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Anthropogenic environmental pressure influence on oak forest biodiversity and *Quercus robur* mitosis pathologies

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Abstract. The significant reduction of shrubs and grass plants biodiversity between urban and reserved territory could be a biomarker of human impact and effect of urbanisation. We could conclude that trees are more resistant to climate change and anthropogenic pressure. The research of cytogenetic parameters for English oak seed progeny in the areas with different levels of anthropogenic pollution was carried out. High level of mitotic pathologies and mitotic activities of seed progeny plantlets from trees growing near freeway was observed. Also high level of variability indices, such as percentage of prophase cells, metaphase cells, mitotic pathologies were seen.

1. Introduction

Ecological balance is practically impossible in the urban ecosystem. In the ecosystems of cities the ratio of producers, consumers and decomposers is disrupted. Regulation of all processes associated with the flow of matter and energy in the city is taken by the individual. The city produces a huge amount of toxic gases and waste polluting the environment. To some extent, this is due to the simultaneous impact on the body of two, three or more harmful factors, each of which has a minor effect, but in combination leads to serious human problems. Urban ecosystems can be characterized by follow traits: dependence (the need for a constant flow of resources and energy); instability (impossibility of achieving ecological balance); accumulation of solid matter due to excess of its import into the city over export (approximately 10: 1). Urban ecosystems are commonly characterized by reduction of biodiversity. Ruderal species suppress plants normally growing in such natural conditions. Normally amount of species growing in urban ecosystems is 2-4 times low than in natural conditions [1]. City territory is commonly characterized by high risk of plant teratogenesis. Fois M. et al. revealed influence of the Human Influence Index (HII) and the proportion of anthropogenic land uses. Such influences were negatively correlated with the number of local endemic taxa [2].

Environmental change is the most important factor, which causes individual and population variability [3], urbanization also influences on habitat and living organism directly [4]. Additional strains affect on plants by trampling soils, gas and dust contamination of the atmosphere in the conditions of the urban territory. Anthropogenic factors can also influence the biogeocenosis by



change in natural areas, soil compaction, reduction of soil aeration, change in water exchange, and polluting urban atmosphere by increase in the amount of vehicle emissions profiles [5]. The estimation of the level of this pollution is necessary by creating special global information system, which assess atmosphere conditions. We also think that such systems should include not only information on contamination and dangerous elements, but as much as possible abiotic and anthropogenic factors and condition of living organisms in analysed area, by using different methods.

Cytogenetic methods have been proven to be both informative and sensitive [6, 7]. Survival in the destabilization requires activation of compensatory mechanisms, which expands the reaction norm of the specie and boosts characteristics variability. Appearance of new variants of characteristics leads to the development of intraspecies variation and consequently increases the adaptive capacity of population. In addition to climate change, which directly influences habitat conditions of some species, urbanization even more forces and exacerbates the process of areal change of species as a result of environmental pollution and habitat fragmentation [8].

It is necessary to study the plants as an important and integral component of the environment under the conditions of constant human changes in the natural areas [9]. To estimate “current” adaptation processes, which occur within a population, it is necessary to constantly estimate different indices in different levels of plants organization, both genetic changes, and phenotypic variation of characteristics. The research of cytogenetic parameters of woody plants is actively conducted [10-12].

Trees are unique biomarkers of anthropogenic influence: they are long-lived in permanent habitat and can accumulate pollutants. Forest trees constitute about 82% of the continental biomass [13] and more than 50% of the terrestrial biodiversity [14]. Oaks (Genus *Quercus*) are dominant woody species throughout the northern hemisphere and are key drivers of terrestrial biodiversity from tropical to boreal landscapes [15].

The purpose of the investigation was to provide analysis of plant biodiversity of oak stand and to reveal the influence of environmental pressures on amount of plant species and cytogenetic parameters of the oak seedlings.

