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Phenotypic variation of generative signs of different reproductive types of *Pseudotsuga menziesii* (Mirb.) Franco in urban environment (in the forest-steppe zone)

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Abstract. The average values and variability amplitude of morphological characters and reproductive traits of cones have been determined under the introduction of *Pseudotsuga menziesii* (Mirb.) Franco trees of different reproduction types. It is shown that the average indicators of the most signs correspond to the control in urban conditions. The level of signs variability is close to control ones, or increased in trees of female and mixed types. The possibility of phenotypic selection of trees of different reproduction types according to their stability in the urban environment is considered, as follows from the variability level of generative sphere parameters.

1. Introduction

The deterioration of the ecological situation in modern metropolitan cities, global climate change necessitates the stabilization and maintenance of the urban environment [1]. The use of green spaces can reduce the impact of technogenic pollution. Considering this, it is fair to say that the issue of optimizing the urban environment by woody species which are resistant to polluting emissions is currently central [2]. The task can be solved by studying the individual variability of woody species to identify resistant species and forms, as the scientific basis for selection.

The study of the regularities of individual plant variability is necessary for assessing the adaptation of a plant organism in the studied conditions. [3-5]. Under the new conditions, the introduced species can have morphological and biological features of plants which are connected with the limiting effect of the growth conditions which are not typical for the species, and also the stress factors of the urban environment [6]. Therefore, an increased polymorphism and organs variability level of the vegetative and generative spheres are often registered [7]. One of the most important regularities of variability is the same level of majority variation of characteristics; this concerns not only different tree species, but also members of different genus and families. Thus, variability is specific for characteristics, but not specific for species [8]. It has been established that the reproductive type of plants has a high genetic dependence. Numerous research works in this sphere [9-11] revealed that reproductive type of plants varies depending on the environmental influences, geographic location, and growth conditions. Environmental stress causes acceleration in the rate of morphosis and their early aging [12, 13].



The aim of the research is to establish the levels of variability of the basic morphometric and reproductive parameters of cones of *Pseudotsuga menziesii* (Mirb.) Franco trees depending on reproductive type in conditions of vehicular pollution. It is necessary to identify biologically stable types, forms, individuals in an urban environment.

2. Objects and methods

The species of interest grow in the Central region of Voronezh (Central part of the Russian Federation), in the zone of intensive traffic. 30 - 40 years planting rows of *Pseudotsuga menziesii* (Mirb.) Franco is located along the motorway. Soils are cinereous, sylvatic and sabulous. Control plantations grow in the central part of the Semiluki nursery. They are represented by artificial plantings with the placement of trees - 4×1.5 m. The number of the induced trees is 9 in the samples and 10 - in the controlled trees.

The reproductive type of trees has been determined according to the number of micro- and macrostrobiles [14, 15]. Structural indicators have been studied: the number of cones on the tree, their length and width, the number of seed scales and seeds in the cone, weight of 1000 seeds and germination ability of seeds. Cones have been taken from four corners of crown (northern, east, western, and southern) on 25 pieces for each tree. All cones were removed from the male trees if the harvest was low.

Seeds have been separated according to their linear dimensions (width and thickness) using a mechanical separator equipped with oscillating sieves to obtain individual dimensional fractions. In this case, metal sieves with round and rectangular apertures have been used [16]. The collected seeds have been germinated in the laboratory setting. Yields and condition of *Pseudotsuga menziesii* (Mirb.) Franco trees have been compared in different periods of 2004-2005 and 2017-2018. The condition of a tree has been assessed according to the scale of visual evaluation by the external characteristics of the tree crown, leaves and needles, sprout and shoots, growth and trunk: 1 point - healthy; 2 points - weakened; 3 points - strongly weakened; 4 points - drying up; 5 points - dry (fresh and old dead). The output yield has been recorded according to the method of A. A. Korchagin [17]. The weight of 1000 seeds has been determined according to All Union State standard [18]. The level of variability of morphological features has been estimated from the empirical scale of variability levels of S.A. Mamaev: very low ($C < 7\%$), low ($C = 8-12\%$), medium ($C = 13-20\%$), elevated ($C = 21 - 30\%$), high ($C = 31-40\%$), very high ($C > 40\%$) [19]. The results have been processed on a computer using Microsoft Excel. The accuracy of the experiment is in the range of 5-6%.

3. Main part

Pseudotsuga menziesii (Mirb.) Franco does not grow naturally in Russia. The native land of this species is western regions of the USA and Canada. This species is promising in the central forest-steppe conditions for introduction into forest cultures and in the gardening of cities, as it grows rapidly and decoratively [20].

