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Effects of Anthropogenic Impact on Sexual Differentiation of Certain Species of the Genus Salix

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***Abstract*—Plant organisms actively respond to changes in the natural components of their environment. A sensitive indicator of the adaptation of the plant organism to the environment is the state of the reproductive system. The article discusses the results of a study of the sex composition of populations and the quality of pollen grains of two species of the genus Salix. The peculiarities of the sexual sphere Salix fragilis and Salix dasyclados revealed during the work show the feminizing effect on the sexual structure of the populations of these types of anthropogenic factors. During the increase of stress conditions the level of vitality and fertilizing ability of pollen grains decreases. The results of the study confirm the possibility of using these plants as bioindicators of the environment.**

***Keywords*—anthropogenic load, bioindicator, palinoindication, pollen fertility and viability, *Salix fragilis L., Salix dasyclados L*.**

I. Introduction

Today, against the backdrop of intense anthropogenic impact on natural ecosystems, a pressing issue in environmental studies is finding new bioindicators and checking their suitability for assessing the ecological state of the environment. Bioindicating methods are widely used as a comprehensive and accessible diagnostic of the state of the environment. The reproductive sphere of plants is highly sensitive to external influences [22], which can be used for bioindication studies.

As one of the environmental indicators to judge the quality of the environment, we chose the sex ratio, fertility and viability of pollen. The definition of sex in plants is understood as the formation of characters under the influence of both genetic factors and the conditions of the external and internal environment [28].

The influence of environmental factors on the manifestation of sex in plants is realized through the impact on the endogenous hormonal system, which in turn interacts with the genetic apparatus [24]. At the same time, the individual has a chromosomal form characteristic of one sex, and another appears in the phenotype [28]. Numerous experiments have shown that all physiologically active substances influence the process of sexual differentiation of plants [24, 28, 29]. Gibberellins promote the development of male genitalia. Auxins and cytokinins contribute to feminization [14].

Various external factors affect the change in the sex of a plant: day length [8, 24, 29], X-rays [22], ultraviolet rays [21], electromagnetic field [24], laser irradiation [19], radiation [5, 12, 13, 24].

The effect of trace elements, mineral nutrition [24, 26], temperature change [5, 29] and water regime [9, 17] on the sexualization of plants has been studied many times.

So, the floor of the plants is not a frozen symptom and may change under the influence of external factors. When adverse factors affect plants, changes can be observed both in the male (pollen) and female (ovule) spheres [22, 27].

The viability of pollen can serve as an indirect indicator of mutagenicity and phytotoxicity of environmental pollutants [3, 15, 20], which underlies the method of palinoindication. The worse the ecological situation, the more defective and sterile pollen is produced by plants [10]. The work carried out by different authors with different plants showed that the number of sterile plants in the zone of influence of factories and roads increases [2, 3, 4, 11, 15].

The study of the sexual structure of populations of some dioecious plants can serve as a bioindicator of the ecological state of the environment. According to the hypothesis V.A. Geodakian, in various dioecious species,

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adverse factors cause a shift of the sexual proportion to the male side, if necessary, adapt to changing conditions, and catastrophic factors - to the female, for the survival of the species [6]. Females are more viable than males.

This circumstance serves as a basis for recommending the use of dioecious plants as bioindicators of the state of the environment [16, 25].

Objective: to study the sex structure of plant populations of the genus Salix (willow brittle — S. fragilis L. and willow – willow willow — S. dasyclados L.), the quality of their male gametophyte, and to assess the possibilities of using changes in the reproductive sphere in biological monitoring of the environment.

II. Materials and methods

As objects of study, we considered several populations of 2 species of the genus Salix. This is Willow fragile (Salix fragilis L.), representing a dioecious tree 15–20 m tall with a marquee, widely spreading crown. It has bare, slightly drooping branches of olive-green color, which by the fifth year of life become brittle at the base. It grows along the banks of reservoirs, in damp places [7].

Fig. 1. *Salix fragilis L.*

Fig. 2. *Salix dasyclados L.*

Woolly willow (Salix dasyclados L.) willow is a dioecious plant, tall shrub or rarely a tree, up to 5 - 8 m high with gray or white woolly young shoots, with large, woolly, ovate-beaked, dark brown buds. It grows along the banks of rivers and lakes.