2. Methods and materials

English oak (*Quercus robur* L.) seed material was collected in the area of the 9th km of Zadonsk highway (part of freeway M 4 DON) in 2017 in Voronezh, Russia. The two experimental oak stands are parts of one population “Voronezh upland oak grove” (state nature wildlife sanctuary of regional importance). The first plot A was located deep in the forestland separated from the road by approximately 2 km. It is a part of naturally reserved territory and we considered it as conventionally “clean” area. Second plot B is located close to the Voronezh State Forestry University campus, right near the freeway with the high level of pollution caused by car exhaust fumes where the MAC (maximum allowable concentration) level is exceeded for nitrogen peroxide, phenol and some other compounds [16, 17]. Another important influence is connected to urban development leading to the waste accumulation and soil destruction. In other words, plot B is characterized by significant anthropogenic pressure. The study of these two experimental areas was necessary to estimate the direct influence of human impact on plant biodiversity and stability of forests in urban ecosystems.

We analyzed 10m × 10m plot of oak forest by 20 for each investigation area. In these 40 plots, we made geobotanical description to assess biodiversity of forest community. The geobotanical description of experimental plots was provided by accepted recommendations [18].

Besides the geobotanical description we estimated quality of oak seed progeny. Approximately 150-200 seeds were collected from each area after which they were germinated in moist sand. When the roots of plantlets reached a length of 2 to 3 cm, they were fixed in the 3:1 mixture of 95% ethanol and glacial acetic acid at 11 p.m., when the peaks of mitotic activity and pathological mitosis usually takes place [19]. The material was kept at 4°C. Roots of the plantlets were stained by acetic haematoxylin, squash preparations were made using the method described earlier [20].

80 preparations were made in general, 40 for each plot. Cytological analysis was performed using a Laboval-4 microscope (Carl Zeiss, Jena) at magnification of $40\times 1.5\times 10$ and $100\times 1.5\times 10$ to study mitosis characteristics and types of pathologies of mitosis in 700 cells.

Based on the obtained data, we estimated mitotic activity (by calculating the mitotic index – the ratio of quantity of dividing cells to the total quantity of analyzed cells). We also calculated mitotic index excluding prophase cells (the ratio of quantity of cells at the mitosis stages of metaphase, anaphase and telophase to the total quantity of analyzed cells – as an express-index of the delays of cells at mitosis stages and functioning of checkpoint-repair system [21]). We calculated the percentage of cells at the stages of prophase, metaphase, anaphase-telophase; the percentage of pathological mitosis out of the total number of cell divisions and the percentage of disturbances at the stages of metaphase, anaphase, telophase to the total number of aberrant cells at these stages of mitosis (as the majority of pathologies are recorded at these stages of mitosis); the percentage of every type of mitotic disturbances out of their total number and the percentage of cells with persistent nucleoli at the stages of metaphase – anaphase of mitosis out of the total number of cells at these stages. The classification of pathological mitosis was made according to Alov [22].

The results were processed statistically using the STADIA software package. The procedure of data grouping and processing were described by Kulaichev [23]. Cytogenetic characteristics of common oak plantlets were compared using the following criteria: the frequency of cells with pathological mitosis, using the Van der Varden rank X-test, as the distribution of these indicators is nonparametric; the mitotic index, the percentage of cells at different stages of mitosis, using the parametric Student's t-test. The percentage of different types of pathologies of mitosis was compared using Z-test approximation for equal frequencies criterion. Coefficient of variation (Cv) was counted according to Lakin [24]. Cv over 25 % is regarded as high, from 10 to 25 % – moderate, under 10 % – low [24].

3. Results and discussion

Results of biodiversity oak stand were received after analysis of 40 plots. The final data are presented below.

Geobotanical description of forest vegetation

Association name: Aegopodium-sedge oak forest

Geographical location: State nature wildlife sanctuary of regional importance “Voronezh upland oak grove”, Russian Federation, Central Federal District, Voronezh Region, Voronezh

Environment: Plot A – 2 km away from a freeway. Plot B – city, freeway, campus and urban development

Relief, Climate, Soil: Relatively warm and dry temperate continental climate. Winters are mild with frosts. The height of the snow cover reaches 30 cm, the transition through 0 °C occurs by April 1. The nature of moistening is unstable; drought and dry wind are frequent. The humidification coefficient is close to 0. Gray forest soils.

Size and shape of the test plot: 10m×10m

Characteristics of the tree group – A

Clarity of crowns plot A – 95%; plot B – 85%

The formula for the composition of the stand: 80% oak, 10% aspen, 5% birch, 5% acer for both analyzed plots.

Characteristics of the shrub group – B

Total projective coverage (in% or marks) Plot A – 75%; Plot B – 50%

Species of the shrub group are presented in Table 1.