Pseudotsuga menziesii (Mirb.) Franco is a monocious species. Micro- and macrostrobiles are formed in the crown of one tree, but the quantitative ratio is not the same: the number of microstrobils dominates over macrostrobiles. According to the ratio of microstrobils to macrostrobils, reproductive types are distinguished. In females, the ratio of generative organs of both reproductive types varies from 4:1 to 25:1, in mixed individuals - from 30:1 to 70:1, in males - from 80:1 to 130:1. The lowest reproductive type index is observed in all trees during the years of reduced output yield. In the long-term cycle, individuals retain their belonging to a particular type, which indicates a high genotypic dependence of reproductive type forms. The greatest output yield of cones is formed on trees of female type (induced trees have from 1100 to 13421 pieces while observed trees have from 150 to 2600), the smallest - on trees of male type (30-300 and 60-1550 pieces). Intermediate position is occupied by trees of mixed type - from 100 to 1900 pieces in the city and 250 to 3000 pieces in the lining-out nursery. Generally, in the female trees the seed-bearing layer occupies 80-90% of the tree crown, in the mixed one it is about 60%, in the male one it is less than 30% [21, 22].

The productivity of cones of female trees is higher in experimental conditions; it is higher at male and mixed types in monitoring. This is probably due to partial shadowing of female trees which affects productivity. It is important to note that experience and monitoring differ on the light, wind and temperature modes. Trees are partially shaded in the nursery, less force of the wind, above soil humidity. Ecological factors of urbanized territories have their specific features such as polluted atmosphere, increased thermal condition, smaller relative humidity of air and soils. The complex of the above factors may both smooth and to strengthen distinctions of experience with control.

The induced trees No. 1, 2 and 5 have been assigned to the female type, No. 3, 6, 7 and 8 to the mixed one, No. 4 and 9 to the male ones. The observed trees No. 4 and 7 have been assigned to the female type, No. 1, 5, 6 and 10 to the mixed, and 2 and 2 and 8 to the male. The results of the analysis according to the morphometric and reproductive parameters of cones are represented in Table 1. Mean values of selection \pm selection average error are given.

The minimum average length of the induced trees cones (4.9 ± 0.1 cm) has been noted in females, the maximum (6.3 ± 0.1 cm) in the mixed, the average value is typical of males (5.1 ± 0.2 cm). The maximum length of the observed trees was also noted in individuals of mixed type (6.0 ± 0.1 cm), for males and females the value was almost identical (5.1 ± 0.2 and 5.5 ± 0.1 cm). The largest cones are marked in mixed-type trees of both induced and observed groups. The width of the observed trees cones in the mixed type was 3.2 ± 0.05 cm while the width of the induced trees cones were 3.7 ± 0.04 . The parameter of this characteristic in trees of the female type in the city was lower (2.8 ± 0.1 cm) than the one observed trees have (3.1 ± 0.1 cm). In male trees, the cones in the city are wider (3.2 ± 0.1 cm) than observed trees cones (2.6 ± 0.03 cm).

Table 1. Morphometric and reproductive descriptors of *Pseudotsuga menziesii* (Mirb.) Franco cones ($\bar{X} \pm S_x$).

Parameter	Induced trees			Observed trees		
	female	mixed	male	female	mixed	male
Cone length, cm	4.9 ± 0.1	6.3 ± 0.1	5.1 ± 0.2	5.5 ± 0.1	6.0 ± 0.1	5.1 ± 0.2
Cone width, cm	2.8 ± 0.1	3.7 ± 0.04	3.2 ± 0.1	3.1 ± 0.1	3.2 ± 0.05	2.6 ± 0.03
Quantity of seed scale, pcs	32 ± 1.0	37.3 ± 0.7	32 ± 0.6	31 ± 0.6	35 ± 0.9	29 ± 0.59
Quantity of developed seed scale, pcs	21 ± 0.8	24 ± 0.5	21 ± 0.62	18 ± 0.6	22 ± 0.8	17 ± 0.5
Quantity of undeveloped seed scale, pcs	11 ± 0.4	13.3 ± 0.4	11 ± 0.22	13 ± 0.5	13.25 ± 0.5	12 ± 0.23
Total number of seeds, pcs	46 ± 1.25	47.6 ± 1.3	43 ± 0.9	43.5 ± 1.5	50.7 ± 1.6	49.5 ± 1.2
Full-grain, pcs	11 ± 1.15	11.3 ± 1.15	21 ± 0.99	21 ± 0.8	23.3 ± 1.0	17 ± 0.76
Full-grain seed efficiency, %	26 ± 1.1	23.5 ± 1.4	50 ± 2.2	58 ± 3.0	52 ± 2.2	50 ± 2.3
Weight of 1000 pcs of seeds, g	9.8 ± 0.7	10.6 ± 0.7	11.4 ± 0.5	9.95 ± 0.8	8.5 ± 0.8	10.36 ± 0.8