In the course of the study, we examined the populations of brittle and willow willow willow in the following areas:

No. 1. The grove near the Nuksenskoye Lake (Mukhtolovo workers' settlement, Nizhny Novgorod Region) - a territory remote from the aggressive influence of anthropogenic factors was a control option. The phytocenosis is composed of such hardwoods as aspen, rowan, brittle willow, goat willow, three-willow willow, woolly willow.

No. 2. The territory of the former Kirillov dump of MSW (closed about 20 years ago) is located in the southeast of the city of Arzamas along the Vladimir-Arzamas regional road (P72), in the direction of the village of Kirillovka. This is a site with a high anthropogenic load (19.3 thousand cars per day). Any territory of the landfill is a carcinogenic-mutagenic zone, which remains such for a long time as a result of the accumulation of heavy metals, polyaromatic hydrocarbons (PAHs), and dioxins. It is an abandoned landscape, overgrown with trees and shrubs. Gas station adjoins the dump.

No. 3. The grove, located behind the Trinity cemetery in the south-east direction from the city of Arzamas, is an area that is 3.5–14 km away from the highways with an intensive level of traffic. The phytocenosis is composed of such tree species as white birch, common aspen, small-leaved linden, Norway maple, fragile willow, white willow, wool-willow willow, goat willow.

No. 4. The coastal territory of the Ramsay pond is a site with a high anthropogenic load, located in the eastern part of the city of Arzamas (May 9 st.). It is a willow-grown area, on the square of which there are species of willow: white, fragile, goat, sherstistoprebegovaya, blackening, ashen. Near the pond there is a highway with heavy traffic (18.1 thousand cars per day), a car wash, parking lots, OAO Arzamas Engineering Plant, emitting about 74 tons per year of 50 names of harmful substances.

No. 5. Turning to Maryevsky spring (Arzamas district) is a plot overgrown with willow on both sides of the inter-settlement road, with a low level of traffic intensity (1.7 thousand vehicles per day).

To study the sexual structure of the population of two willow species, we counted the number of males and females during the flowering period (April 2018) on the experimental and control plots of 400 m2. The values obtained were converted to percentages and the ratio of the sexes in the populations was determined. The sample for different areas ranged from 50 to 100 copies. The significance of differences between the control and experimental variants was evaluated by the method χ^2 [18].

$$\chi^2 = \Sigma \frac{(O-E)^2}{E},$$

where Σ is the sum of the results for all the classes observed in the experiment, O is the observed splitting, E is the expected splitting. In experiments, the number of degrees of freedom is K-1, where K is the total number of classes.

When organizing palinoindication studies in different areas, similar weather conditions were selected, the degree of maturity of pollen and the time of collection were taken into account. Collected pollen or anthers were stored in tubes closed with a stopper in the refrigerator at a tempo of + 2º - + 5º and humidity 50 - 70%. The acetocarmine method was used to determine pollen fertility [23]. The number of dyed and unpainted pollen grains was counted in five fields of view, in triplicate. Pollen fertility was determined by the number of colored pollen grains (percent) (Fig. 3).

Fig. 3. Willow pollen brittle, **colored with** acetocarmine

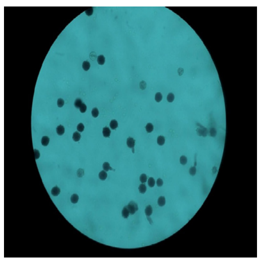

Fig. 4. Willow pollen brittle, colored with isatin.

Pollen viability was detected using an isatin reagent or a proline test [1]. Viable pollen reacts with isatin and turns intensely dark blue, while non-viable remains colorless. The viability of pollen was determined by the number of colored pollen grains (percent).

