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A simple technique of random leaf collecting for biometric studies in a tree stand

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Abstract: The method reflects proportions in the number of leaves of different sizes and shapes, which appear in a tree stand. The study was carried out during autumn leaf fall, in about a hundred years old tree stand, dominated by beech. Leaves were collected three times, altogether with assessment of foliage density. For all gathered leaves, the width and length were measured and the data were statistically analyzed. Differences among all three samples were significant, which indicates different size of falling leaves in the following periods. Thus, only the research carried on at the end of leaves falling allows collecting a sample which represents proportions among leaves of different sizes in the tree stand.

Key words: *Fagus sylvatica*, random sampling, plant variation, biometry, leaf blade

VARIABILITY, TAXONOMY AND PHYLOGENY

1. Introduction

Leaves within a tree crown are differentiated by the shape, size and thickness of leaf blade (Beaudet & Messier 1998; Cowart & Graham 1999; Barna 2004). In case of phanerophytes it has been proved that the size of leaf blade may be changed by many factors, connected with light intensity which is different in varied parts of the tree crown and is related to the position of the tree in a tree stand (Barna 2004) as well as to the density of stand (Jack & Long 1991) or crowns (Frazer *et al.* 2000). As those factors operate simultaneously and with a varied force, their influence on the size of leaf blade is often difficult to define (Barna 2004). Moreover, the size distribution of leaves within the tree crown of various tree species may be dissimilar (Cowart & Graham 1999; Osada *et al.* 2003; Barna 2004). Proportions among leaves of different size and shape may also vary among individuals of the same species, even if they grow close to each other within the same tree stand.

Shape and size of leaves is an attribute frequently used in taxonomical or ecological research and it may be used in estimation of negative impact of environmental factors on trees and their condition (fluctuating asymmetry) (Premoli 1996; Møller 1999; Hódar 2002; Black-Samuelsson & Andersson 2003; Freenam *et al.* 2005). Thus, the appropriate method of leaf sampling is very important, often crucial for interpretation of the results. In some papers concerning intra-species leaf diversity, sampling was made only within particular part of the tree crown and particular fragment of the branch, without considering a status of the tree in the tree stand (e.g. Danielewicz 1993; Danielewicz & Maciejewska 1994; Białobrzeska & Staszkiewicz 1997). There are also publications, in which the procedure of leaf collecting is described very briefly or it is not mentioned at all (e.g. Jennions 1996; Nagamitsu *et al.* 2004).

Choosing the accurate method of sampling may be easy while it is based on the knowledge of leaf differentiation within the tree (Barna 2004). Some research concerning leaf diversity require using a sample representing proportions among leaves of varied size and shape which appear in the crowns of particular tree stands. Such a sample is easy to obtain by cutting down the trees (Barna 2004), but it is rather an impractical method. Another technique of sampling is dividing the tree crown into several areas and collecting equal subsamples of leaves separately from each area (Cowart & Graham 1999). But even in that case it may be difficult to keep proportions among varied leaf size classes, as there are differences in size between leaves growing close to the base and close to the top of stems (Cowart & Graham 1999; Svoboda 1972 after Barna 2004).

Another issue is the time of leaf sampling. In some papers regarding changes in size and shape of leaves the authors don't give any information about the time of sampling (Cowart & Graham 1999; Hódar 2002; McDonald *et al*. 2003; Nagamitsu *et al*. 2004). In consequence, it is difficult to find out whether sampled leaves were fully developed and finished their growth. Only in a few papers authors considered that factor by collecting leaves in the time of their discoloration (Niinemets & Kull 2003).

In most of papers referring to fluctuating asymmetry or comparison among individuals of the same species it was very important to analyze leaves coming from the same areas of a tree crown and growing in similar light conditions (e.g. Møller 1999; Lempa *et al*. 2000; Hódar 2002). Thus, correctly carried procedure of leaf sampling didn't have to concern the whole range of their diversity or to keep the proportions among different classes of size and shape of leaves within the tree crown. The main aim of the paper is to present a method of a random leaf sampling that expresses proportions in leaf quantity of different sizes and shapes, which appear in the tree stand.

