STUDIES REGARDING SOME MORPHOMETRIC AND BIOMASS ALLOCATION PARAMETERS IN THE URBAN HABITAT ON *PLANTAGO MAJOR*

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Abstract. *The present paper presents data obtained from a biomonitoring study conducted in the summer and fall of 2015 on Plantago major in Timișoara, Romania. P. major is a common perennial herb used as a bioindicator due to its adaptability to environmental conditions and occurrence in many urban habitats. Therefore, this species became of interest in habitat quality assessing. The studied parameters were leaves areas (LA) and lengths using Digimizer software, which allows a nondestructive, cheap and quick approach. Through other methods Specific Leaf Area (SLA), Total Dry Mass (TDM) and Dry Biomass Allocation Ratio (DBAR) were calculated. While Leaf Area presented significant variations among seasons and study zones, SLA values did not range so much. Furthermore, a strong association between leaves lengths and areas was obtained. On the other hand, TDM presented the highest values for the samples collected from Green Forest during the summer and the smallest values for the probes sampled from Urban area during the autumn. Biomass allocation ratios had also variations, and appeared that plants invested differently in aboveground or underground structures depending on season and habitat type.*

Keywords: *biomonitoring, Digimizer, Leaf Area, urban zone, biomass allocation ratio.*

INTRODUCTION

Air pollution is a topic of global concern and many studies have suggested the necessity of using bioindicators to monitor air quality (KLUMPP et al., 1994). Advantages of biomonitoring have been frequently discussed (WITTIG, 1993). Biological responses can be considered more representative than data supplied by chemical or physical detectors, in that they are spatially and temporally extensive; moreover, they allow for estimating both the levels of pollutants and, even more importantly, the impact on biological receptors (CALZONI et al., 2007). A large variety of organisms, such as lichens (LARSEN et al., 2007), herbs and trees, have already been used in the biomonitoring of air pollution (HIJANO et al., 2005). Herbs can be used in habitat quality assessment due to their wide distribution and high accessibility (KARDEL et al., 2009).

Plantago major L. (common or greater plantain), member of the Plantaginaceae family, is a perennial herb with rosette leaves. It is a very familiar weed and may be found anywhere on roadsides, meadow-land, cultivated fields, waste areas, and canal banks (GALAL AND SHEHATA, 2014). This species has already been used in biomonitoring in Timișoara, Romania (IANOVICI et al., 2009). There are studies that compare the anatomical particularities and the ecological adaptations of Plantago species from Romania (IANOVICI et al., 2011; IANOVICI, 2011), but Plantago also was studied for its interactions with bacteria, viruses and micoritic fungi (BLASZKOWSKI et al., 2006). Ecophysiological parameters such as Leaf Relative Water Content – LRWC – were determined for this species (DATCU, 2014). The first studies about quantifying the degree of colonization by the vesicular-arbuscular mycorrhizas were realized on the species of the Plantago genus (IANOVICI, 2010). In 2010, a review synthetized the Romanian specialty literature in regards to Plantago species (IANOVICI et al., 2010).

Less attention has been given to morphological and plant biomass parameters as indicators of long-term habitat (urban) change, although parameters such as the specific leaf area (SLA) have been recognized to vary depending on microclimatic conditions (BALASOORIYAA et al., 2009). Leaf area (LA) is an important variable for most eco-physiological studies in terrestrial systems regarding light interception, photosynthesis efficiency, response to irrigation or fertilizers and yield of crop plants (BLANCO AND FOLEGATTI, 2003). Determining this parameter with the Digimizer software is faster and cheaper, allowing the user to store and process data, and review photos. It is also a non-disruptive technique (IANOVICI et al., 2015).

The specific leaf area (SLA, foliar area per dry mass unit) is an important feature in plant ecology because it is associated with many critical aspects of plant growth and survival, which can lead to variations in the relative growth potential rate and plant behavior (LI et al., 2005). Numerous authors have provided wide-ranging reviews of biomass allocation among plants (e.g. REICH, 2002), the aboveground / underground biomass allocation ratio being a parameter of interest in such studies.

The aim of this study was to apply an easy and fast method of calculating leaves areas and specific leaf area on plants with bioindicator potential and to calculate and compare the total dry mass and aboveground / underground biomass allocation ratio for Timişoara, Romania.