Table 1. Species of the shrub group.

Name of the shrub	Numbers of plants		Height, cm		Plot A - deep in the forest	Plot B – near freeway
	Avg.		Avg.	Max.		
	Plot A - deep in the forest	Plot B – near freeway				
<i>Euonymus verrucosus</i>	3	-	80	120	+	-
<i>Crataegus laevigata</i>	1	-	120	210	+	-
<i>Corylus avellana</i>	2	1	150	280	+	+
<i>Acer tataricum</i>	2	4	140	350	+	+
<i>Viburnum opulus</i>	1	-	152	270	+	-
<i>Sambucus racemosa</i>	2	3	125	140	+	+

Characteristics of the lichen-moss layer

Most often lichens and moss are presented in Table 2.

Table 2. Most often lichens and moss.

Name of plant	Abundance	
	Plot A - deep in the forest	Plot B – near freeway
Lichen		
<i>Parmelia sp.</i>	+ medium	+ many
<i>Cetraria islandica</i>	+ sporadically	-
<i>Evernia prunastri</i>	+ rarely	+ sporadically
<i>Xanthoria sp.</i>	+ medium	+ many
Moss		
<i>Pleurozium schreberi</i>	+ many	+ many
<i>Dicranum undulatum</i>	+ medium	+ rarely
<i>Polytrichum commune</i>	+ medium	+ rarely
<i>Climacium dendroides</i>	+ rarely	+ sporadically
<i>Mnium hornum</i>	- sporadically	-

Characteristics of the grass layer – C

Total projective coverage (in% or marks) Plot A – 90%; Plot B - 70%.

Species of the grass layer are presented in Table 3.

Table 3. Species of the grass layer.

Name of plant	Abundance	
	Plot A - deep in the forest	Plot B – near freeway
<i>Equisetum pratense</i>	+ rarely	-
<i>Pteridium aquilinum</i>	+ rarely	-
<i>Convallaria majalis</i>	+ medium	-
<i>Polygonatum multiflorum</i>	+ medium	+ rarely
<i>Carex pilosa</i>	+ medium	+ many
<i>Gagea lutea</i>	+ medium	+ rarely
<i>Agropyron tenuis</i>	+ medium	+ rarely
<i>Dactylis glomerata</i>	+ rarely	+ medium
<i>Elytrigia repens</i>	+ rarely	+ medium
<i>Melica nutans</i>	+ rarely	-
<i>Millium effusum</i>	+ medium	-
<i>Poa nemoralis</i>	+ medium	- rarely
<i>Asarum europaeum</i>	+ medium	+ many
<i>Arctium minus</i>	+ rarely	+ medium
<i>Taraxacum officinale</i>	+ sporadically	+ medium
<i>Impatiens noli-tangere</i>	+ rarely	+ many
<i>Pulmonaria obscura</i>	+ many	+ many
<i>Berteroa incana</i>	-	+ medium
<i>Dianthus deltoides</i>	+ medium	-
<i>Stellaria nemorum</i>	+ medium	+ many
<i>Glechoma hederacea</i>	+ rarely	+ medium
<i>Lamium maculatum</i>	+ medium	+ many
<i>Prunella vulgaris</i>	+ medium	-
<i>Chelidonium majus</i>	+ medium	+ many
<i>Plantago major</i>	+ rarely	+ medium
<i>Lysimachia nummularia</i>	+ many	+ rarely
<i>Anemonoides ranunculoides</i>	+ many	+ many
<i>Scilla siberica</i>	+ many	+ many
<i>Potentilla argentea</i>	+ rarely	+ medium
<i>Rubus idaeus</i>	+ medium	+ rarely
<i>Melampyrum nemorosum</i>	+ medium	-
<i>Veronica chamaedrys</i>	+ many	- medium
<i>Urtica dioica</i>	+ medium	+ many
<i>Viola mirabilis</i>	+ many	+ medium
<i>Aegopodium podagraria</i>	+ medium	+ many
<i>Corydalis marschalliana</i>	+ rarely	+ sporadically
<i>Corydalis halleri</i>	+ many	+ many
<i>Campanula sp.</i>	+ rarely	+ sporadically

