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Original article

ASSESSMENT OF ANTHROPOGENIC LOAD BY THE LEVEL OF FLUCTUATING ASYMMETRY OF *BETULA PENDULA* LEAVES (USING THE EXAMPLE OF THE DON STATE TECHNICAL UNIVERSITY TRAINING AND EXPERIMENTAL SITE)

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Abstract

Background. This study aimed to assess the level of anthropogenic stress on the territory of the Don State Technical University training and experimental site (Rassvet, Rostov Oblast) using the fluctuating asymmetry method of *Betula pendula* Roth. A comparative analysis of the integral index of fluctuating asymmetry was conducted between zones with different levels of expected impact ("Site 1" and "Site 3") and a conventional background zone ("Roshcha"). The level of fluctuating asymmetry in the anthropogenic impact groups was significantly higher than in the background zone, and the overall environmental condition of the study area corresponded to the "alarm" category. The most stress-sensitive traits were the length of the second vein and the angle between the veins. The fluctuating asymmetry method has proven its effectiveness for bioindicating complex anthropogenic stress in urban ecosystems.

Purpose. The aim of the study was to quantitatively assess the level of anthropogenic stress on the territory of the Don State Technical University training and experimental site by analyzing the fluctuating asymmetry of the leaves of *Betula pendula* Roth and identifying the most sensitive morphological features.

Materials and methods. The study focused on *Betula pendula* Roth growing in anthropogenically altered habitats, with special attention to the fluctuating asymmetry of its leaf morphological traits in the leaf blade of *Betula pendula* as an indicator of developmental instability. A method for assessing fluctuating asymmetry of the leaf blade was employed. Standard biometric methods were used to measure five leaf blade traits, followed by calculation of the relative asymmetry value for each trait and the integral fluctuating asymmetry index for each leaf. Statistical data processing included descriptive statistics, testing for normality (Shapiro-Wilk test), and homogeneity of variance (Levene's test). One-way analysis of variance (ANOVA) with Tukey's post hoc test was used to compare groups.

Results. The integral fluctuating asymmetry index (IIFA) for the combined sample was 0.093, which corresponds to the “alarm” zone according to Zakharov’s scale. Statistical analysis revealed significant differences ($p < 0.001$) between the conventional background zone “Roshcha” (IIFA = 0.065 ± 0.004) and the impact zones “Site 1” (0.110 ± 0.007) and “Site 3” (0.104 ± 0.006). At the same time, no significant differences were found between the two sites ($p = 0.721$). Analysis of the contribution of individual traits showed that the “Length of the second vein” (0.152) and “Angle between veins” (0.095) exhibit the greatest sensitivity to anthropogenic stress.

Conclusion. The study demonstrates that fluctuating asymmetry in *Betula pendula* leaves is an effective tool for diagnosing complex anthropogenic stress. The data indicate an unfavorable environmental situation within the Don State Technical University training and experimental site, where developmental disturbances reach alarming levels. Spatial differentiation of FA values confirms the hypothesis of higher stress in areas immediately adjacent to infrastructure. Identifying the most sensitive morphological features allows for the optimization of bioindication monitoring programs for such urbanized areas.

Keywords: fluctuating asymmetry; bioindication; *Betula pendula*; anthropogenic stress; urban ecosystems

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Научная статья

ОЦЕНКА АНТРОПОГЕННОЙ НАГРУЗКИ ПО УРОВНЮ ФЛУКТУИРУЮЩЕЙ АСИММЕТРИИ ЛИСТЬЕВ *BETULA PENDULA* (НА ПРИМЕРЕ УЧЕБНО-ОПЫТНОГО ПОЛИГОНА ДГТУ)

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Аннотация

Обоснование. Исследование направлено на оценку уровня антропогенной нагрузки на территории учебно-опытного полигона ДГТУ (п. Рассвет, Ростовская область) методом флуктуирующей асимметрии листовой пластинки берёзы повислой (*Betula pendula* Roth). Проведён сравнительный анализ интегрального показателя флуктуирующей асимметрии между зонами с предполагаемой разной степенью воздействия («Полигон 1», «Полигон 3») и условно-фоновой зоной («Роща»). Установлено, что уровень флуктуирующей асимметрии в группах антропогенного воздействия достоверно выше, чем в фоновой зоне, а состояние среды на всей исследованной территории соответствует зоне «тревоги». Наиболее чувствительными к стрессу признаками являются длина второй жилки и угол между жилками. Метод флуктуирующей асимметрии подтвердил свою эффективность для биоиндикации комплексной антропогенной нагрузки в урбоэкосистемах.