2. Methods

The study was carried out in the phytocoenosis of the acidophilus lowland beech forest *Luzulo pilosae-Fagetum* localized in the Trójmiejski Landscape Park, in the surroundings of Gdańsk-Wrzeszcz. That phytocoenosis is situated on the hill-side with mean slope of 15° and south-eastern exposure. In about a hundred years old tree stand, dominated by beech, two tree layers – canopy and sub-canopy – had height of 30 and 18-20 m, respectively. Cover of the highest tree layer was about 75%, while of sub-canopy layer – 20%. There was no beech undergrowth in the described patch of community, probably due to a great density of the tree crowns. Within the studied area, a 200 m long transect from the slope base up to the hill top was established. A handful of leaves was collected from the surface of the litter every 2 m along the whole transect. After tossing the leaves, 200 pieces (not concerning damaged ones) were drawn for measurement. Sampling was made three times: on the October 28th and November 13th and 23rd 2005. Each time a few selected trees were photographed to record changes in the crown foliage. On the basis of those photos, drawings of trees in the following collecting terms were made (Fig. 1). During the first visit, the tree crowns had quite dense foliage. Only the highest shoots of the top part of the crown were leafless (Fig. 1a).

After 16 days, during next sampling, the crowns were thinned and the lack of leaves was observed on the shoots of the top part of the crowns as well as on the side, distal fragments of branches. Most of leaves were situated in the central part (closest to the trunk) and in lower parts of the crowns (Fig. 1b). During the last visit only few leaves in the central and lower parts of the crowns were noticed (Fig. 1c).

For all gathered leaves, the width was measured in its widest part and length of leaf blade – from the petiole base to the blade top (Fig. 2). All measurements were made by the same person, using a ruler with 1 mm accuracy. Collected data were then analyzed statistically, according to the methods described by Zar (1996) and using Statistica 6.0 programme (StatSoft 2001).

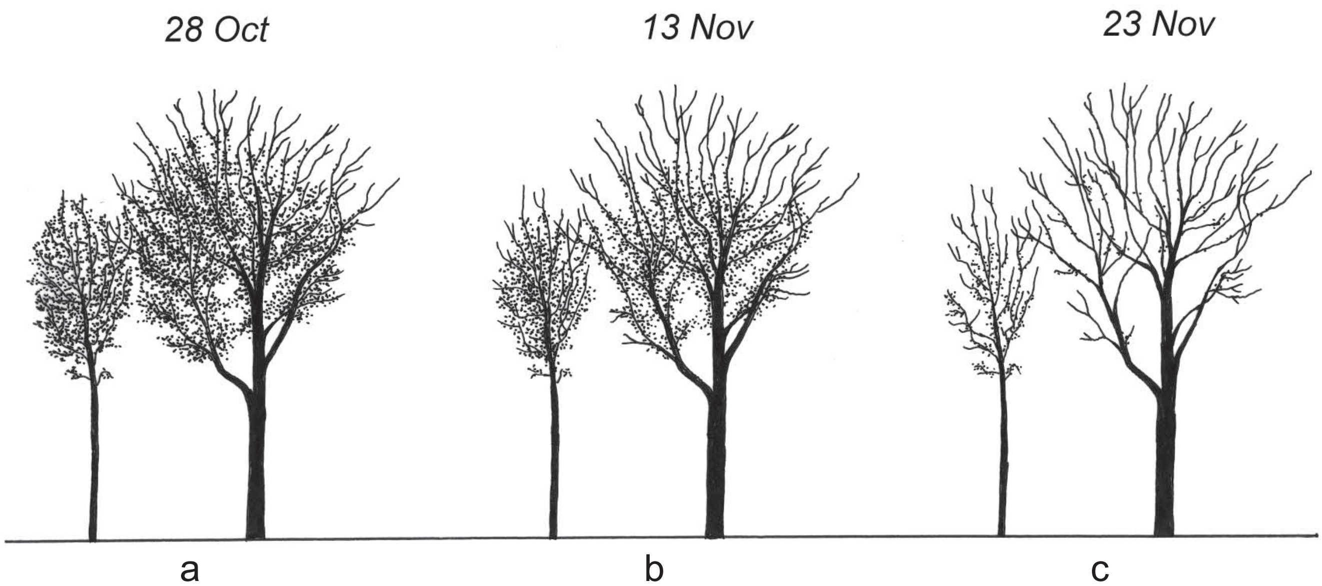

Fig. 1. Tree crown foliage in the studied beech stand in the terms of sample collecting (drawn by Tomasz S. Olszewski)

