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METHODS OF BIOMONITORING IN URBAN ENVIRONMENT: LEAF AREA AND FRACTAL DIMENSION

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ABSTRACT
In urban conditions, we investigated several leaf traits (leaf area, specific leaf area, fractal dimension and specific leaf weight) on Taraxacum officinale, Tilia tomentosa, Aesculus hippocastanum and Ambrosia artemisiifolia. The analyzed organs were mature leaves, on the first indications of senescence. This study used an exact, inexpensive and efficient in terms of costs alternative methods for determining the leaf parameters. On the other hand, this paper presents an application of the leaf area and fractal dimension in the analysis of leaf shape. Our results show that leaf area and fractal dimension are sensitive parameters that can be effectively used in biomonitoring.
KEY WORDS: *biomonitoring, architecture of plant, phenology, Taraxacum officinale, Tilia tomentosa, Aesculus hippocastanum, Ambrosia artemisiifolia*

INTRODUCTION

Phenology has become of major interest within the fields of conservation, ecology, evolution and agronomy (Scheifinger *et al*, 2002; Böhm *et al,* 2001; Ianovici *et al*, 2011). Primary, phenological data have been used to support the scheduling of agricultural works (Chuine *et al,* 2004). Plants vary in their sensitivity to pests or frost depending on their state of development and informations provide important support in the warnings and recommendations (Zadoks *et al,* 1974; Koslowski & Glaser, 1999; Jevrejeva, 2001). Various types of networks were designed: phenological networks observing wild plants, agricultural observation systems and measurements of the airpollen concentrations (Defila & Clot, 2001; Ianovici & Faur, 2001). Phenology is an easy-to-observe and cost-efficient instrument for the early detection of changes in the ecosystems and can be viewed as integrative measurement device for the environment. It is known the relationship between plant development, climate and weather. Phenological phases show great interannual differences and also large spatial fluctuations (Ianovici *et al,* 2012; Ianovici, 2012). Individual and environmental factors influence plants (Sparks *et al,* 2001; Ianovici *et al,* 2010). In recent years phenology is very valuable integrative parameter to assess the impact of climate change (Menzel, 2002). Phenological stages are recognized as globally coherent ecological fingerprints of climate change (Schröder *et al*, 2014).

Phenological data in urban habitats reflect biological response to climate and can be used for climate biomonitoring (Parmesan, 2007; Ianovici *et al,* 2009). Urban habitats are often disturbed, heterogeneous, highly dynamic and usually feature high degrees of soil sealing, heavy traffic, noise and vibration pollution, air and soil pollution, soil compaction, more light, high CO_2, NO, NO_2, CO, O_3, SO_2 and particulate matter levels, high nitrogen deposition, and low water availability (McKinney 2008; Ianovici *et al,* 2008). Urban soils are more alkaline than rural soils, with higher NaCl concentrations. Temperatures are higher compared to their rural surroundings ('urban heat island'). Wind conditions in cities are also highly variable (Klumpp *et al.* 2009).

The architecture of plant is the result of developmental processes and is a dynamic notion. Plant architecture addresses two important concepts: the shape and its structure. It has long been recognized that the in situ morphology of a plant can be complex and may vary greatly, even within a species, reflecting the interplay between developmental processes and environmental constraints (Hodge et al, 2009; Ianovici & Latiș, 2015).

The interactions between different plant species and urban habitat quality were extensively investigated by different researchers. We tested several methods useful in biomonitoring urban habitat quality: the concentrations of airborne pollen (Ianovici & Faur, 2001), the concentrations of fungal spores in the atmosphere (Ianovici & Faur, 2003), the viability of pollen (Ianovici *et al,* 2008), quantification of colonization with vesicular arbuscular mycorrhizae (Ianovici, 2010), the density of stomata and density of trichomes (Ianovici *et al,* 2009), leaf relative water content and leaf ash content (Ianovici, 2011a), leaf relative saturation deficit and succulence (Ianovici, 2011b), leaf water loss, specific leaf area and specific leaf weight (Ianovici, 2011c), leaf thickness and leaf thickness lost (Ianovici *et al,* 2012), density of foliar tissue (Ianovici & Latiș, 2015). In this work we tested the leaf area and fractal dimension of leaves for the first time. The aim of this study is to compare several foliar parameters of plants (*Taraxacum officinale*, *Tilia tomentosa, Aesculus hippocastanum* and *Ambrosia artemisiifolia*) from urban areas, in the immediate vicinity of road traffic.