Цель. Целью исследования была количественная оценка уровня антропогенного стресса на территории учебно-опытного полигона ДГТУ путём анализа флуктуирующей асимметрии листьев берёзы повислой и выявления наиболее чувствительных морфологических признаков.

Материалы и методы. Объект данного исследования берёза повислая (*Betula pendula* Roth) в условиях антропогенно-преобразованных территорий. Предметом исследования выступила флуктуирующая асимметрия морфологических признаков листовой пластинки *Betula pendula* как показатель нарушения стабильности развития. В работе применялся метод оценки флуктуирующей асимметрии листовой пластинки. Использовались стандартные биометрические методы измерения пяти пластинчатых признаков листа с последующим расчётом относительной величины асимметрии для каждого признака и интегрального показателя флуктуирующей асимметрии для каждого листа. Статистическая обработка данных включала описательную статистику, проверку распределения на нормальность (критерий Шапиро-Уилка) и однородность дисперсий (тест Левена), для сравнения групп применялся однофакторный дисперсионный анализ (ANOVA) с последующим пост-хок тестом Тьюки.

Результаты. Интегральный показатель флуктуирующей асимметрии (ИП ФА) для объединённой выборки составил 0,093, что соответствует зоне «тревоги» по шкале Захарова. Статистический анализ выявил достоверные различия ($p < 0{,}001$) между условно-фоновой зоной «Роща» (ИП ФА = 0,065 ± 0,004) и зонами воздействия «Полигон 1» (0,110 ± 0,007) и «Полигон 3» (0,104 ± 0,006). При этом значимых различий между двумя полигонами не обнаружено ($p = 0{,}721$). Анализ вклада отдельных признаков показал, что наибольшую чувствительность к антропогенному стрессу проявляют «Длина второй жилки» (0,152) и «Угол между жилками» (0,095).

Заключение. Проведённое исследование демонстрирует эффективность метода флуктуирующей асимметрии листьев берёзы повислой для диагностики комплексной антропогенной нагрузки. Полученные данные свидетельствуют о напряжённой экологической обстановке на территории учебно-опытного полигона ДГТУ, где уровень нарушений развития достигает уровня «тревоги». Пространственная дифференциация значений ФА подтверждает гипотезу о более высоком стрессе в зонах непосредственной близости к инфраструктуре. Выявление наиболее чувствительных морфологических признаков позволяет оптимизировать программы биоиндикационного мониторинга для подобных урбанизированных территорий.

Ключевые слова: флуктуирующая асимметрия; биоиндикация; *Betula pendula;* антропогенный стресс; урбоэкосистемы

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Introduction

Urban tree stands are a critical component of urban ecosystems, providing a wide range of ecosystem services, including carbon sequestration, improved air quality, microclimate regulation, and biodiversity conservation. However, the viability and functionality of these stands are threatened by the chronic impact of a complex array of anthropogenic stressors. These include chemical pollution of the soil and atmosphere, physical degradation and compression of soil cover, disruption of symbiotic interactions in soil biota, and specific microclimatic conditions [1; 5].

The cumulative effect of these factors is reflected in fundamental disturbances in the physiology and ontogenesis of woody plants. Research has documented accelerated development, leading to a two- to threefold reduction in lifespan, premature senescence and leaf shedding, as well as the activation of compensatory mechanisms at the molecular and cellular levels [2]. Therefore, monitoring the condition of urban dendropopulations requires the development of integrated diagnostic methods sensitive to the overall stress response of the organism, as opposed to traditional approaches that measure the concentrations of individual pollutants.