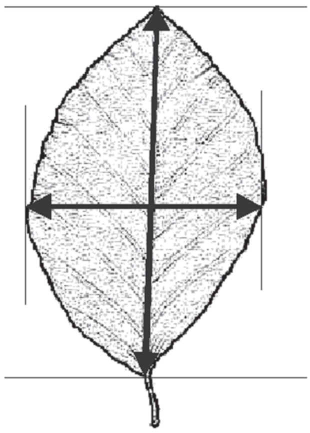

Fig. 2. Beech leaf measurements taken in this study

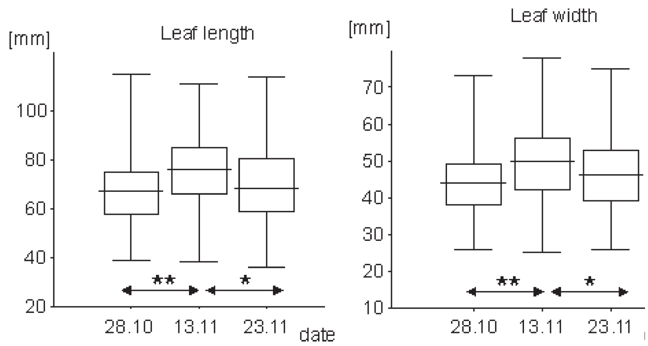

Fig. 3. Changes in leaf blade length and width of beech for the consecutive sampling dates.
Explanations: horizontal line – median, rectangular – quartile deviation, vertical line – range. Significant differences among samples (Dunn's test) are shown by arrows, significance levels: * – p<0.05, ** – p<0.0001

3. Results

Differences in variances of leaf blade length and width among three collected samples were highly significant (Levene's test, $F_{2,597}$=9.51, p<0.0001 for leaf length and $F_{2,597}$=7.47, p<0.0001 for leaf width). Also distributions of the leaf blade length and width in the first and third sample differed significantly from normal distribution (Shapiro-Wilks' W test, p<0.05). For that reason, for comparison among the samples nonparametric

