash content of the leaves of spring was significantly lower than that of the leaves of autumn. The density of foliar tissue was significantly higher in old, senescence and sick leaves of *Tilia.* On average, spring samples for *Juglans* leaves contained a higher LRWC than autumn (p= 0,032077). The statistical comparison of the values for SLA from both seasons shows significant differences among the leaves values at the 0.05 significant level. Results show that the spring samples have contained considerably lower LAC than the autumn samples (p= 0,00000002). The density of foliar tissues levels showed that an increase in old, senescence and sick leaflets of *Juglans regia.* The ash content (LAC) in *Aesculus* leaflets is high in autumn. LRWC in spring leaves was greater than that found in autumn samples. SLA of spring samples was the highest than SLA of leaves sampled at autumn. Results show that the spring samples have contained considerably lower density of foliar tissues (D) than the autumn samples (p= 0,00000677). In autumn was a negative correlation between LRWC and LAC (r= –0,29388 for Aesculus, r= –0,55213 for Juglans, r= –0,15871 for Tilia). Correlation analysis showed negative correlation between the LRWC and density of foliar tissue in spring. In this case, the leaflets of *Aesculus* (r= –0,58004) and *Juglans* (r= –0,77592) were more strong negative correlations than in the *Tilia* leaves.

Table 1. Analysis of significant differences (with p-values) between the leaves collected in spring and autumn

	FW(g)	TW(g)	DW(g)	Ash content (g)	LRWC (%)	LAC (%)	SLA (cm2/g)	D (g/kg)
Tilia	0,00479920*	0,00350990*	0,00023510*	0,00013655*	0,01849900*	0,00000001*	0,00188681*	0,00000287*
Aesculus	0,19390400	0,13272600	0,01339200*	0,00656300*	0,00362900*	0,00179100*	0,00102900*	0,00000677*
Juglans	0,00307100*	0,00224500*	0,00082600*	0,00020000*	0,03207700*	0,00000002*	0,03571936*	0,00006520*

Measurements of LRWC were used to assess the state of water balance of a plant (Fig. 4).

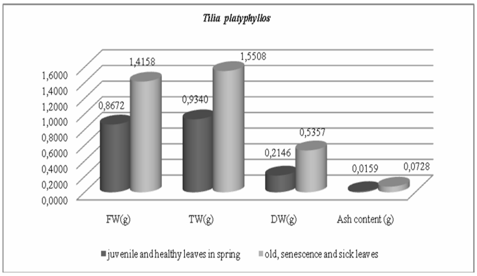

Figure 1. Mean values for measurements on *Tilia platyphyllos* leaves

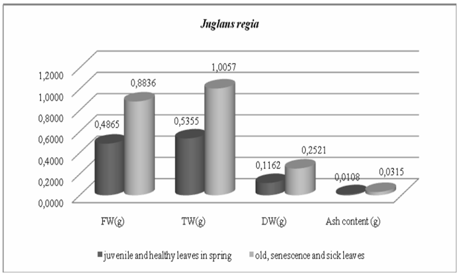

Figure 2. Mean values for measurements on *Juglans regia* leaves

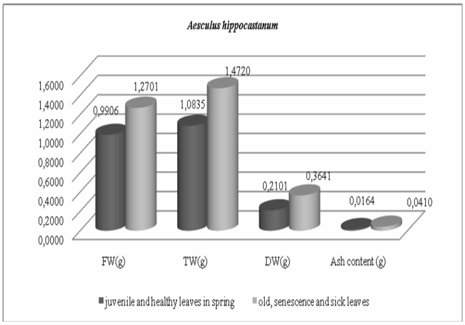

Figure 3. Mean values for measurements on *Aesculus hippocastanum* leaves

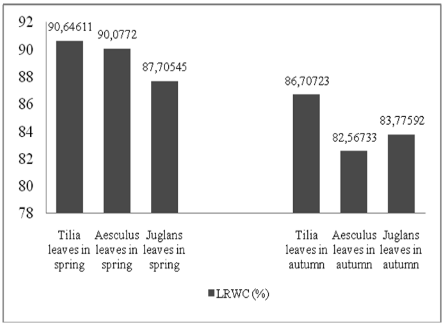

Figure 4. The mean values for leaf relative water content (LRWC)