A promising tool in this area is assessing developmental stability based on the magnitude of fluctuating asymmetry (FA) of bilateral organs. FA, defined as small, non-directional deviations from ideal symmetry, is a consequence of the organism's inability to buffer disturbances during ontogenesis [4]. Its level correlates with the intensity of stress – both genetic and environmental – making FA a universal indicator of environmental conditions [9]. The effectiveness of this approach has been confirmed for a wide range of stress factors: from heavy metal pollution [6] and thermal stress to the impact of radical changes in environmental conditions at the boundaries of habitats [10].

Thus, given the complex nature of anthropogenic stress in urbanized environments, fluctuating leaf asymmetry represents a theoretically sound and methodologically robust bioindicator parameter. However, under optimal conditions, low levels of fluctuating asymmetry will be observed as a manifestation of ontogenetic noise [8].

Purpose

The aim of the study was to assess the level of anthropogenic load on the territory of the educational and experimental site of the Don State Technical University (Rassvet, Rostov Region) based on the analysis of fluctuating asymmetry of the leaf blade of the *Betula pendula* Roth. To achieve this goal, *Betula pendula* leaf material was sampled in areas with expected varying degrees of anthropogenic impact (“Site 1”, “Site 3”) and in the conditional background zone “Roshcha”. The integrated index of fluctuating asymmetry (IIFA) was calculated for each study group based on five lamellar leaf traits. To qualitatively process the obtained data, a comparative statistical analysis of the obtained IIFA values was conducted between the groups to identify significant differences. Based on the statistical analysis data, the morphological leaf traits most sensitive to anthropogenic stress were determined. As a result of the study, the state of the environment in the study area was evaluated according to the Zakharov scale.

Materials and methods

The study was conducted using a model species, *Betula pendula* Roth. This species was chosen as a bioindicator due to its widespread distribution, high sensitivity to anthropogenic impact, and ease of leaf sampling.

The study was conducted at the Don State Technical University training and experimental site in the village of Rassvet, Aksay District, Rostov Region. The site represents an anthropogenically transformed ecosystem typical of the southern European part of Russia and includes areas with varying levels of recreational and anthropogenic pressure. Three groups of *Betula pendula* trees, growing 100–200 meters apart, were selected for the study. This allowed us to cover areas with differentiated anthropogenic pressure: zones in the immediate vicinity of educational and laboratory buildings and infrastructure (“Site 1” and “Site 3”) and a background zone, remote from the main sources of pressure (Roshcha). The climate of the area is temperate continental with arid features, characterized by hot summers with frequent dry winds and moderately cold, low-snow winters. Average annual precipitation is approximately 500-600 mm, with high interannual variability and a pronounced summer minimum [3].

To ensure representativeness of the data and allow for comparative analysis, material was collected at three sites with different expected levels of anthropogenic load:

1. “Site 1”: A site in close proximity to the landfill infrastructure, characterized by potentially high recreational load.
2. “Site 3”: A site adjacent to technological zones or utility lines, where anthropogenic impacts are expected.
3. “Roshcha”: A site consisting of a forest belt or natural tree stand within the landfill, remote from the main sources of impact and considered a background (control) site.

Leaf collection for the study subject was carried out uniformly from the middle canopy layer (at a height of 1,5–2 m) to minimize the influence of age-related and microclimatic gradients.

To assess developmental stability, the fluctuating asymmetry method – small, non-directional deviations from strict bilateral symmetry – was used. Five lamellar features were measured on each leaf (Fig. 1) on the left and right halves of the leaf blade:

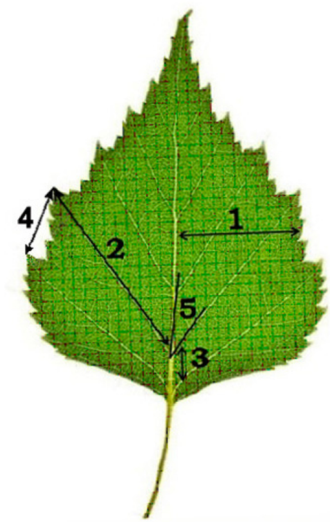

Fig. 1. Measured leaf parameters [7]

1. Width of leaf half.
2. Length of second vein from base.
3. Distance between bases of first and second veins.
4. Distance between ends of first and second veins.
5. Angle between main and second veins.