Study of tree biodiversity has shown no significant difference between plots. However, at the level of shrubs and grass one can see a significant decrease of existence and abundance of spices at the territory with high level of anthropogenic pressure. The significant reduction of shrubs and grass plants biodiversity between urban and reserved territory suggests a significant impact of urbanisation on plant biodiversity, while trees are more resistant to climate change and anthropogenic pressure. We

can also suppose that changing of oak stand is manifested not so much by decrease biodiversity as inner conditions of trees in the wildlife national reserve with regional importance “Voronezh upland oak grove”. On the other hand the decrease of trees biodiversity can take more time than the change in the species composition of shrubs and grass plants. Kirik et. al also provided estimation of biodiversity and anthropogenic pressure on Voronezh upland oak forest. It was found that the quantity of *Quercus robur* increases the competitiveness degree of the community. The contribution of other trees in the amplification of this population strategy is insignificant. [25].

In order to estimate the oak trees inner physiological processes we studied cytological properties of the oak seedlings from the trees growing in the areas with different levels of environmental pollutions caused by urbanization. The results of cytological characterization of the oak seedlings are shown in Table 4.

Table 4. Cytogenetic parameters of English oak seedlings from trees growing in the areas with different levels environmental pollution by highway influence.

Cytogenetic parameters	Plot A – deep in the forest		Plot B – near freeway	
		Cv, %		Cv, %
Mitotic index, %	7.5±0.1*	14.6	8.9±0.3*	21.3
Mitotic index excluding prophase cells, %	15.3±0.4**	7.8	22.2±3.5**	7.2
Prophase cells, %	32.1±1.8	48.2	35.7±1.6	28.0
Metaphase cells, %	25.3±1.5*	38.3	20.5±1.6*	29.8
Anaphase-telophase cells, %	42.6±2.0	22.7	43.8±1.8	24.2
Mitotic pathologies, %	10.3±1.2*	60.1	12.6±1.0*	41.2
Mitotic pathologies excluding prophase cells, %	15.1±1.8**	51.6	19.5±1.6**	51.3

* $p < 0.05$, ** $p < 0.01$

In the course of research of English oak seedlings’ cytogenetic parameters for such indices as “the percentage of cells at the stages of prophase, metaphase”, “pathologies of mitosis, calculated both including prophase cells and excluding them” the coefficient of variation Cv exceeds 25 %. This might indicate the genetic heterogeneity of common oak seed progeny.

Plot A (deep inside the forest) is characterized by following properties: plantlets are characterised by the low percentage of cells with pathologies of mitosis, the narrowed spectrum of pathologies of mitosis and the high percentage of metaphase cells. Plot B (actively explored urban territory) is characterised by high level of pathologies of mitosis, mitotic index and the percentage of prophase cells.

General trends of cytogenetic properties of oak seedlings in long-term researches were revealed. We observed a high level of mitotic pathologies, the spectrum spreading of pathologies of mitosis (the appearance of agglutination of chromosomes, asymmetric and tripolar mitosis) in 2001, 2007, 2009 and 2012 years investigations [26, 27].

4. Conclusion

The study of anthropogenic pressure on biodiversity of forestry ecosystems was carried out. Study of tree biodiversity has shown no significant difference between actively explored and preserved part of

“Nagornaya dubrava” oak forest, which is growing inside the borders of one-million city (Voronezh). However, anthropogenic pressure causes significant decrease of biomass production and biodiversity of shrubs and grasses. Such significant reduction of shrubs and grass plants biodiversity between urban and reserved territory could be a biomarker of human impact and effect of urbanisation. Plants have different mechanisms of adaptation to stress conditions such as extremal temperature, salinity, and xenobiotics [28-31], but environmental changes in urban ecosystems could overload adaptation potential of grass plants. In the same time, creation of artificial ecosystems can enhance local biodiversity in biotechnosphere by restoration of critical and necessary environmental conditions for species that are on the verge of extinction or by introduction of new species [32].

We could conclude that trees are more resistant to climate change and anthropogenic pressure. On the other hand, we have observed significant changes of cytogenetic parameters for English oak seed progeny in the areas with different levels of anthropogenic pressure. High level of mitotic pathologies and mitotic activities of seed progeny plantlets from trees growing in zone with high anthropogenic pressure was defined. High level of variability indices, such as percentage of prophase cells, metaphase cells, mitotic pathologies were observed for such trees. We also showed possible decrease of seeds quality that could cause oak forest degradation in the long-term scale.

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