Article

Changes in the Fluctuating Asymmetry of the Leaf and Reproductive Capacity of *Betula pendula* Roth Reflect Pessimization of Anthropogenically Transformed Environment

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Abstract: We have estimated the reproductive capacity of *Betula pendula* Roth and its relationship with an integrated measure of developmental stability, i.e., fluctuating asymmetry (FA) of the leaf. On the territory of a city with moderate anthropogenic pollution, a change has been detected in the integrated fluctuating asymmetry (IFA) of the morphology of the female reproductive sphere and reproductive capacity of *Betula pendula*. In conditions of anthropogenic stress, the birch is observed to produce a large yield of fruits annually, which is not subject to year-to-year fluctuations. Morphological variety of size and shape of fruit-producing organs increases along the gradient of industrial and transport pollution; part of morphotypes of infructescences and seeds is characterized by lowered or zero capacity for reproduction determined by seed quality (germination energy and germination capacity). The statistical data processing involved correlation, Shapiro–Wilk test, Levene’s test, factorial ANOVA, Scheffe test, Kruskal–Wallis ANOVA, Mann–Whitney test, $\chi^2$ method. Analysis of IFA has allowed us to reasonably well assess the state of the plant organism and to characterize environmental quality. A negative correlation between IFA and quantitative parameters of the functions of the reproductive sphere of *B. pendula* (infructescence diameter, seed quality) has been found, and positive correlation with qualitative parameters (the number of morphs of infructescences and seeds, the share of rare morphs of infructescences). Pessimization of urban environment creates the conditions for an increase in the share of defective infructescences and non-germinating seeds; a compensatory mechanism for this is an increase in reproductive effort of *B. pendula*. The consistency of responses in the vegetative and reproductive spheres reflects the disturbances in developmental stability of plants in urban communities.

Keywords: fluctuating asymmetry; developmental instability; silver birch; *Betula pendula*; urban area; reproductive ability; reproductive capacity; fruit producing; seed germination energy; seed germination

1. Introduction

The problem of environmental pollution and the response of living organisms to anthropogenic factors remain relevant. In conditions of urban ecosystems, plants are constantly subjected to action of various chemical substances present in the atmosphere and soil. The use of instrumental methods for analysis of air quality or soil composition, in conjunction with plant bioindicative characteristics, allows one to obtain more detailed information on their response to exposure to pollutants and to assess environmental quality [1–7].
Numerous studies have shown the effectiveness of using fluctuating asymmetry (FA) as an indicator of environmental health [8–15], including our own research by the example of the leaf of the silver birch [16]. The possibility of using FA for assessment of environmental health derives from the fact that it responds to the cumulative effect of the stress, reflecting the disturbances in developmental stability of the organism. This raises the question how developmental stability is related to the population ability to reproduce itself.

Studying the reproductive capacity of different species of trees in conditions of urbanization is important both for understanding the natural recovery of their populations, and for assessment of the impact of anthropogenic factors on the adaptation processes of plants existing in conditions of anthropogenic stress. It is known that woody plants in conditions of air pollution can show a decrease in the efficiency of their vegetative and reproductive spheres [1,5,6,17–21]. Environmental pollution affects the reproductive process both directly, by causing negative changes in the reproductive structures of woody plants, and indirectly, by weakening their life potential. The nature and extent of this impact depends on the natural environment, pollutant types, and their concentration level. In the anthropogenically transformed environment, tree species often demonstrate decline in abundance of flowers and fruits, forming underdeveloped reproductive organs or their premature abscission, or decreased seed quality. It is known that in city trees, their infructescences more often are observed being damaged by pest insects or fungal infections [22–24]. Other researchers have revealed another trend: contamination of plants with air pollutants has an adverse action on insects, leading to a lower insect species composition and abundance [25–27]. On the other hand, in stress-inducing conditions, trees can demonstrate another tendency in the reproductive sphere development: earlier and more intensive fruit producing [28–30]. One of the indicators of plant adaptation to the conditions of technogenic environment is their ability to bloom and form normally developed seeds. Phytoindication-based studies often use such parameters of trees as fruit abundance and seed quality and germination capacity [1,31–35].

The goal of our research is to assess the reproductive capacity of the silver birch in conditions of urban ecosystems and its correlation with fluctuating asymmetry of the lamina.

2. Materials and Methods

Research object: *Betula pendula* Roth is widely distributed on the territory of Russia and is often used in city street plantings [36,37]. The leaves of *B. pendula* are arranged alternately; on long shoots the leaves are triangularly rhomboidal, while on short shoots they are rhomboidal. *B. pendula* forms two types of inflorescences depending on the flower sex, i.e., whether they bear staminate or pistillate flowers. The fruits are aggregated into narrow cylindrical infructescences, in which they are arranged in groups of three fruits in the bases of three-lobed bract scales. The fruit of *B. pendula* is a samara with elongated elliptical shape and dark-yellow color, with two lighter wings. The seed is also an elongated ellipse, dark-yellow, and consists of a thin membranous seed coat, small endosperm, and white embryo [38–40].

We have studied trees of the middle-aged ontogenetic state. In this age, the trees have a pyramid-shaped crown with a rounded top, with the cortex covering the trunk to the height of 1–2 m [41].