MATERIALS AND METHODS

The study was conducted in Timişoara, Romania. The biological material to be analyzed was represented by samples belonging to the species *Plantago major.* The specimens were harvested in the 2015 summer, when the plants were in the flowering phenophase, and autumn season respectively, corresponding to the fructifying phenophase. The research was realized in two types of sites: urban area (U zone), represented by Titu Maiorescu Street, from Timişoara, Romania and Green Forest (GF zone), located in the North-East part of the city, representing an Urban Green area. The plants were brought in the laboratory, then washed and organs were separated using a scalpel. From each plant a leaf was scanned, using a scanner (HP Scanjet G3010) along with a piece of millimeter paper. The resulting images were processed using the Digimizer Free Image Analysis Software, resulting the leaf area (LA) (cm^2). Digimizer also offers the possibility to analyze lengths of the leaves. Therefore a regression was realized between lengths and areas of the leaves.

To determine the specific leaf area (SLA), the scanned leaves were then placed in an oven (Sauter Model) for 2 hours at 85° C, then weighed using the analytical balance (Kern Model), in order to determine Dry Weight (DW). SLA was calculated by dividing dry leaf weight to leaf area ($g * cm^{-2}$).

On the other hand, for the calculation of the biomass parameters, the organs of the harvested samples were detached with a scalpel, washed and weighted using an analytical balance, resulting FW – fresh weight. The samples were then placed in an oven for two hours at 85° C. After drying, the organs were weighted obtaining the dry weight (DW) for each organ.

TDM (g) was calculated as the sum of all organs dry weights.

DBAR - Dry biomass allocation ratio- was calculated by dividing the sum of all dry weights specific to the aboveground organs to dry root weight (POORTER, 1999).

The statistical processing was realized using the GraphPad Prism 6 software.

RESULTS AND DISCUSSION

A wide range of LA values was obtained for the *P. major* samples collected from the investigated areas and between the studied seasons (Figure 1). The highest values of this parameter were found for the samples which were sampled in summer from GF. Overall, leaf area was bigger for the GF collected samples. Regarding U area, the biggest values for this parameter appeared also for the samples harvested during summer. A t test indicated that summer collected samples had significant higher values ($p < 0.05$) when compared to that specific for fall. We also remarked a significant bigger value of this parameter for the samples belonging to GF area ($p < 0.01$) when compared to those specific for U area.

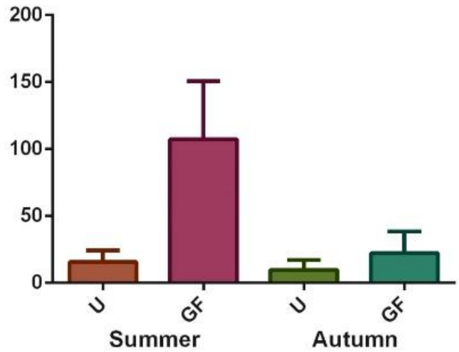

Figure 1. Mean ± SD of LA (cm^2) depending on investigated seasons and zones

Figure 2 represents the linear regression between foliar lengths (cm) and areas (cm^2). Following the completion of statistical analysis, a strong association between this two parameters was noticed ($r^2 = 0.9026$).

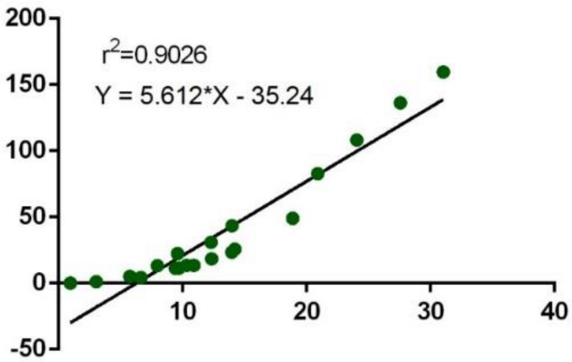

Figure 2. Regression between lengths of the leaves (cm) and LA (cm^2)

Specific leaf area (SLA) presented small variations among the investigated seasons and areas for the analyzed species (Figure 3). Thus the smallest mean values of this parameter were obtained for the samples collected during summer. For this season, a slight grow was recorded for the GF collected samples, by comparison with those from U area. The highest average values appeared to GF samples collected during autumn.

After completion of t test, no significant differences were found between the SLA from the both seasons ($p > 0.05$). Also the values from the investigated sites were not significantly different ($p > 0.05$).