The maximum number of seed scales in the cone is typical for large cones. The scales quantity of induced trees of mixed reproductive type is 37.3 ± 0.7 and of observed trees is 35 ± 0.9 pcs. This characteristic coincide with female and male trees of both induced (32 ± 1.0 and 32 ± 0.6) and controlled (31 ± 0.6 and 29 ± 0.59 pcs) groups.

According to the number of undeveloped seed scales of both induced and observed trees, cones of different tree types do not differ, the parameter varies from 12 ± 0.23 pcs in males, to 13.25 ± 0.5 pcs - in mixed types, 13 ± 0.5 pcs - in females. The characteristic of the induced trees has the following values: trees of female and male type have 11 ± 0.4 pcs and 11 ± 0.22 pcs, mixed type trees have 13.3 ± 0.4 pcs.

The female and mixed induced trees have approximately the same total number of cone seeds - 46 ± 1.25 and 47.6 ± 1.3 pcs, the male trees have slightly lower quantity - 43 ± 0.9 pcs. As for the controlled trees, male and mixed types have increased parameters - 49.5 ± 1.2 and 50.7 ± 1.6 pcs, a low number of seeds is noted in trees of female type: 43.5 ± 1.5 pcs.

The number of full-grain seeds of induced female (11 ± 1.15 pcs.) and mixed (11.3 ± 1.15 pcs) trees is twice lower in comparison with the observed group (21 ± 0.8 and $23.3 \pm 1, 0$). The male trees have higher values of this characteristic in an urban setting (21 ± 0.99 pieces) ($t_f 2.8 > t_{st} 2.0$ at $P - 0.95$).

Full-grain seed efficiency has been defined as the percentage of the number of full-grain seeds in the cone to the doubled number of developed (fertile) scales in the cone. Induced trees of female and mixed types have lower parameter (26 ± 1.1 and 23.5 ± 1.4) than observed trees (58 ± 3.0 and 52 ± 2.2); male-type trees have the same values ($50 \pm 2.3\%$).

On an average, the cone contains from 43 up to 60 seeds. Normally developed seeds are formed in the fertile cone zone. The induced trees seed efficiency in posse varied from 23.5 to 50%, the observed trees seed efficiency was higher - 50 to 58%.

Perhaps the low full-grain seed efficiency is connected with cross weather during the pollen dispersion and as a result of self-pollination. It has been established that the full-grain seed efficiency in posse, the actual number of seeds and the total quantity of all seeds depends strongly on the influence of external factors that determine effective pollination [23]. The lowest full-grain seed efficiency (0.9 and 0.7%) was found among the induced trees No. 1 and No. 3. The reason for this phenomenon is the particular formation of cones in these individuals. On the whole tree number 1, and on the eastern side (from the road side) on the tree number 3, together with normally developed cones, a cluster of small underdeveloped cones with empty seeds was formed. Basically, they developed from the sprouts located in the axils of the needle, and not from the sprouts at the ends of the shoots. Such a cone consists of an axis at the base of which several seed and bract scales are located, and microsporophylls are located on its apex. According to the literature data, the phenomenon of the hermaphrodite flower formations and aglets in species that have dioecious flowers and aglets is described for different species of *Larix*, *Pinus* and *Picea*. Most of these anomalies refer to the introduced species [24].

The tree number 1 didn't have this peculiarity up until the age of 25 and the tree number 3 up until the age of 20-23. Perhaps, this abnormality in fruit-bearing is connected with the impact of the vehicle pollution. Previously, other researchers at this facility did not register a similar phenomenon. Perhaps, under the influence of environmental factors - emissions of vehicles - a mutation occurred, which is corrected by the location of trees and their genotype. Slowly developing cones reach receptivity at the time of the end of the pollen dispersion, and therefore they do not form full-grained seeds.

In 2017 and 2018, the formation of small cones was observed among female trees No. 1 and 5 and mixed type trees No. 8 taking into account the yield of cones. The length of these cones varies from 1.2-2.0-2.5 up to 3.0 (Figures 1-3).

Cones form bunchlike from axillary sprouts on shoots (Figure 2). The number of seed scales in these cones is from 8 to 12 pieces. Small seeds are formed, their size is not typical - up to 3.5 mm in length and with a short wing up to 3.0 mm long, and the seeds are empty (without germ).