The material was collected during 1999–2019 on the territory of Yoshkar-Ola, the capital of the Republic of Mari El, located in the eastern part of the East European Plain. The city has a machine industry, instrument engineering, wood processing, pharmaceutical, and food industry enterprises. The greatest contribution to the air pollution is made by motor vehicles, up to 70–85%. Compared to other cities of the Russian Federation, the potential air pollution is low. To evaluate the anthropogenic impact, we ranked traffic, industrial, and anthropogenic loads using data from the environmental committee and air pollution monitoring service [42,43]. For each site, an average score was calculated. We estimated not separate pollutant levels but anthropogenic impact on the whole. Four sites with different industrial and traffic loads were studied. The ecotopes are ranked in the order of increase in the total anthropogenic load, taking into account traffic density, proximity to industrial enterprises, and the negative effect of these enterprises. In detail, we have discussed the calculation of the complex anthropogenic load.
load in Shadrina et al. [16]. Site 1, nature protected territory “Sosnovaya roscha”. Natural biotope, no traffic and industrial loads. Site 2, residential area, low traffic load (up to 499 vehicles/h). As far as 1000 m away from the industrial zone (construction materials plant). Site 3, in the center of the city, moderate traffic load (500–1499 vehicles/hour). Site 4, industrial zone, in the vicinity of a pharmaceutical plant, moderate traffic load. Thus, site 1 is characterized by the minimal anthropogenic load, site 4 by the maximal, and sites 2 and 3 by a moderate load.

**Material collection and processing:** The leaves were collected using the method developed by Zakharov et al [44], according to what is necessary to have 100 leaves (10 leaves from 10 trees) to characterize one site. The measure of FA of each leaf was determined by five characteristics of the lamina structure and venation. We collected only intact leaves because the laminae damaged by pests or diseases can skew the final outcome in the direction of overestimated FA [16]. The full description of the method of collecting the leaves, material processing, and calculation of fluctuating asymmetry level is given in our article [16]. For each of the characteristics, its FA value was calculated as the absolute value of the ratio of the difference between the measurements of the left and right halves of the leaf to their sum. Integrated fluctuating asymmetry (IFA) was calculated as the five characteristics’ mean. We calculated the average IFA for each of the examined trees and for the site as a whole. Data for 4 years have been analyzed (2000, 2001, 2016, 2019).

During 1999–2002 and 2009–2019, the intensity of fruit producing of the plantings of **B. pendula** was assessed visually on a five-point scale proposed by V. G. Kapper [45]. Seed quality of **B. pendula** was studied using characteristics of germination [46–48] during two years, 1999–2000. Seeds for analysis were collected from 5 trees in each ecotope. Before germination, the seeds were kept at +15...+20 °C, one month before the germination the seeds were subjected to stratification at +1...+5 °C. Seeds were germinated on filter paper dampened with settled tap water in Petri dishes at a temperature of +25 °C in a thermostat, with 2 replications of 100 pcs. in each. According to standard methods, **B. pendula** seeds are germinated in three replications. In our experiment, we increased the number of replications to 10 (5 trees, 200 seeds from each of them). Due to that, the quality of 1000 birch seeds was examined in each ecotope. We determined germination energy (the proportion of normally germinated seeds (%) that have germinated by the 7th day from initiating the experiment) and germination capacity (the proportion of normally germinated seeds (%) that have germinated by the 15th day from initiating the experiment). The seeds are considered normally germinated when their seedlings have a root whose length is no less than the seed’s length. For further statistical analysis, relative data (%) were converted to 2 arcsin x.

To study the variability of the reproductive organs, in late August, we collected the infructescences of **B. pendula** in the amount of 20–50 per tree [49]. The variability of **B. pendula** infructescences was examined by three parameters: infructescence length, width, and the ratio of its length to thickness. Some morphological polyvariety was noted in the shape of infructescences and seeds [50]. Four varieties of the infructescence shape were found: typical elongated; atypical elongated with a narrow wedge-shaped dry base; elongated with a narrow tapered dry top; atypical rounded. Additionally, we took note of manifestations of intraindividual variability of infructescences and occurrence of defective infructescences.

Analyzing morphological polyvariety of **B. pendula** seeds, we distinguished 7 morphs: typical elongated elliptical (1), obovate (2), elliptical (3), rounded (4), elongated (5), rhombic (6), and narrow obovate (7) (Figure 1).
Figure 1. Morphological variety of the seed shape in B. pendula: 1, elongated elliptical; 2, obovate; 3, elliptical; 4, rounded; 5, elongated; 6, rhombic; 7, narrow obovate.

For the assessment of morphological variety of shapes, we used indices proposed by L.A. Zhivotovsky [51,52], \( \mu \), average number of morphs; \( h \), proportion of rare morphs; and \( r \), similarity between populations in polymorphic traits; which were calculated by the following formulae:

\[
\mu = \left( \sum_{i=1}^{m} \sqrt{p_i} \right)^2
\]

(1)

where \( \mu \), the average number of morphs; \( p_i \), morph occurrence; \( m \), the amount of the morphs found.

\[
h = 1 - \frac{\mu}{m}
\]

(2)

where \( h \), the proportion of rare morphs; \( \mu \), the average number of morphs; \( m \), the amount of the morphs found.

Error for \( \mu \) and \( h \) was calculated by these formulae:

\[
S_\mu = \sqrt{\frac{\mu - (m - \mu)}{N}},
\]

(3)

\[
S_h = \sqrt{\frac{h(1-h)}{N}},
\]

(4)

where \( S_\mu \) and \( S_h \), the error for \( \mu \) and \( h \), respectively; \( N \), sample volume.