Measurements were taken using a digital caliper and a protractor with an accuracy of 0,1 mm and 1°, respectively.

For each trait on each leaf, the relative asymmetry value was calculated using the formula:

$$\text{FA val} = \frac{|R - L|}{|R + L|}$$

where *R* and *L* are the values of the trait on the right and left sides of the leaf, respectively.

The integral index of fluctuating asymmetry for each leaf was calculated as the arithmetic mean of the asymmetry values for all five characteristics:

$$\text{IIFA leaf} = \frac{\sum \text{FA val}}{5}$$

To describe the data for each group, the arithmetic mean (M), standard error of the mean (±m), standard deviation (SD), minimum, maximum, and coefficient of variation (CV, %) were calculated (Table 1).

Data distribution was tested for normality using the Shapiro-Wilk test, and homogeneity of variances was tested using Levene's test. Since the basic assumptions for using parametric tests (normality and homogeneity of variances) were not violated, a one-way analysis of variance (ANOVA) was used to compare mean values of the IIFA between the three groups. If statistically significant differences were identified, pairwise comparisons between groups were performed using Tukey's post-hoc test for multiple comparisons. The level of statistical significance was set at $p < 0,05$.

To rank the features based on their contribution to overall asymmetry, the mean asymmetry value for each feature was calculated across the entire pooled sample.

Environmental conditions were assessed based on the IIFA using the generally accepted Zakharov scale.

Results of the research

The integral index of fluctuating asymmetry (IIFA) for the combined sample of 30 leaves was 0,093, which, according to Zakharov's scale, corresponds to the “alarm” zone and indicates the presence of moderate anthropogenic stress.

To further clarify this conclusion, a comparative analysis of three groups of trees was conducted: two from the hypothetical impact zone (“Site 1” and “Site 3”) and one from the conventional background zone “Roshcha.”

The initial data analysis revealed that the IIFA distribution in the “Site 1” and “Site 3” groups deviated slightly from normality (Shapiro-Wilk test, p > 0,05), while in the “Roshcha” group, the distribution was normal. The group variances were homogeneous (Levene’s test, p = 0,152). Given that the parametric assumptions were met, a one-way analysis of variance was used to compare the groups.

Table 1.

Descriptive statistics of the integral index of fluctuating asymmetry of birch leaves in different groups

Group	n	Average (M)	Standard error (±m)	Standard deviation (SD)	Min	Max	CV, %
Site 3	10	0,104	0,006	0,020	0,074	0,134	19,2
Site 1	10	0,110	0,007	0,023	0,080	0,152	20,9
Roshcha	10	0,065	0,004	0,012	0,047	0,082	18,5

Descriptive statistics (Table 1) demonstrate that the lowest values of the IIFA were found in the “Roshcha” group (0,065 ± 0,004), while in the “Site” groups, the values were significantly higher and approximately the same (0,104 ± 0,006 and 0,110 ± 0,007). The coefficient of variation in all groups was below 25%, indicating acceptable homogeneity of the samples.

The results of the analysis of variance revealed statistically significant differences between at least one pair of groups (ANOVA: F (2, 27) = 21,45, p < 0,001).

Table 2.

Results of pairwise comparison of IIFA groups using Tukey’s post-hoc test (p-value)

Comparison groups	p-value
Site 3 — Roshcha	<0,001
Site 1 — Roshcha	<0,001
Site 3 — Site 1	0,721

Pairwise comparison using Tukey’s test (Table 2) confirmed that both “Site” groups differed significantly (p < 0,001) from the “Roshcha” background group. However, no statistically significant differences were found between the “Site

1" and "Site 3" groups (p = 0,721). A box plot (Fig. 2) was constructed to visualize these differences.