Statistical processing of the research results was carried out by assessing the difference between the sample fractions of the general population using Student's t test [18]. The ratio of this difference to its error gives a random variable t = d / Sd, which follows t - Student's distribution. But the hypothesis or assumption that p1 = p2 is rejected if

tф ≥ tst for a certain level of significance P = 5%, 1%, 0.1% and the number of degrees of freedom k = n1 + n2 - 2 Error of the difference between the fractions taken from approximately equal-sized samples (when the number of groups differ by no more than 25%) are calculated by the formula:

$$Sd = \pm\sqrt{\frac{p_1 * g_1}{m_1} + \frac{p_2 * g_2}{m_2}},$$

m1 is the total number of controls, m2 is the total number of experience. p1 = m1 / (m1 + m2); p2 = m2 / (m1 + m2); g1 = 1 - p1, tf = d / Sd, d = p1 - p2

The value of tst for k = 28 at different levels of significance: p = 5% – 2,05 (*); p = 1% – 2,76 (**);
p = 0,1% – 3,67 (***).

III. Results and discussion

In the course of studying the sex structure of different populations of 2 willow species, a shift in the sex ratio to the female side was established in plants growing in two areas exposed to the most intense anthropogenic load (Table 3, Table 4).

The sex structure of Salix fragilis and Salix dasyclados in the control plot, in the area of the cemetery and spring is not statistically different from the ideal 1: 1 ratio, which indicates favorable environmental conditions for the growth of populations.

Table 3
CORRELATION OF FLOORS IN SALIX FRAGILIS POPULATIONS

Study area	♂/♀	*f* ♂:♀	*f*' ♂:♀	$\chi^2_ф$	χ^2_{st} at p=0,05
1. Grove Lake. Nuksen (control)	0,91	39:43	41:41	0,20	3,84
2. The former Kirillov dump	0,54	21:39	30:30	5,40	
3. Trinity cemetery	0,84	41:49	45:45	0,71	
4. Ramsay pond shore	0,45	13:29	21:21	6,10	
5. Maryevsky spring	0,84	65:77	71:71	1,01	

Table 4
CORRELATION OF FLOORS IN SALIX DASYCLADOS POPULATIONS

Study area	♂/♀	*f* ♂:♀	*f*' ♂:♀	$\chi^2_ф$	χ^2_{st} at p=0,05
1. Grove Lake. Nuksen (control)	0,87	52:60	56:56	0,57	3,84
2. The former Kirillov dump	0,47	21:45	33:33	8,73	
3. Trinity cemetery	0,82	27:33	30:30	0,60	
4. Ramsay pond shore	0,38	11:29	20:20	8,10	
5. Maryevsky spring	0,91	42:46	44:44	0,18	

At these sites, a reduced anthropogenic load is noted, which does not significantly affect sexual differentiation in the populations of S. fragilis and S. dasyclados. Statistically significant deviations in the secondary sexual structure compared to the control were found in the populations of S. fragilis and S. dasyclados in areas 4 and 5, where the actual chi-square value is significantly higher than the standard value 2 = 3.84. On the territory of the landfill for S. fragilis 2 it is 5.4, and for S. dasyclados - 8.7, which is the largest deviation of the values found. On the coastal area of the Ramsay pond, the matching criterion is equal for S. fragilis 6.1, for S. dasyclados - 8.1.

In general, the actual difference between the c2 values of the control and anthropogenically loaded areas: No. 2 and No. 4 in S. dasyclados is slightly larger (8.16 and 7.53) than the similar difference in the chi-square values of these areas in S. fragilis (at 5.20 - 5.90), respectively (Table 3, Table 4).

This indicates that severely adverse growing conditions of plants significantly affect the sexual imbalance of the population, increasing its feminization.

In all studied populations of the genus Salix, the numerical advantage was found in females. The ratio of male to female specimens for S. fragilis is shown in Figure 5, for S. dasyclados in Figure 6.

For S. fragilis, the ratio of males to females in the population in the landfill site (plot No. 2) is 0.54, in the coastal area of the Ramzai pond - 0.48 (plot No. 4).