The similarity between populations in polymorphic traits was calculated by this formula:

\[
r = \left( \sum_{i} \sqrt{p_i q_i} \right)^2
\]

(5)

where \( r \), population similarity index; \( p \), occurrence of the morph in the population 1; \( q \), occurrence of the morph in the population 2.

The statistical data processing was carried out in Statistica 10 software package using parametric and nonparametric statistics, Shapiro–Wilk test, Student’s t-test, factorial ANOVA (model 1, since ecotope and year factors are fixed), Scheffe test, Kruskal–Wallis ANOVA, Mann–Whitney test, Spearman’s coefficient, method \( \chi^2 \). ANOVA hypothesis of the homogeneity of variance was tested using Levene’s test. If the variances were homogeneous (\( p > 0.05 \)), then factorial ANOVA was used, with heterogeneity of variance (\( p < 0.05 \))—Kruskal–Wallis ANOVA [53].
Material amount: A total of 1600 birch leaves were analyzed, 16,000 measurements of the right and left sides of leaves were taken. Variability of 2800 infructescences and 2000 seeds was studied, parameters of seed quality of 8000 seeds of *B. pendula* were determined.

3. Results and Discussion

3.1. Fruit Producing of *B. Pendula*

*B. pendula* belongs to species with stable fruit production and with relatively frequent and abundant fruit yields [49]. The intensity of fruit producing of *B. pendula* trees in relatively clean environments (sites 1 and 2) was modest or moderate (grades II and III). Grade II of fruit producing intensity designates a modest quantity of infructescences on a small number of branches of a specimen, mainly in the upper and middle parts of the crown, especially at its south side; grade III, the trees have an average number of infructescences arranged uniformly or in groups on a significant number of branches in the upper and middle parts of the crown, especially at its south side. In the central park (site 3), we have recorded a greater intensity (grade IV) of fruit producing in the trees of *B. pendula*, which means that infructescences are arranged almost over the entire crown, especially at its south side. A very high intensity of fruit producing (grade V) was observed in *B. pendula* trees found in the vicinity of a pharmaceutical plant (site 4); in their crowns, there was observed a very significant amount of infructescences. Consequently, fruit producing of *B. pendula* increases in conditions of environmental pollution.

3.2. Variability in Seed Quality of *B. pendula*

Biological properties of seeds: seed germination energy (SGE) and seed germination capacity (SG) in a significant degree characterize the resistance of plants, their population stability, and self-reproduction ability [46]. Table 1 presents Spearman’s rank correlation of the examined traits of *Betula*. We used Spearman’s rho because the traits have different dimensions. These characteristics are highly intercorrelated: Spearman’s $r = 0.97$, $p < 0.05$ (Table 1).

| Infructescence, $L$ | Infructescence, $D$ | Infructescence, $L/D$ | SGE | SG |
|---------------------|---------------------|-----------------------|-----|----|
| Infructescence, $L$ | -                   | -                     | -   | -  |
| Infructescence, $D$ | 0.54 *              | -                     | -   | -  |
| Infructescence, $L/D$ | 0.96 *              | 0.08                  | -   | -  |
| SGE                 | 0.54 *              | 0.55 *                | 0.35| -  |
| SG                  | 0.54 *              | 0.42                  | 0.43| 0.97 *|
| IFA                 | -0.24               | -0.52 *               | 0.00| -0.62 *|

* $p < 0.05$.

Below we present the histograms with the occurrence of germination of birch seeds in different sites and the respective bell curves (Figure 2). They show the density function of the normal distribution with the average value and the standard deviation. The figure shows that the distribution of occurrence of SG in the ecotopes 1–3 is close to the normal distribution, which is indicated by the Shapiro–Wilk test ($W = 0.92–0.93$, $p > 0.05$), which is used for evaluating distribution parameters of small samples. The distribution series of SG occurrence in the ecotope 4 has a tail on the right ($W = 0.92–0.93$, $p < 0.01$). The test on seed germination has revealed a repeatable very low germination in this habitat.
To reveal the effect of different factors on the studied parameter, we performed analysis of variance. Two-factor ANOVA showed no dependence between the parameters of quality of *B. pendula* seeds collected in Yoshkar-Ola in 1999–2000 and the characteristics of the year \((p > 0.05)\), so the following are the average data (Table 2). In our experiment on germinating *B. pendula* seeds, in all collection sites, low, i.e., not exceeding on average 20%, SGE and SG were registered. However, it was found that as the pollution level increases, seed quality significantly drops \((F = 8.15, H = 31.69, p < 0.001,\) respectively). If ANOVA (or Kruskal–Wallis ANOVA) revealed a significant effect of the factor on the parameter, then pairwise comparison of the average values was performed with Scheffe’s multiple comparison test (or Mann–Whitney test), to determine which of the analyzed pairs differ significantly.

In the specimens found in the vicinity of the pharmaceutical plant, the parameters of the seed quality are the lowest and significantly differ from those in other ecotopes (Scheffe test or Mann–Whitney test \(p < 0.001–0.01\)) (Table 2). The correlation coefficient between the germination energy, germination capacity, and infructescence size (length and diameter) is average (Spearman’s \(r = 0.54, 0.55, p < 0.05\) (Table 1).
Table 2. The parameters of seed quality of *Betula pendula* on the territory of Yoshkar-Ola.

| Ecotope | n    | SGE, M ± m (%) | SG, M ± m (%) |
|---------|------|----------------|---------------|
| 1 2000  | 10.27 ± 1.541 | 13.12 ± 1.897 |
| 2 2000  | 11.02 ± 1.247 | 14.57 ± 1.635 |
| 3 2000  | 8.93 ± 1.705  | 12.67 ± 1.705 |
| 4 2000  | 2.70 ± 0.665  | 3.93 ± 0.888  |

**Factorial ANOVA**

Intercept: \( F = 141.71, \ p < 0.001 \)

Ecotope \( F = 8.15, \ p < 0.001 \)

Year: \( F = 1.14, \ p = 0.288 \)

**Kruskal–Wallis ANOVA**

Ecotope \( H = 31.69, \ p < 0.001 \)

Year: \( H = 0.01, \ p = 0.905 \)

Note: M, the arithmetic mean; m, standard error.