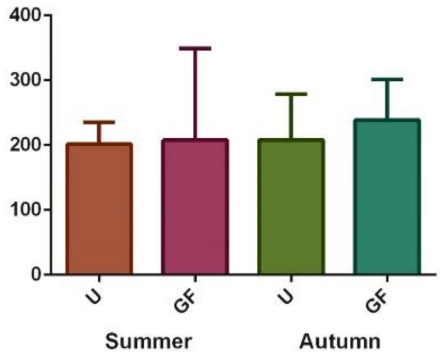

Figure 3. Mean ± SD of SLA (g * cm^{-2}) depending on investigated seasons and zones

The samples from GF area showed for both seasons higher values than those from U zone (Figure 4). Also, TDM for both studied areas, was higher for samples harvested in the summer. Through t test it was revealed that the samples collected in the summer had a significantly higher TDM ($p < 0.05$), compared to the autumn collected samples. Probes collected from the GF area in both seasons did not show significant differences compared to those in the U area ($p > 0.05$).

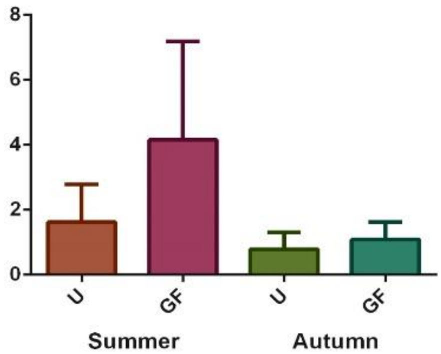

Figure 4. Mean ± SD values of TDM (g)

The highest biomass allocation ratio was obtained for the summer collected samples from GF area (Figure 5), the mean value in this case being 7.202. For this probes the aboveground part was the most developed in comparison with the subterranean part, represented by the root.

On the other hand, biomass allocation to aboveground in comparison with underground was the smallest, with an average value of 2.618, for *P. major* individuals collected from the U area in the summer. The plants which were collected during fall invested more in aboveground structures, especially the ones that represent U area. For GF area there was a bigger interval of variation for this parameter.

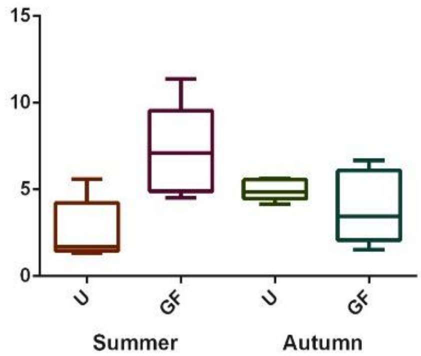

Figure 5. Min to max values of dry biomass allocation ratio for both investigated areas and seasons.

Leaf area is an important parameter for studies realized on light interception, photosynthesis efficiency, the response to irrigation or fertilizers and the yield of crops (BLANCO AND FOLEGATTI, 2003). Estimation of leaf area is also important in studies on plant nutrition and competition between plants, plant-soil-water relationships, measures to protect plants and heat transfer in plants (PANDEY AND SINGH, 2011). There are different techniques used to determine LA: planimeters methods, photogravimetric methods (the gravimetric method based on the weight of the paper cut-out of the silhouette leaf compared to the weight of known areas on the same paper) and area-length regressions (IANOVICI et al., 2015). Scanning leaf with is a quick and cheap technique which provide the possibility for automatic calculation of this parameter.

Models of optimal biomass allocation in plants predict decreasing root allocation with increasing nutrient availability (BLOOM et al., 1985). Regarding the allocation of all environmental factors, it is known that irradiance has the strongest effect, but nutrient levels also have a large effect, which becomes more pronounced after a size correction, as does the effect of temperature. However, in most cases, important foliar functional traits (SLA) are far more variable than allocation traits across species, a statement that, with the exception of nutrient stress, extends to most environmental effects (POORTER et al., 2012). Moreover, plants change their reproductive allocation patterns in response to competition. When plants are not crowded, they behave more like ‘r-selected’ species, allocating a large proportion of their biomass to reproductive structures (high reproductive effort). When plants are crowded, on the other hand, they behave more ‘K’ like, allocating less of their biomass to reproductive structures and a greater proportion to competitive structures such as stems and leaves (WEINER, 2004). Therefore, further studies on crowded and not crowded are useful.

CONCLUSIONS

Digimizer Free Image Analysis Software is a useful analysis tool that allows a quick and easy approach of the samples. By aggregating the data obtained with this software with DW for each leaf, the specific leaf area can be calculated very quickly.

Leaves areas had the biggest values for the samples collected in summer from GF zone.

There is a strong association between LA and lengths of the leaves.

Although, in this study, LA has shown large variations depending on the vegetation season, the SLA grew slightly in Plantago during autumn.

TDM and biomass allocation ratio had also the biggest values on summer GF probes. In conclusion, it was noticed that *P. major* individuals show a phenotypic plasticity.

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