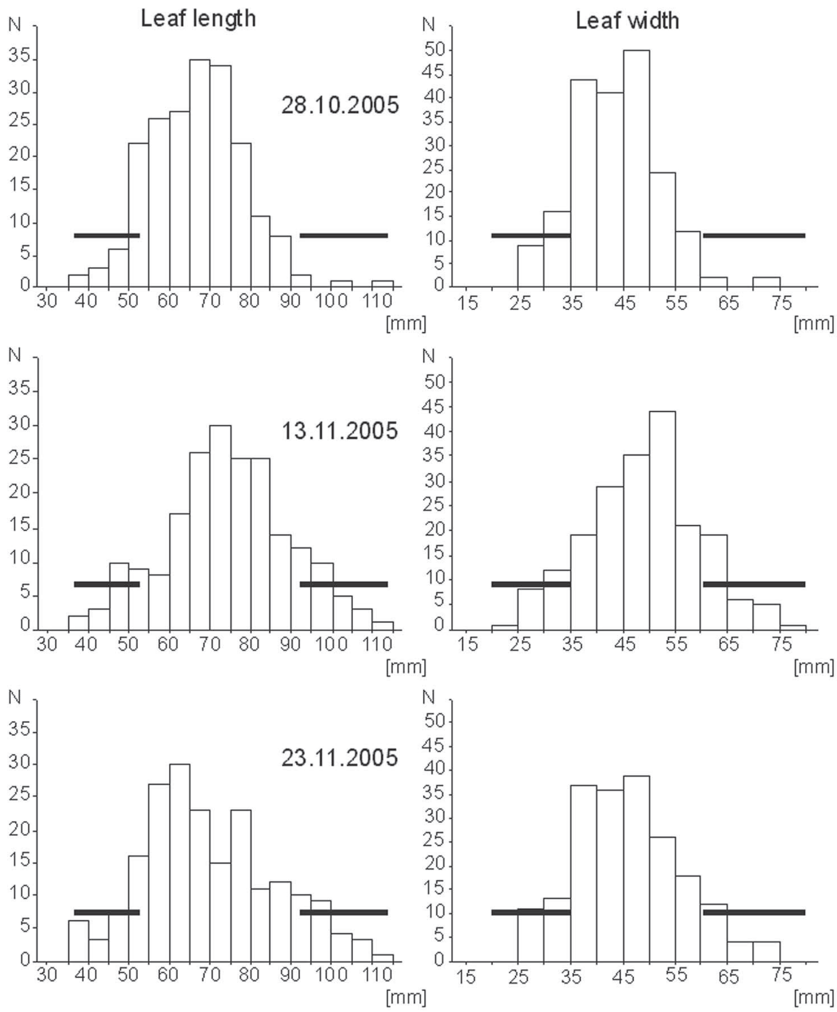

Fig. 4. Distributions of leaf blade length and width of beech collected on the consecutive sampling dates
Explanation: horizontal lines – range of the 10^{th} and 90^{th} percentile of distribution after joining data of the three samples

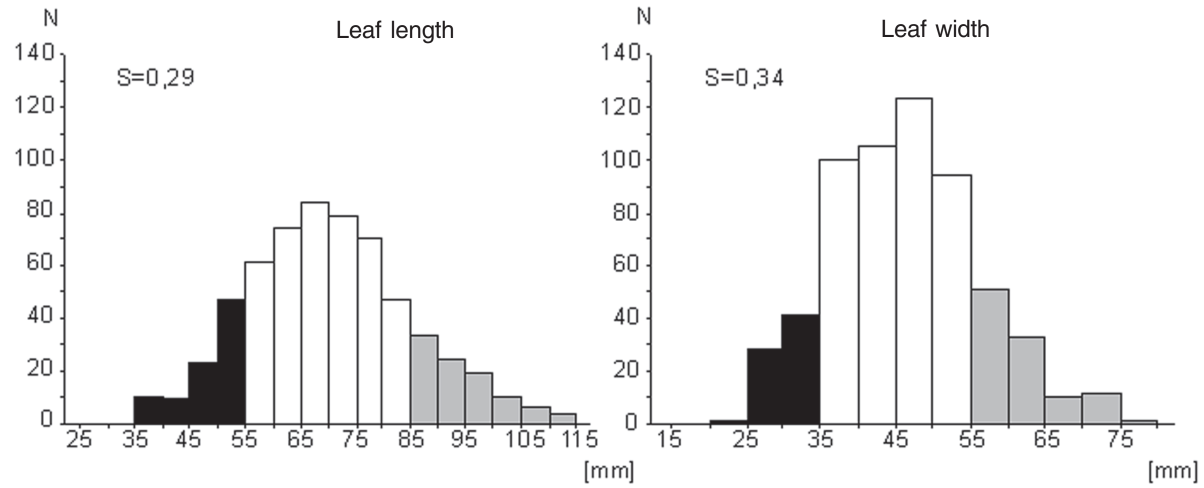

Fig. 5. Distributions of leaf blade length and width of beech from all samples
Explanations: in black – small leaves, in grey – large leaves, S – skewness coefficient

tests were used. The medians of leaf blade length and width were significantly higher in the second collected sample, while they did not differ between the first and third sample (Fig. 3).