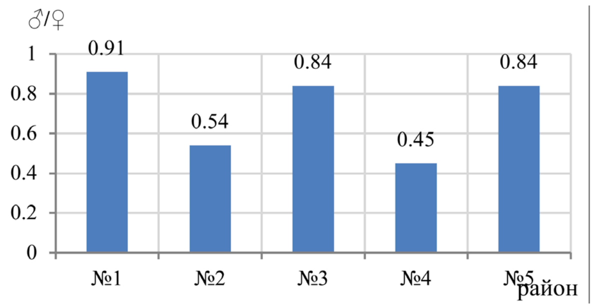

Fig. 5. Sex ratio in populations of Salix fragilis

A similar relationship between the ratio of females and males in these territories was also found for Salix dasyclados populations (Fig. 6). The coefficient of the secondary sexual proportion in stressful conditions (sections 2 and 4) is 0.47 and 0.38, respectively.

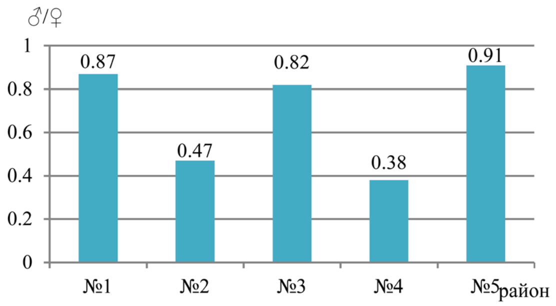

Fig. 6. Sex ratio in populations of *Salix dasyclados*

So, in the course of the study, no specific features of changes in sexual differentiation in the populations of the genus Salix depending on the species were found - similar trends in the dynamics of the sex structure were observed in all areas.

As a result of studying the pollen fertility, a significant difference in the quality of the pollen grains of 2 species of the Salix genus in the experimental areas from the control (Nuksenskoe grove), which is not experiencing intense anthropogenic impact, was shown. Control pollen fertility rates are the highest: 91.4% for S. fragilis and 94.1% for S. dasyclados (Table 5, Fig. 7).

Table 5

FERTILITY OF PLANT POLLEN (%) IN RESEARCH AREAS

Plant type / Study area	*Salix fragilis*	*Salix dasyclados*
1. Grove oz. Nuksenskoe (control)	91,4 ± 0,7	94,1 ± 0,6
2. The former Kirillov dump	47,8 ± 0,8***	53,6 ± 0,8***
3. Trinity cemetery	84,2 ± 1,0*	83,1 ± 0,7**
4. Ramsay pond shore	67,2 ± 1,0***	63,1 ± 0,8***
5. Maryevsky spring	90,1 ± 1,0	91,6 ± 0,7*
The average value of the studied areas	76,1	77,1

*P ≤ 0,05; ** P ≤0,01; *** P ≤0,001

The closest to the control indicators of the fertilizing ability of pollen grains are determined at site No. 5 (Maryevsky spring). The pollen fertility of S. fragilis here is 90.1%, which is only 1.3% less than control. The differences in the S. dasyclados fertility rates between this and the control sites are significant, but relatively small and differ by only 2.5%. Consequently, favorable conditions for the growth and reproduction of plants have developed in this area, and the low intensity of traffic on the asphalted village road adjacent to the studied area does not carry a significant anthropogenic load.

The lowest fertility of pollen in both studied species was found on the territory of the former Kirillovsk dump: 47.8% in S. fragilis, 53.6% in S. dasyclados, which differs significantly from the control area by 43.6% and 40.5%, respectively ($P \leq 0.05$).

Low pollen fertility values are also noted in area No. 4 - the coast of the Ramsay pond. The site is subject to high anthropogenic load: a large industrial enterprise (AMZ), a parking lot and a road with heavy traffic are located nearby. In S. fragilis, the pollen fertility here differs from the control area by 24.2% and is 67.2%. In S. dasyclados, the difference in fertility between the control and plot No. 4 is significantly higher - 31.0%, the percentage of fertility in the study area is 63.1%.

The pollen fertility rate in S. fragilis at site No. 3 (grove near the Trinity cemetery) is 84.2%, in S. dasyclados the similar value is 83.1%. The difference with the control plots in the first population is 7.2%, in the second - 11.0% can be caused by a negative effect on the male gametophyte of cadaver poisons and a small distance from the city.

When studying the viability of pollen collected from plants of different habitats, such a decrease was found in all areas of the study compared with the control of both Salix species (Table 6, Fig. 8).