3.3. Morphological Variability of the Reproductive Organs of *B. pendula*

Morphological polyvariety of *B. pendula* infructescences was studied by the following quantitative and qualitative parameters: infructescence size (length: L, diameter: D, length to diameter ratio: L/D), infructescence shape, and the presence of defects. The quantitative parameters of the infructescence size are intercorrelated: Spearman’s \( r = 0.54, 0.86, \ p < 0.05 \) (Table 1). The analysis of the study materials (1999–2000) in Yoshkar-Ola showed that weather and climatic conditions of these two years had no effect on the studied traits in *B. pendula* (\( p > 0.05 \)) (Table 3). In the Table 3, we give the average morphometric data on the infructescence size of the silver birch in Yoshkar-Ola. The variability in infructescence length and diameter, L/D index, is affected by specific ecotopes (\( H = 13.86, H = 20.29, H = 9.23, \ p < 0.05–0.001 \), respectively), with the trends of the changes in these parameters being directed oppositely. Maximal infructescence L and D are recorded in sites 1 and 3; minimal measurements, in sites 2 and 4 (Table 3). Within the natural habitat and park zone, birch infructescences are larger and are comparable in size. In the central park of the city (site 1.3), we observed the maximum length of infructescences.

Table 3. Infructescence size of *Betula pendula* on the territory of Yoshkar-Ola.

| Ecotope | n | Linear Dimensions, cm |
|---------|---|-----------------------|
|         |   | Infructescence, L, M ± m | Infructescence, D, M ± m | Infructescence, L/D, M ± m |
| 1 700   | 3.15 ± 0.068 | 0.68 ± 0.004 | 4.64 ± 0.088 |
| 2 700   | 3.02 ± 0.033 | 0.65 ± 0.004 | 4.69 ± 0.038 |
| 3 700   | 3.21 ± 0.034 | 0.66 ± 0.005 | 4.87 ± 0.063 |
| 4 700   | 3.01 ± 0.069 | 0.64 ± 0.008 | 4.73 ± 0.088 |

**Kruskal–Wallis ANOVA**

Ecotope \( H = 13.86, \ p < 0.01 \)

Year: \( H = 0.02, \ p = 0.637 \)

Ecotope \( H = 9.23, \ p < 0.05 \)

Note: M, the arithmetic mean; m, standard error.

Infructescence variability was found also in the qualitative traits. The following variations in the shape of infructescences of *B. pendula* were found: typical elongated (84% specimens); atypical: elongated with a narrow wedge-shaped dry base (12%); elongated with a narrow tapered dry top (3%); rounded (1%). Unusual rounded infructescence shape in *B. pendula* is a rare occurrence. Infructescences of such a shape develop from androgynous (bisexual) infructescences, which can be found in *Betula* L. species in extreme conditions and after intentional introduction [46]. We registered the rounded infructescence shape in a single tree in the vicinity of a pharmaceutical plant. In parameters of intrapopulation morphological variety and the share of rare morphs, ecotope 4 dramatically stands out, while ecotopes 1–2 show a tendency towards monomorphism and do not demonstrate significant differences from each other (Table 4).
Along the pollution gradient, there is also an increase in the occurrence of defective infructescences, as observed in specimens growing in the ecotopes of the moderate pollution zone. The trees from sites 1 and 2 demonstrate a higher percentage of defective infructescences compared to the control and low pollution levels. Specifically, the trees from sites 1 and 2 show dark-brown, seedless (parthenocarpic) fruits with reduced wings and dark color, while exactly these fruits have been observed in the vicinity of the pharmaceutical plant (Table 4). Among the primates, we have found that the percentage of individuals exhibiting intraindividual variability increases from 0 in the natural biotope to 15 in the vicinity of the pharmaceutical plant, with a significant increase in intraindividual variability (Table 5).

It is worth noting that within one tree, typical elongated infructescences and infructescences with an unusual shape can be found, with the percentage of individuals exhibiting intraindividual variability increasing from 0 in the natural biotope to 15 in the vicinity of the pharmaceutical plant (Table 4). Along the pollution gradient, there is also an increase in the occurrence of defective infructescences, both in the total number and in the percentage of trees with defects.

The increase in the share of defective infructescences with the increase in anthropogenic impact intensity is characterized by high significance. The increase in intraindividual variability is also significant but does not change monotonically with the increase in the anthropogenic load; instead, two pairs of biotopes stand out: the control and low load on one side and two biotopes with a relatively high anthropogenic load on the other side (Figure 3). The birches encountered in the natural biotope were found to have no defective infructescences and zero intraindividual variability. Factorial ANOVA has shown that the ecotopes significantly affect the occurrence of defects, while the year and the tree age do not (Ecotopes: \( F = 17.14, p < 0.001 \). Year: \( F = 2.85, p = 0.09 \), Age: \( F = 2.77, p = 0.10 \)).