The following samples differed also in the shape of measurement distributions. In cases of leaf blade length and width, the kurtoses of the first sample were much higher than the next two (leaf length: K=0.77, K=0.03 and K=-0.09, leaf width: K=0.59, K=0.08 and K=-0.04, respectively for the following sampling terms). The Skewness parameters indicated that all distributions were moderately right-skewed (leaf length: S=0.32, S=0.25 and S=0.29; leaf width: S=0.35, S=0.33 and S=0.34, respectively for the following sampling terms). Frequency of large leaves in the earliest sample (in the 90^{th} percentile of the sample distribution), was clearly lower than in both samples collected later (G test, for leaf length: G=26.19, $p<0.001$, for leaf width: G=24.34, $p<0.001$), while small leaves (in the 10^{th} percentile of the sample distribution) showed similar frequencies in each sample (G test, for leaf length: G=1.98, p=0.37, for leaf width: G=0.42, p=0.81) (Fig. 4). After combining the three samples, distributions of leaf blade length and width were right-skewed (Fig. 5). That points at a higher frequency of biggest leaves in the studied tree stand than it would be expected in the case of a normal distribution.

4. Discussion

Leaves of phanerophytes within the tree crown are differentiated by size and shape. If they don't be collected randomly for biometric studies, the results may lead to wrong interpretations due to omitting the intra-individual diversity (Cowart & Graham 1999). Presented method of leaf collecting after its falling solves that problem. Moreover, it allows to estimate the diversity of leaf size and shape within the whole tree stand as well as for particular individuals growing singly, although it does not allow to compare leaves from particular trees within the selected population. One of advantages of the presented method is possibility of estimation of the proportion among particular leaf size classes in the tree stand, which may be important for example in studies on tree stand biomass and productivity (Osada *et al.* 2001, 2003).

In the tree stand, leaves that fell earliest mostly came from the tops of the tree crowns and from distal fragments of shoots in the upper part of crowns. Strong lighting in those parts of trees results in apparently smaller leaves with thicker epidermis (Lichtenthaler *et al.* 1981; Barna 2004). In consequence of a great amount of such leaves altogether with small frequency of big leaves, the median of leaf blade length and width distributions in the first sample was lower. In the second and third sample, bigger leaves appeared more frequently. Probably, they grew in the middle and lower part of tree crown, where weaker light is compensated by larger area of assimilation organs. It has been proved by Barna (2004) on the example of beech, that on the trees forming canopy layer the largest leaves grew in lower parts of crowns, while on the individuals appearing in sub-canopy layer leaves in the lower part of crown were smallest. In the studied tree stand, frequencies of small leaves in all three samples were similar. Smaller, late falling leaves might come from the part close to the trunk. In that crown area the weakest light affects small sizes of beech leaves (Svoboda 1972 after Barna 2004). The later falling time of those leaves is connected with the limited penetration of crown interior by the wind when the crowns still had tight foliage.

Distributions of leaf blade length and width are still quite right-skewed after joining data from all three samples. Most probably such distributions represent real proportions of particular size classes of leaves in the studied beech stand. Due to suitable habitat conditions, amount of nutrients might not influenced significantly leaf size and the main feature affecting leaf blade size in the studied stand was an access to light. Higher proportion of large leaves was probably a consequence of a great quantity of leaves that grew in relatively weak light condition, due to high density of tree crowns and a minor role of sub-canopy layer.

The results of our research indicate that in order to collect a sample which represents proportions among leaves of different sizes from the whole tree stand one should collect it at the end of leaf falling. Only in the case of species with a fragile leaf blade, which may quickly decay in moist litter, it is better to collect samples in at least three terms. However, in that method it may be difficult to avoid collecting old leaves that lay in the litter for a long time. They may be covered by newly fallen ones as well as brought into the transect area by the wind. Instead of collecting leaves from the litter, samples may be gathered from sheets hung under the crowns along the transect, on which leaves would fall. If placed on the suitable height, a chance of bringing other leaves to the sample by the wind is rather minimal. Moreover, it seems that most of indigenous trees, especially the species of such genera as: *Fagus*, *Quercus*, *Betula*, *Carpinus* and *Alnus*, have thick leaves and their decomposition is slow enough to allow collecting only one sample after all leaves have fallen in the studied tree stand.

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