MATERIALS AND METHODS

In the fall of 2014 (October) we harvested integral and mature leaves of *Taraxacum officinale*, *Tilia tomentosa, Aesculus hippocastanum* and *Ambrosia artemisiifolia*. Foliar samples came from urban areas (Timisoara), immediately adjacent to the road traffic. The biological material was transported to the laboratory, where we determined the following parameters: SLA (specific leaf area), SLW (specific leaf weight - SLW, leaf mass per area - LMA or specific leaf mass - SLM), LA (leaf area) and fractal dimension (FD).

The leaves can have complex shapes, making the determination of leaf area be inaccurate sometimes. The leaf, whose area was measured, was placed on a white background next to an object with known area. The leaf was laid as flat as possible and parallel to the known object. Photographical distance was adjusted so as to contain only the background, the leaf and the known object. Photographs taken were processed with the Digimizer program. The digital method is faster and cheaper, it allows storing and processing of data and reanalyzing the photos. Also it is nondestructive.

Typically, the fractal dimension of a real-world object is calculated using the box-counting method. The fractal dimension (FD) can be used as a simple, single index for summarizing properties of real and abstract structures in space and time (Berntson & Stoll, 1997; Ioanes & Isvoran, 2006). To obtain the box-counting fractal dimension of leaves architecture we made slides when plants were completely developed. Slides were taken at the same distance, focusing on the centre of the leaves. Images were captured with Adobe Photoshop with a resolution of 3072/2048 pixels. Because the thickness of the lines within digitized images can have a large impact on fractal dimension, images were preprocessed similarly, by selecting a window size of 1024/1024 pixels, then filtering, converting to greyscale, and adjusting to the same intensity. A specific software (ImageJ) was use for fractal analysed the data (Escos *et al,* 2000).

To determine the SLA we pierced 15 disks with known area. The sample was dried at 60 - 70°C for at least 24 hours until constant weight. The SLA (cm^2 g^{-1}) of each leaf was calculated by dividing the area of the leaf to its dried weight. Specific leaf area (SLA) describes the efficiency with which the leaf captures light in relation to biomass invested in the leaf. SLA is positively related to growth rates, relative concentrations of foliar nutrients and photosynthetic capacity (Cornelissen *et al,* 2003; Kardel *et al,* 2009; Patel & Saravanan, 2010; Ianovici, 2011c). Specific leaf weight in g/cm^2 is the inverse of SLA. Specific leaf weight (SLW) has become a widely used parameter as an indicator of leaf hardness when attacked by herbivores (Landsberg, 1990; Abbott *et al,* 2000; Steinbauer, 2000; Ianovici, 2011c).These gravimetric methods are faster and less expensive (Jonckheere *et al,* 2004; Ianovici *et al,* 2012).

Statistical analysis we performed using the following tests: Pearson correlations, one-way ANOVA, Tukey and Levene. In statistics, Levene's test (for homogeneity of variances) is used to evaluate the equality of variances for a variable calculated for the two or more groups. Among the methods we used for multiple comparisons we used the Tukey procedure which involves independent testing of observations and equal variation between them.

RESULTS AND DISCUSSIONS

Leaf area, specific leaf aria, specific leaf weight and fractal dimension are the key parameters for studying many physiological processes associated with urban habitats (Peper & McPherson, 1998). They are also extremely rapid and less tiring than traditional methods.

We identified the highest average value of LA in *Aesculus hippocastanum* and the lowest in *Ambrosia artemisiifolia* (Figure 1). We found significant differences among the analyzed samples (F=229.8, p = 8,326E-18). Levene's test is statistically significant and consistent variances hypothesis is rejected (p = 0.005989). Tukey's procedure revealed that only the values of LA in *Taraxacum officinale* leaves are not significantly different from *Ambrosia artemisiifolia*.

We have identified the highest average value of the SLA in *Taraxacum officinale* and the lowest in *Aesculus hippocastanum* (Figure 2). One-way ANOVA indicated significant differences among the samples analyzed (F=10.79; p=0.0001114). In this case the Levene's test is statistically significant and the hypothesis of the homogeneous variations is rejected (p=0.0365). Tukey's procedure showed that the SLA values of the *Taraxacum officinale* leaves are significantly different from the values of all other species.

The highest average SLW value we identified was in *Ambrosia artemisiifolia* and the lowest in *Taraxacum officinale* (Figure 3). There aren't any significant differences among the analyzed samples.

We identified the highest average value of FD in *Tilia tomentosa* and the lowest in *Ambrosia artemisiifolia* (Figure 4). One-way ANOVA indicated significant differences among the samples analyzed (F= 22,31; p= 0,00000006883). In this case the Levene's test is statistically significant and the hypothesis of the homogeneous variations is rejected (p=0. 0,0000000594). Tukey's procedure showed that the FD values of the *Taraxacum officinale* and *Ambrosia artemisiifolia* leaves are significantly different from the values of *Tilia tomentosa* and *Aesculus hippocastanum*.