The morphological polyvariety of *Betula pendula* seeds in ecotopes of Yoshkar-Ola was studied using qualitative parameters (Figure 1). The typical elongated elliptical shape of seeds was found in 27% of specimens; obovate, in 27%; elliptical, in 20%; rounded, in 9%; elongated, in 7%; rhombic, in 6%; and narrow obovate, in 4%. The color of *B. pendula* seeds varies from light- or dark-yellow to dark-brown. The fruits that develop from bisexual inflorescences are narrow obovate in shape, flat, seedless (parthenocarpic), with reduced wings, and dark color. Exactly these fruits have been observed in specimens growing in the ecotopes of the moderate pollution zone. The trees from sites 1

### Table 4. Variation in shape and occurrence of defective infructescences in *Betula pendula* on the territory of Yoshkar-Ola.

| Site | \( m \) | \( \mu \) | \( h \) | 1  | 2  | 3  | 4  |
|------|------|------|------|---|---|---|---|
| 1 2  | 2 2  | 0.46 ± 0.02 | 0.60 ± 0.01 | 1.07 ± 0.04 | 1.59 ± 0.04 | 0.89 ** | 9.19 ** |
| 2 2  | 1.07 ± 0.02 |  |  |  |  | 1.41 | 11.63 ** |
| 3 2  | 1.11 ± 0.02 | 0.44 ± 0.01 | 0.89 |  | 1.41 | 10.73 ** |
| 4 4  | 1.59 ± 0.04 | 0.60 ± 0.01 | 6.26 ** | 9.90 ** | 11.31 ** |

Note: \( 1–4, \) sites; \( m, \) the amount of the morphs found; \( \mu, \) the average number of morphs; \( h, \) the proportion of rare morphs; above the diagonal, bold font, values of the Student’s \( t \)-test for comparisons the sites, by \( \mu \) (average number of morphs); below the diagonal, italic font, values of the Student’s \( t \)-test for comparisons by \( h \) (proportion of rare morphs); significant differences, ** \( p < 0.001 \).
and 2 demonstrate 4–5 variations of seed shape, and those from sites 3 and 4 demonstrate 7 variations ($\chi^2 = 185.15, v = 18, p < 0.01$). The increase in morphological variety in polluted ecotopes of the city is clearly demonstrated by the parameter $\mu$; starting from the sites 3.3–3.8 it nearly doubles, with the occurrence of different morphs being fairly high, so the share of rare morphs $h$ in the seed shape in polluted biotopes decreases (Table 5).

Table 5. Variation in the shape of seeds of *Betula pendula* on the territory of Yoshkar-Ola.

| Site | $m$ | $\mu$ $\pm$ 0.01 | $h$ $\pm$ 0.01 | Statistical Significance of Differences between the Sites, Student’s $t$-Test |
|------|-----|-------------------|-----------------|----------------------------------|
| 1    | 5   | 3.79 ± 0.11       | 0.24 ± 0.02     | ![Table entries](#)               |
| 2    | 4   | 3.33 ± 0.06       | 0.17 ± 0.02     | ![Table entries](#)               |
| 3    | 7   | 6.61 ± 0.07       | 0.06 ± 0.01     | ![Table entries](#)               |
| 4    | 7   | 6.44 ± 0.08       | 0.08 ± 0.01     | ![Table entries](#)               |

Note: 1–4, sites; $m$, the amount of the morphs found; $\mu$, the average number of morphs; $h$, the proportion of rare morphs; above the diagonal, bold font, values of the Student’s $t$-test for comparisons by $\mu$ (average number of morphs); below the diagonal, italic font, values of the Student’s $t$-test for comparisons by $h$ (proportion of rare morphs); significant differences, * $p < 0.05$, ** $p < 0.001$.

In this case, an increase in the variety of achenes is indicative of adverse effects in the process of their formation. For example, round and narrow obovate achenes in 5% of *B. pendula* specimens were non-germinating. This shape of fruits with reduced wings was found in infructescences that had developed from bisexual inflorescences and therefore had seedless (parthenocarpic) achenes. Thus, with the increase in the level of environmental pollution with industrial and transport emissions, the variety in the seed shape increases.

On the whole, the comparison of morphological variety of birch trees found in four Yoshkar-Ola ecotopes with different anthropogenic loads found that the ecotopes differed from each other (Table 6). The dependence on the anthropogenic load is observed most clearly in the infructescence shape: only at site 4 all four morph variations have been found, whereas for the other three sites, the differences were found only in the ratio of the typical morph and morph 2 (typical elongated; atypical elongated with a narrow wedge-shaped dry base). As the result, sites 1 and 2 are almost identical to each other, with similarity varying from 1.00 (full identity) to 0.99 (very high identity). At the same time, the similarity between site 4 and the three first ones were 0.86–0.87. Concerning the variety of the seed shape, the results are more ambiguous; all the sites are not only characterized by the different anthropogenic load but are also located separately. Relatively high similarity is noted between the sites with higher anthropogenic load (3 and 4); a lower similarity, between sites 2 and 4. The similarity of the site 4 with the three others greatly varies, while the morphological variety of the seed shape in sites 1–3 is characterized with closer levels of similarity.