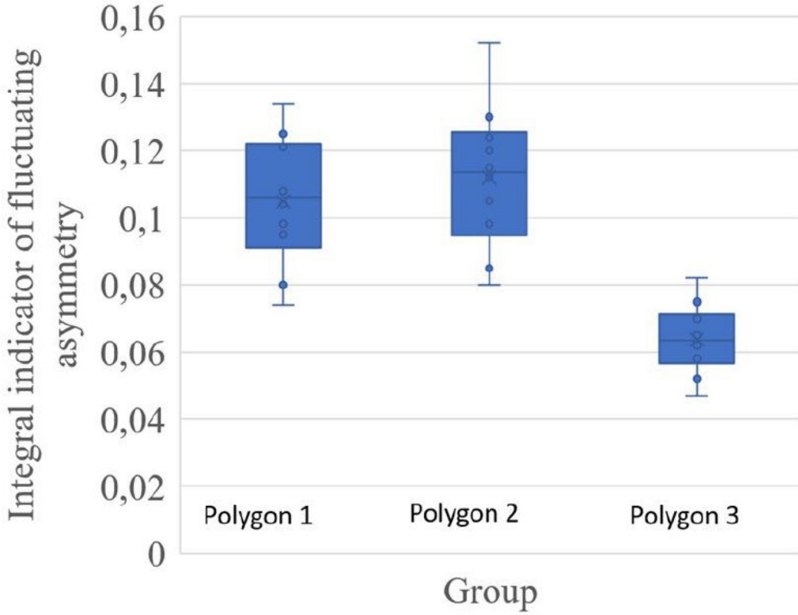

Fig. 2. Distribution of the integral index of fluctuating asymmetry in groups of *Betula pendula* leaves

Note: The figure shows the median, quartiles, and range of values. The "Site 1" and "Site 3" groups are not statistically different from each other (p> 0.05), but both significantly exceed the "Roshcha" group (p < 0,001).

To identify the most sensitive impact markers, an analysis of the mean asymmetry values for each of the five measured traits across the entire sample was conducted. Traits were ranked in descending order of contribution to the overall asymmetry:

1. Vein 2 Length: 0,152
2. Vein Angle: 0,095
3. Vein Tip Distance: 0,088
4. Leaf Halves Width: 0,057
5. Vein Base Distance: 0,037

The traits "Vein 2 Length" and "Vein Angle" make the greatest contributions to the overall asymmetry. This is consistent with literature data indicating that meristematic traits (such as vein length) and traits dependent on complex growth processes throughout the season (vein angles) often exhibit greater plasticity and, therefore, greater sensitivity to stress factors compared to more stable traits such as leaf width.

Conclusion

Thus, the conducted analysis allows us to draw a number of important conclusions. First, it has been confirmed that the level of fluctuating asymmetry in silver birch is an informative indicator of environmental conditions. The average IIFA value obtained (0,093) corresponds to the "alarm" zone on Zakharov's scale (values ≥ 0,055 indicate a critical level of disturbance), indicating the presence of persistent anthropogenic pressure in the sampling area from the testing sites.

Second, a comparative analysis of the groups revealed clear spatial differentiation. Significantly lower IIFA values in the "Roshcha" group compared to the "Site" groups convincingly demonstrate that the trees in the grove experience less stress, allowing us to consider this area as a conditionally background zone. The absence of significant differences between the two testing sites may indicate a similar level of impact in these areas, both in strength and nature.

The identified structure of the contribution of traits to asymmetry has important methodological implications. For future research and comprehensive monitoring, it is advisable to focus on the most sensitive traits (vein length and intervein angle), which may improve the effectiveness of the study.

In our study, the sample size was 10 leaves per tree, which is sufficient to detect gross differences. To conduct a more detailed analysis of the influence of microclimatic and soil variations, a comprehensive monitoring study will be conducted, collecting larger samples and including a larger number of trees in each group.

Based on the integral fluctuating asymmetry index (0,093), the environmental condition in the studied areas is assessed as unsatisfactory and corresponds to the "alarm" zone. The study revealed significant differences between the "Roshcha" background zone and the impact zones ("Site 1" and "Site 3"), confirming the reliability of FA as a bioindication method. The most sensitive characteristics of the silver birch leaf blade to anthropogenic impact are the length of the second vein and the angle between the main and second veins, which make the greatest contribution to the overall asymmetry.

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AUTHOR CONTRIBUTIONS

All authors made an equivalent contribution to the preparation of the article for publication.

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