The LA values in *Taraxacum officinale* and *Ambrosia artemisiifolia* correlate very poorly with the other parameters. The LA values in *Tilia tomentosa* and *Aesculus hippocastanum* correlate positively with the values of the SLW, but negatively with the values of the SLA. The FD values in *Tilia tomentosa* and *Aesculus hippocastanum* correlate negatively with the values of the SLA. The FD values in *Taraxacum officinale* and *Ambrosia artemisiifolia* correlate negatively with the values of the SLW.

These results are explained by the different leaves life. For herbaceous plants present in our study (*Taraxacum officinale* and *Ambrosia artemisiifolia*), new leaves appear throughout the vegetation season. For woody plants present in our study (*Tilia tomentosa* and *Aesculus hippocastanum*), the leaves appear in early spring and were approaching senescence at the harvest time. Differences between the fractal dimension of leaves is caused by two phenomena: the different developmental stages of every leaf and the process of morphogenesis of the leaf (Bradbury *et al,* 1984; Vlcek & Cheung, 1986; Borkowski, 1999; Jonckheere *et al,* 2006; Gazda, 2013).

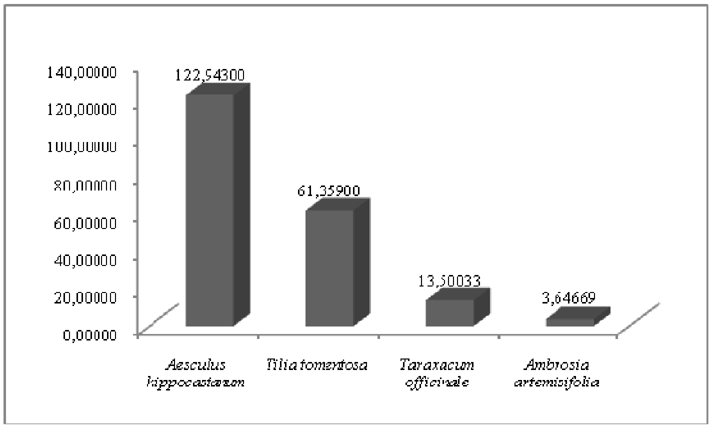

FIG. 1. The average values for the leaf area (LA)

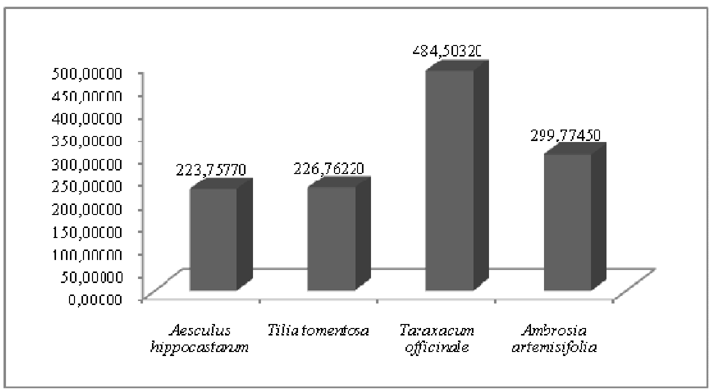

FIG. 2. The average values for the specific leaf area (SLA)

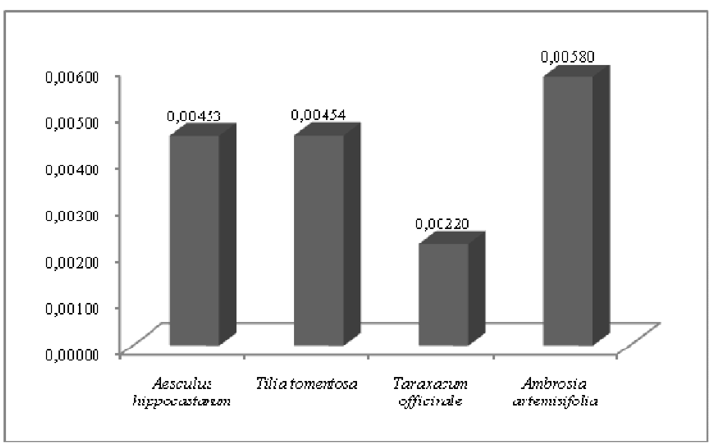

FIG. 3. The average values for the specific leaf weight (SLW)