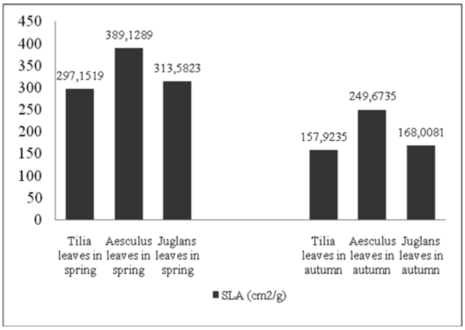

Figure 5. The mean values for specific leaf area (SLA)

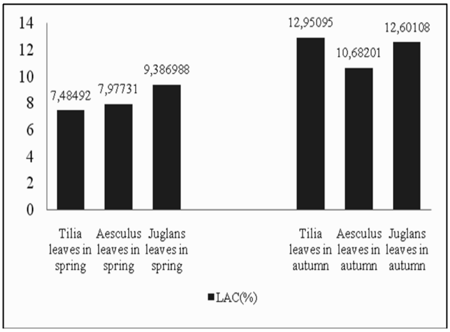

Figure 6. The mean values for leaf ash content (LAC)

This parameter indicates a greater ability to allow young leaf tissue soaking water. LRWC expresses the absolute amount of water which the leaf requires to reach full artificial saturation, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance. Leaf structures reflect the effects of water stress more clearly than those of stems and/or roots [60; 61]. In autumn, leaf water content was lower in the leaves of *Aesculus*. Leaf relative water content (LRWC) is higher in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures. In the present study, leaf senescence is accelerated by a decrease in the leaf water content [62]. In this study SLA decreased during senescence phase for all trees (Fig. 5). A lower SLA is usually a consequence of an increase in the density or thickness of foliar tissue and normally occurs when the costs of the assimilatory apparatus are increased [63]. SLA is not constant during plant development, due to different leaf adjustments to light, temperature, or to partitioning of carbohydrate into leaves. SLA is as a measure of the photosynthetic production-translocation balance. For woody plants, changes in SLA with leaf or plant age have been reported by several authors [64, 65]. Many species exhibit phenotypic plasticity in SLA: low light, soil water availability, high temperatures, low atmospheric CO2 concentrations, lesser nutrient levels in soil induce high SLA [66]. We do not know if plasticity in SLA confers a fitness advantage, or is a maladaptive response to resource limitation or other stresses [67]. In this study, the outside leaves probably reflects stress rather than plasticity. Our results shows that leaf ash content significantly increased in September, for all species investigated (Fig. 6).

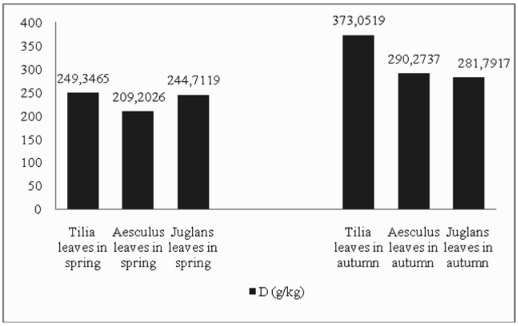

Figure 7. The mean values for density of foliar tissue (D)

The accumulation of mineral or ash content in vegetative tissues appears to be explained through the passive transport of minerals via xylem driven by transpiration [68]. In vegetative organs, several studies report that the organic contents decrease while the ash or mineral content increases [69]. In our study, the reduction in specific leaf aria under autumn conditions was due to an increase in density of foliar tissue. Changes in D and SLA resulted from a change in leaf anatomy. Density of foliar tissue increased as specific leaf aria decreased for all species included in the present study. Density of foliar tissue may be related to age of leaves (Fig. 7). On the other hand, this variability can be attributed to differences in the efficacy of the defense mechanisms used by these species [60].

4. Conclusion

A leaf physiological parameters were determined for *Juglans regia, Aesculus hippocastanum* and *Tilia platyphyllos* at different developmental stages. Ash contents were higher in the autumn leaves than the spring leaves for all trees. All three species studied showed increased density of foliar tissue. LRWC and SLA values have declined significantly for old, senescence and sick leaves. Complex research is needed to determine if changes reported in this study are related to age or are correlated with urban environmental influences or consequences of the attack of pests.

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