By outward signs, the marked trees have a weakened state and have 2 points on a 5-point scale. The crown is slightly open, with the drying of individual branches. Needles live for 2-3 years. The phenological development of trees of different reproduction types has corresponded to previous observations. The pollen dispersion in 2017 was noted at the end of the second - the beginning of the

third decade of April (April 22 - 26), ending of the pollen dispersion which lasted 6-7 days was noted in late April (April 28-30).

The weight of 1 000 seeds of induced trees individuals with different reproduction types conform to the requirements. Among the induced trees this parameter varies from 9.8 ± 0.7 g in the female type, to 11.4 ± 0.5 g - in males. In individuals of mixed type this parameter has an intermediate value of 10.6 ± 0.7 g. Among the observed trees, the minimum value is typical for trees of mixed type 8.5 ± 0.8 g, maximum 10.36 ± 0.8 g - for males, in trees of female type this parameter was 9.95 ± 0.8 g. The weight of 1000 pieces of seed does not depend on the reproductive type of the tree; this feature is highly individual and functionally determined [25].



Figure 1. Normal cone of the female tree No. 5.



Figure 2. The form of normal and abnormal cones and seeds of the mixed type tree No. 8.



Figure 3. Abnormal cones in the crown of a female tree No. 5.

The germinating seed ability of the *Pseudotsuga menziesii* different reproduction types is not the same. It largely depends on the seeds weight. Its influence is stronger than the reproduction type in both induced trees and observed trees. So, in the nursery, the seeds of female trees with the highest weight (10-14 g -) were distinguished by increased germination (52 - 83%). Low germination values (11-23%) are typical for smaller seeds of mixed and masculine individuals (6.3-7.6 g).

The low germination of the seeds of individual induced trees may occur due to the small number in the plantation, and inevitably, as a result of self-pollination. In the harvest years, the germination of seeds in the city (41%) was almost half less than in the nursery (83%). At the same time, the quality of seeds depends on the degree of differences between the genotypes for productivity (type of reproduction), the phase of pollination of macrostrobiles, the fertilizing ability of the tree pollinator pollen [15], the content of the population and habitat conditions.

Considering the induced trees, motor vehicle emissions disrupt normal ontogenesis of plants, causing them the appearance of pathological reactions and processes (mutations in the generative sphere of No. 1 female tree and No. 3 and 8 of the mixed type trees), which is the reason for the decrease in the quality of the seeds of *Pseudotsuga menziesii* trees.

The level of phenotypic variability of cones in relation due to the reproductive type of trees is represented in Table 2.

The phenotypic variability of the morphometric features of the cones in the *Pseudotsuga menziesii* (Mirb.) Franco trees of different reproductive types are not the same. The length and width of cones are highly genetically determined features, so their variability level of both induced and observed trees remained low. In the city, individuals of mixed and masculine types have the most constant cone sizes, features vary at a very low level (CV = 6 - 11%). Up to the average level, female trees have an increased variability of linear parameters of cones (CV = 5-18%), it is possible due to the negative influence of vehicular pollution and developmental challenges of small cones.

The number of seed scales is associated with the process of the fate differentiation, and it limits the total number of seeds or their maximum yield. This feature is also under the strong genetic control of the mother tree and changes at a fairly low level. Male trees feature varies (CV = 7-12%) in comparison with the observed trees at the same low level, mixed type individuals increase the variability up to the average level (CV = 10-13%) and female trees increase it from low to high one (CV = 9-26%). The variability in the number of developed and undeveloped scales is consistent with the variability of the total number of seed scales. Mixed and male trees vary the feature at the low-medium level (CV = 10-19%). Females increase the variability number of developed scales from low to higher level (CV = 12-29%), and the variability number of undeveloped scales - from low to high (CV = 12-35%).

Table 2. Variability of morphometric parameters of *Pseudotsuga menziesii* (Mirb.) Franco cones (CV, %).

Parameter	Induced trees			Observed trees		
	female	mixed	male	female	mixed	male
Cone length, cm	8-18	6-9	9-11	7-10	5-9	8-14
Cone width, cm	5-16	4-8	6-7	7-10	5-6	4-8
Quantity of seed scale, pcs	9-26	10-13	9-10	6-8	7-12	7-12
Quantity of developed seed scale, pcs	12-29	13-14	10-13	8-14	9-14	11-16
Quantity of undeveloped seed scale, pcs	12-35	11-19	13-19	12-15	8-17	6-8
Total number of seeds, pcs	15-24	15-35	10-25	11-37	12-19	8-15
Full-grain, pcs	93-200	26-218	16-60	17-72	17-34	34-38
Full-grain seed efficiency, %	77-84	24-106	13-56	13-77	18-38	22-38
Weight of 1000 pcs of seeds, g	16-18	5-17	12-13	16-17	8-11	13-15