Table 6. The similarity of ecotopes on the territory of Yoshkar-Ola in the morphological variety of the shape of seeds and infructescences of *Betula pendula* ($r$, index of similarity by polymorphic traits).

| Ecotopes | 1     | 2     | 3     | 4     |
|----------|-------|-------|-------|-------|
| 1        |       | 0.59 ± 0.02 | 0.62 ± 0.02 | 0.67 ± 0.02 |
| 2        | 1.00  |       | 0.58 ± 0.02 | 0.42 ± 0.02 |
| 3        | 0.99 ± 0.01 |       |       | 0.87 ± 0.01 |
| 4        | 0.86 ± 0.01 | 0.86 ± 0.01 |       |       |

Note: 1–4, sites; above the diagonal, bold font, index of similarity in variety of seed shape; below the diagonal, italic font, index of similarity in variety of infructescence shape.

3.4. Correlation between Reproductive Capacity and IFA of *B. pendula*

IFA of the leaves of the silver birch on the territory of Yoshkar-Ola was studied during 4 years. Table 7 presents the average data on IFA. Two-factor ANOVA revealed a significant effect of
environmental characteristics on developmental stability of \( B. \) pendula \((p < 0.001)\). Despite the weather fluctuations, we found no statistically significant effect of the “year” factor on IFA \((p > 0.05)\). Therefore, in the 20 years, the environmental quality, as characterized by the integrated developmental stability index of \( B. \) pendula, remained at the same level. The Scheffe test revealed significant differences in IFA between the groups of \( B. \) pendula from sites 1–4 \((p < 0.001)\). The data on air pollution from stationary posts (sites 1–4) also indicate that fluctuations of pollutant emissions differed over the years insignificantly \([42,43]\).

**Table 7.** Average integrated fluctuating asymmetry IFA of \( Betula \) pendula and the rank of environmental health \([44]\) on the territory of Yoshkar-Ola.

| Ecotope | \( N \) | IFA, M ± m | Rank | Factorial ANOVA |
|---------|--------|------------|------|----------------|
| 1       | 400    | 0.037 ± 0.001 | I    | Intercept \( F = 6257.18, p < 0.001 \). |
| 2       | 400    | 0.040 ± 0.001 | II (I) * | Ecotope \( F = 25.17, p < 0.001 \). |
| 3       | 400    | 0.047 ± 0.001 | III  | Year \( F = 1.54, p = 0.20 \). |
| 4       | 400    | 0.050 ± 0.001 | IV (III) * | |

Note: M, the arithmetic mean; m, standard error; * the ranks corrected for error.

During the 4 years of research, IFA of \( B. \) pendula at site 1 ranged between 0.036–0.039. According to the 5-grade scale of developmental stability \([44]\), the condition of these trees of \( B. \) pendula corresponds to relatively normal (rank I). IFA of the trees in site 2 varies within 0.037–0.041, which indicates minor disturbances in development of these specimens (rank II). IFA of the trees found in site 3 indicate average disturbances in development of \( B. \) pendula (rank III). At site 4, IFA of \( B. \) pendula indicated significant developmental disturbances (rank IV). The observed maximum of the IFA apparently evidences a continuing impact not only of emissions from the chemical plant, but also from vehicles. Consequently, adverse conditions in the zone of moderate pollution of Yoshkar-Ola affected the morphological structure of \( B. \) pendula leaves by increasing the IFA.

No effect of the year factor on variability of parameters of seed quality, infructescence size, and IFA within any given ecotope was found \((p > 0.05)\) (Tables 2, 3 and 7). This later allowed us to perform a comparative analysis of the average values of the obtained figures.

Correlation analysis showed a negative relationship between IFA and such parameters of the reproductive sphere of \( B. \) pendula as the diameter of infructescences, germination energy, and germination capacity (Spearman’s \( r = -0.52, -0.62, -0.50, p < 0.05 \), respectively) (Table 1). Below, we present scatter plots on the highest significant correlations (Figure 4). In the natural biotope, we registered low IFA, which indicates stable development of the silver birch. The diameter of infructescences and seed quality parameters are rather high compared to the trees in the urban ecotopes. In conditions of moderate environmental pollution in Yoshkar-Ola, \( B. \) pendula germination energy and germination capacity decrease and its development stability experiences disturbances, which results in an increase in IFA. However, in size, the infructescences can be comparable with those of the specimens in the natural biotope or even enlarged.
In addition, we conducted a correlation analysis of the relationship between IFA and the parameters of intrapopulation variety of morphs of reproductive organs of the silver birch. We found a positive correlation between IFA and such parameters as the average number of morphs of infructescences and seeds and the share of rare morphs of infructescences (Spearman’s $r = 0.95, 0.60, 0.63, p < 0.05$, respectively) (Table 8). IFA is negatively correlated with the share of rare morphs of seeds (Spearman’s $r = −0.80$). Consequently, with an increase in IFA also rises morphological polyvariety of shapes of reproductive structures of the birch, but the share of rare morphs of seeds ($h$) decreases.

### Table 8. Spearman’s rank correlation of IFA and parameters of variability in the shape of *Betula pendula* reproductive organs on the territory of Yoshkar-Ola.

|        | IFA | Infructescence, $\mu$ | Infructescence, $h$ | Seed, $\mu$ | Seed, $h$ |
|--------|-----|----------------------|--------------------|-------------|----------|
| IFA    | -   | -                    | -                  | -           | -        |
| Infructescence, $\mu$ | 0.95 ** | -                    | -                  | -           | -        |
| Infructescence, $h$    | 0.63 ** | 0.33                 | -                  | -           | -        |
| Seed, $\mu$       | 0.60 ** | 0.74 **             | 0.32               | -           | -        |
| Seed, $h$        | −0.80 ** | −0.74 **            | −0.32              | −0.80 **    | -        |

Note: ** $p < 0.05$.