Table 6

VIABILITY OF PLANTS OF PLANTS (%) IN RESEARCH AREAS

Study area	Plant type	
	Salix fragilis	*Salix dasyclados*
1. Grove oz. Nuksenskoe (control)	62,2 ± 0,5	65,7 ± 0,5
2. The former Kirillov dump	37,8 ± 0,6***	38,7 ± 0,6***
3. Trinity cemetery	49,7 ± 0,6***	47,1 ± 0,6***
4. Ramsay pond shore	37,9 ± 0,8***	40,6 ± 0,7***
5. Maryevsky spring	59,5 ± 0,9*	61,5 ± 0,8*
The average value of the studied areas	49,4	50,7

*P ≤ 0,05; ** P ≤0,01; *** P ≤0,001

Pollen viability indicators in the control region in S. fragilis — 62.2% and in S. dasyclados — 65.6%.

Values of pollen viability at station No. 5 (Maryevsky Spring) are observed close to the control: for S. fragilis here is 59.3%, and for S. dasyclados - 61.5%. These figures differ from the control in just 2.7% and 4.2%, but statistically significant. This makes it possible to assume that the growing conditions here are most close to favorable, and the anthropogenic load is insignificant.

In section 3 (grove near the Trinity cemetery), the pollen viability index for S. fragilis is 49.7%, the difference from control is 12.5%; for S. dasyclados - 47.1%, the difference with the control - 18.6%.

The lowest viability of pollen was noted at sites No. 2 and No. 4. Here, the values differ from the control in S. fragilis by 24.4% - 24.8%, the percentage of viable pollen grains in this species in the former landfill is 37.8%, and in the coastal area of the Ramzai pond - 37.9%. In S. dasyclados in section 2, viability was 38.7%, in section 4, 40.6%, the difference from control was 26.9% and 25.1%, respectively. These areas are subject to the greatest anthropogenic load among the studied.

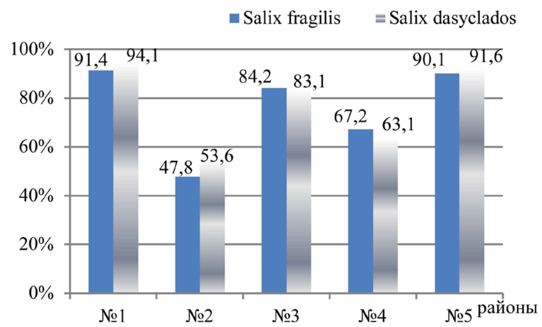

Fig. 7. Pollen fertility dependence,% of the study area

The average viability of pollen in all sites in S. fragilis is 49.4% and in S. dasyclados - 50.7%, that is, it does not differ significantly. The average fertilizing ability of pollen grains in the studied Salix species is also not significantly different in all areas: in S. fragilis - 76.4% and in S. dasyclados - 77.1%.

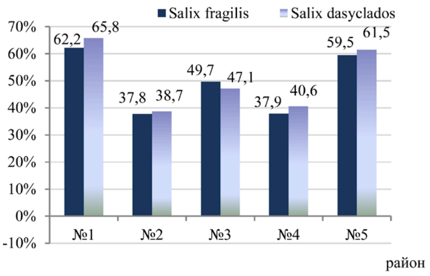

Fig. 8. The dependence of the viability of pollen,% of the study area

Therefore, S. fragilis and S. dasyclados are equally influenced by anthropogenic factors on the process of microsporogenesis.

IV. Conclusion

The study revealed that intense anthropogenic stress has a feminizing effect on the sexual structure of populations of 2 species of the genus Salix.

No peculiarities of changes in sexualization in the Salix populations depending on the species were found.

It was shown that with increasing stress conditions, the level of viability and fertilizing ability of pollen grains decreases. This can be viewed as a manifestation of the functional displacement of the sex ratio towards the female sphere.

Specific features of changes in the quality of pollen grains among the studied species of the genus Salix were not identified.

It has been established that changes occurring in the reproductive sphere of S. fragilis and S. dasyclados under the influence of anthropogenic influence can be used in the bioindication of the state of the environment.

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