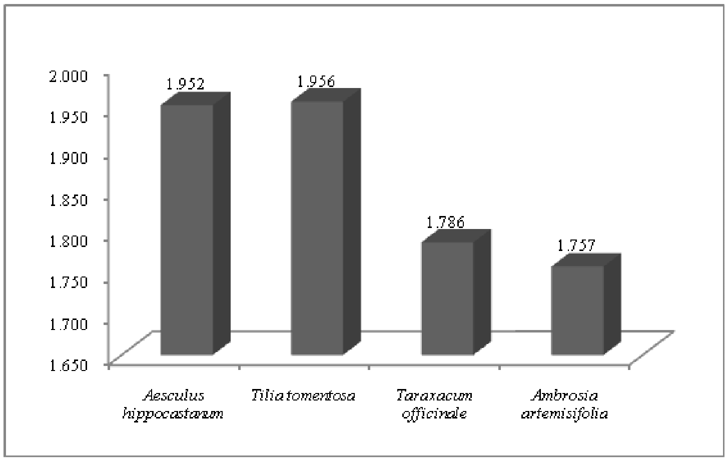

FIG. 4. The average values for the fractal dimension (FD)

Leaf area is an important variable for most ecophysiological studies which are made in terrestrial systems on light interception, photosynthesis efficiency, the response to irrigation or fertilizers and the yield of crops (Blanco & Folegatti, 2003). Estimation of leaf area is important in studies on plant nutrition and competition between plants, plant-soil-water relationships, measures to protect plants and heat transfer in plants (Sousa *et al,* 2005; Pandey & Singh, 2011). It is an important parameter in understanding photosynthesis, respiration, evapotranspiration, water and nutrient use and crop yield (Ugese *et al,* 2008). Measurements of leaves area are useful for studying the primary production in plants (Sestak *et al,* 1971). Ecologists use the relationships between the leaf area to elucidate the competition between different plant species (Harper, 1977).

Several papers have focused on developing appropriate techniques for measuring of leaf area. The techniques normally used (e.g. Ackley *et al,* 1958; Kvêt & Marshall, 1971; Coombs *et al,* 1985; Korva & Forbes, 1997) are: 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. The most common methodology includes counting squares on millimeter graph paper (Faur & Ianovici, 2004). Direct methods are restricted to the use of an automatic area-integrating meter. A mathematical model can be obtained by correlating the leaf length, width or length x width to the leaf area of a sample of leaves using regression analysis (Blanco & Folegatti, 2005).The alternative method uses a hand scanner linked to an computer. The image of the leaf generated by the scanner is transformed to area by an appropriate software program, which is based on the pixel counts of the image (Styer Caldas *et al,* 1992). The methods involving image processing based on video camera images and computer programs for analysis of these images, have been proposed (Hargrove & Crossley, 1988; Hagerup *et al,* 1990). These methods permit automatic calculation of leaf areas, and of areas lost to disease or herbivores, depending on the computer programs used.

Plant architecture can be described by synthetic descriptors such as fractal dimensions (FD), which represent the space filled by the plants (Tatsumi *et al,* 1989). The fractal dimension is having applications in ecology, neurobiology, species diversity, landscape structure, taxonomy and plant architecture (Oancea, 2006; Fodor & Hâruţa, 2008; Araujo Mariath *et al,* 2010; Bayirli *et al,* 2014). As much as plants grow, the FD increases (Nielsen *et al,* 1998). Fractal dimension has been correlated with plant topology and plant architecture. Differences in FD have been noted among species of dicots and monocots, as well as among genotypes (sorghum, rice and common bean). FD has been observed to vary with N availability for corn, and P acquisition from low P soils in common bean (Walk *et al*, 2004). Also, shoot ratio was correlated with variation in FD of roots. A large number of studies have demonstrated that FD increases under different kinds of stress, including genetic, temperature, food deficit, parasitic and pathogenic, and pesticide stress, among others (Escos *et al,* 2000). Fractal analysis are providing a quantitative characterization of the dynamics of plant spatial patterns in response to stress. The changes in the fractal dimension may indicate a substantial change in the processes that generate plant spatial patterns: edaphic parameters fluctuations, variations in plant secondary metabolites etc.

CONCLUSIONS

We measured several leaf traits (leaf area, specific leaf area, specific leaf weight) and leaf complexity (fractal dimension) for *Taraxacum officinale*, *Tilia tomentosa, Aesculus hippocastanum* and *Ambrosia artemisiifolia*. Leaf area was determined using digital photos. The LA and the SLA parameters are found to be sensitive, reflecting the living conditions of the plant and the different development modes during the seasons. The FD proved to be a powerful attribute that quantified the complexity of the leaf morphology. The development of leaves is characterized by a succession of stages each of which may involve distinctive morphological and physiological patterns. For a better understanding of the differences between species it is necessary to analyze these parameters throughout an entire year, at least. The present study provides a good basis for further research on impact of the anthropogenic influences on anatomical structure, morphology and physiology of the plants in urban habitat.

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