### 3.5. Changes in the Reproductive and Morphological Parameters of *B. Pendula* Resulting from Deterioration in Environmental Conditions

It is known that vegetative growth and reproductive development of plants are closely connected. For example, in years with abundant blossoming, fruit producing trees show lower total growth of shoots. Excessive blossom and fruit set inhibits the growth of shoots, leaves, and reproductive organs. This reduces the seed yield of the following year, weakens the organism, and reduces winter hardiness [34]. However, in *B. pendula*, in conditions of moderate environmental pollution in Yoshkar-Ola, we noted a high extent of functioning in both vegetative and reproductive spheres. For example, the average growth of shoots is increased to 1.3–1.5 times and the lamina area up to 1.1–1.2 times ($p < 0.01–0.05$). This trend can be explained by pollutants being used by the plant as an additional source of mineral nutrients [15].

The analysis of the extent of fruit producing in the research object is complicated by the fact that it depends on weather conditions, both in the vegetation year, and the year when the reproductive organs were being formed [54]. However, if frosts had periodically occurred, they would have had an impact on the silver birch in all the ecotopes of Yoshkar-Ola. So, in the subsequent discussion, we are going to exclude the possibility of the negative impact of weather conditions on the extent of fruit producing. It is known that mineral nutrition deficiency, particularly concerning nitrogen,
which is often the case in urban plants, and an increased concentration of carbon dioxide in the air accelerate the plant reproduction [23,54], which could affect the intensity of blossoming in urban trees. Some chemical compounds can intensify blossoming in plants. For example, it was found that in the presence of sulfur dioxide, B. pendula produces more fruit [31]. Stationary observation posts of ecotopes 3 and 4 in the years of the study found SO\textsubscript{2} in the industrial and transport emissions, and its content exceeds maximum permissible concentration by 1.5–2.5 times [55]. Consequently, the increased fruit producing by B. pendula that we observed in conditions of industrial and transport emissions, probably is associated with a deficit of soil mineral nutrition and pollutants acting as boosters of blossoming and fruit producing. Intensive fruit producing in a related species, alder Alnus fruticosa, was registered in our studies previously, in conditions of industrial pollution on the territory of a coal strip mine in Yakutia, where it also was accompanied by an increase in the asymmetry level of this species leaves [56].

Navashin assessed the normal level of germination capacity of B. pendula as 10–20% [57], Turskii assessed it as 60–80% [58]. Our materials demonstrate figures similar to those obtained by Navashin [57], but we also registered a significant decrease in germination capacity of seeds collected at the sites subjected to higher anthropogenic load. It is worth noting that on the territory of another city, experiencing a similar anthropogenic load but in more severe climatic conditions, B. pendula is characterized by higher figures of germination capacity and germination energy, which are comparable with the Turskii results [58], but with the same statistically significant trends being observed: a decrease at the sites with high anthropogenic load [59]. In addition, there are studies showing a negative impact of an anthropogenically transformed environment on the male reproductive sphere of Betula [60].

The reason for low germination capacity of Betula seeds is development of parthenocarpic fruits due to the fact that its male flowers are often damaged by late spring frosts, which prevents the pollination of ovaries. Another reason for low germination could be development in the fruit of a parasitic fungus Sclerotinia [57]. Additionally, birch seeds can be damaged by various insects such as Betulapion simile Kirby, Protapion fulvipes Geoffroy, Curculio venosus Grav., Semudobia betulae Winn [26].

It is known that in the years of low yield, a decrease in seed mass, germination capacity, and other such figures is observed [57]; however, our study shows the opposite trend. The trees producing a large amount of fruits in conditions of moderate pollution by industrial and transport emissions have low seed quality, which may be caused by seed germination inhibitors [61], or the effects of fungal diseases, or by air pollutants [22]. Consequently, the minimal seed germination of B. pendula specimens growing in less favorable conditions is compensated by their maximal reproductive capacity: high extent of fruit producing and long infructescences, which contain more seeds.

Elevating reproductive capacity is one of the most important mechanisms of adaptation to adverse conditions. Schwartz [62] showed that in nature, high fertility in mammals compensates for increased mortality in Subarctic conditions, which is also confirmed by our data [63]. Our studies in a zone of anthropogenic impact showed that small mammals in the course of adaptation to continuing anthropogenic stress also demonstrate an increase in individual fertility, which can be regarded as an evidence of anthropogenic pessimization of the environment. Additionally, there can be observed an increased occurrence of pregnancy disorders and an increase in FA of cranial features in offspring [56]. One can assume that the stress associated with the increased reproductive effort leads to a destabilization of ontogeny, which in its turn limits the distribution of the species in its pessimal zone (natural or anthropogenic).

Thus, our own data and literature indicate that pessimization of the urban environment creates a background for an increase in the reproductive effort of B. pendula, leading, on the one hand, to an increase in fruit producing, on the other hand, to an increase in the proportion of defective infructescences and non-germinating seeds.

In conditions of moderate environmental pollution in Yoshkar-Ola, an increase in morphological polyvariety in the reproductive sphere of B. pendula can be observed, which manifests itself in 4 variations of infructescence shape and 7 variations of achene shape. In both groups of traits, we observed a statistically
significant increase in their variety at the site with higher industrial and traffic load. An increase in intrapopulation morphological variety in stress-inducing conditions is an ambiguous phenomenon, it may reflect both positive and negative effects [64]. Phenotypic flexibility plays an important role in population functioning; it is the main way of plant adaptation to different environmental conditions, and reflects the evolutionary fitness of the species [65,66]. Changes in the phenotypic structure of the population under stress is known for different animal and plant species [67,68]. There is evidence that genotypic richness in plants covaries with phenotypic variations of functionally significant traits [69,70]. Note, that there are studies showing both a consistent increase in genetic and phenotypic variety in new and adverse conditions [71,72], and the opposite effect [73,74]. According to our data, the phenotypic variety of seeds and infructescences of the silver birch in adverse conditions increases.

Cities play an important role in contemporary evolution, accelerating phenotypic changes in nature, including animals, plants, fungi, and other organisms [75]. Note that in urban conditions, the potential for rapid changes of traits may be amplified by simultaneous action of several selection factors [76]. Within cities in animals, there can be observed an increase in a variety of morphological traits and changes in occurrence of different morphs [77,78], and changes in morphometric parameters [79]. In plants found at technogenically polluted sites, there can be observed an increase in variability of morphometric parameters of leaves [80], or changes in the leaf shape. We suppose that an increase in polyvariety of shape of infructescences and seeds of B. pendula in conditions of urban ecosystems that we have found is an indication of adverse conditions, which, along with an increase in FA, demonstrates destabilization of the organism development.

It should be noted that part of the phenotypic variability we observed comes from the morphs associated with decreased or zero viability of the offspring: Makhnev [46] believes the rounded infructescence shape is characteristic of abnormal androgynous inflorescences, and two seed shapes (rounded and narrow obovate) were non-germinating. Usually such achenes are seedless (parthenocarpic). These morphs were not registered in the natural ecotope and most often are found in ecotopes with high anthropogenic load where the trees are characterized by a high FA level. Diaz et al. [81] noted that for the oak Quercus ilex, a direct relationship is observed between the FA level of the leaf and fruit abortion. Aborted acorns more often than the normal ones were characterized by higher asymmetry in their shape. The authors supposed that stressed trees selectively interrupt the reproductive act of low viability and that evolutionary selection has an effect on the level of developmental instability of fruits [81]. It is possible that in the case of the birch, we are also dealing with a differential mortality of the offspring of the trees growing in adverse conditions.

B. pendula trees in conditions of some ecotopes of Yoshkar-Ola are subject to complex adverse anthropogenic impact. An increase in IFA indicates a disturbance in morphogenetic processes and the homeostasis of the organism developmental stability on the whole. Morphological variability of infructescences of B. pendula is connected with forming low-quality, inviable seeds. Of course, in the urban environment, when artificial tree plantings are made, the natural population renewal is halted. However, this parameter can indicate the adaptation capacity of the species and so can be used for bioindication. Thus, not any variability can be considered as an adaptation or a mechanism that ensures population resistance. How can one determine the invisible limit of an environmental factor that can be safely overcome by the specimens of a certain species using various adaptations so that the population can survive? How can one determine the limit of variability that contributes to the population stability? This remains one of the topical questions in both population biology and applied ecology.

4. Conclusions

As compared to other cities of Russia, the registered air pollution level in Yoshkar-Ola was moderate, which is attributed to the landscape features and climatic conditions propitious for dispersion of pollutants; which we wrote about in Shadrina et al. [16]. However, anthropogenic stressors (chronical impact of industrial and transport emissions) exceed the limit of adaptation capacity of the silver birch, which may lead to disturbances in developmental stability. On the territory of a city with
moderate anthropogenic pollution, changes in the IFA level of the lamina, in the morphology of the female reproductive sphere, and in reproductive capacity of Betulapendula were found. In conditions of anthropogenic stress, abundant annual seed yield of the birch can be observed, not subject to year-to-year fluctuations. An increase in seed production and variety of fruit-producing organs in size and shape indicates poor quality of the environment and of the seeds themselves, because part of the morphotypes of infructescences and seeds is characterized by reduced (or zero) reproductive ability. Complex anthropogenic impact not only increases the variability of reproductive organs in B. pendula, but also impairs the quality of seeds, i.e., decreases their germination energy and germination capacity. Poor seed quality may reduce the possibility for natural population reproduction in an urban ecosystem, which is partially compensated for by a higher seed production. Consistency of reactions of the vegetative and reproductive spheres reflects the disturbances in developmental stability of plants in urban ecosystems.

An increase in the seed production of the birch with the increase in occurrence of disturbances in the reproductive sphere, morphological variety, and fluctuating asymmetry are links in the chain that reflects the strain put on the plants in urban conditions. An increase in seed production is achieved by increasing the reproductive effort and, consequently, energy expenditure for reproduction. Against this background, a decrease in seed germination takes place; thus, an increase in reproductive effort cannot completely compensate for the adaptation to pessimal conditions. An increase in the tension in mutual relations between the organism and its environment leads to a destabilization of ontogeny, which manifests itself, among other things, in a disbalance in the morphological structure of the population: an increase in the share of unviable morphotypes and the level of fluctuating asymmetry.

Thus, urban populations of plants can be a model, illustrating the differences in the processes that take place in nature in the optimum and the pessimum zones. The increased energy expenditure allocated to reproduction contributes to population survival, but increases the pressure experienced by the individual, which intensifies ontogeny destabilization, thus increasing the share of individuals with lowered viability, which in its turn negatively affects population reproduction. The balance between the well-being of the population and the individual defines, in the end, the adaptation potential of the population both in natural and in anthropogenic ecosystems.

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