The total number of seeds in the cones is a very variable feature in individual trees of the same population and in different populations. Mamaev S.A. [19] established a very high degree of variation in the number of seeds in cones of *Pinus sylvestris* (CV = 38 - 90%). In his opinion, this is due to the fact that the differences in trees by the size of the cones also increase the level of variability in the population by the content of seeds in it. However, he pointed out other causes as well, including the mosaic distribution of pollen in the forest. The formation of well-developed, full-grained seeds depends on the presence of pollinating trees during the blooming of megastrobiles, favorable climatic conditions during pollen dispersion, that is, from a number of different environmental factors. The number of potential seeds in cones is genetically programmed; therefore the variability of this feature is comparatively low in natural plantations.

In the urban environment, compared to the observed trees, male trees increase the variability of the total number of seeds from low to high (CV = 10-25%), mixed - from medium to high (CV = 15-35%), and female trees vary the signs on the average-elevated level (CV = 15-24%). The variability of this feature depends strongly on the nature of the pollination, especially in artificially created, small plantations.

The variability of the full-grain seeds quantity in the cone among mixed and male observed trees varies at the medium-high level (CV = 17-38%), females increase it from medium to very high (CV = 17-72%). In the city, male trees have more constant parameter (CV = 16-60%), individuals of mixed and female type increase the variability of the feature from elevated to very high (CV = 26-218%).

Full-grain seed efficiency in posse (%) is a very unstable feature, which depends on the number of pollinated ovules. The high viability of the pollen does not yet guarantee a large yield of full-grain seeds from the cones. It largely depends on the nature of the pollination, which in turn is due to the placement of pollinating trees, a change in direction and wind force, the presence of various kinds of shielding obstacles, the density of the plantation, meteorological conditions and other factors. Pollinization of cones is influenced by their position in the crown, the state of female strobilus during the period of pollen dispersion. It is also important that the pollen of *Pseudotsuga menziesii* is heavy - it does not have wings. It is necessary to take into account the fact that even after fertilization, the death of the ovules may occur. The level of variability of the yield of full-grain seeds in posse (%) coordinates with the level of variation in the number of full-grain seeds (pcs). Under conditions of vehicle pollution, in comparison with observed trees all trees of different reproductive type has increased the level of variability from medium to very high (CV = 13-106%). The highest absolute values of the variation coefficient for these two characteristics are typical for the female and mixed trees; the minimum is typical for the males.

The weight of 1000 seed pcs is a more constant feature. It is highly genetically conventional and weakly dependent on environmental conditions. Varying of this characteristic conform to the requirements. The parameter changes at a low-medium level (C.V. = 8-17%) among the observed trees, and at a very low-average level among the induced trees (C.V. = 5-18%).

4. Summary

In the conditions of vehicle pollution in Voronezh, different reproductive types of *Pseudotsuga menziesii* (Mirb.) Franco have average values and the variability amplitude of morphological character and reproductive trait of cones. It is established that the average parameters of most features conform to the requirements in urban conditions. The level of features variability is close to the conformed requirements, or increased among the trees of female and mixed type.

The variability of the morphometric features of the cones is influenced by the intensity of the yield: the greater the yield of cones on the tree, the lower the level of variability is. This pattern is well observed among the observed trees as well as among the characters under strong genetic control: the length and width of cones, the number of developed and undeveloped seed scales, their total number, the number of potentially developed seeds, and the weight of 1000 pieces of seeds.

The yield of full-grain seeds from potentially possible ones, the number of empty and full-grain seeds, and their total number is very dependent on the influence of external factors contributing to

effective pollination. There is an increase in the level of variability among the females of the following features: length and width of cones, the number of developed and undeveloped scales, their total number, the number of full-grain seeds in urban conditions. Most of the studied features among the individuals of mixed and male types are constant. Only the amount of full-grain seeds, the yield of full-grained seeds and the total number of seeds in cones of mixed-type trees varies more among the induced trees. The quality of seeds in the trees of all studied reproductive types among the induced trees is lower than among the observed trees.

The levels of endogenous variability of cone structural features in trees of different reproductive types have been established. Linear features (length, width) vary low, weight (weight 1000 pieces of seeds), number of seed scales - on average, quantitative (content of seeds) - high and very high. The obtained data, generally, correspond to the peculiarities of individual conifer variability in natural plantations established by S.A. Mamaev [19, 26].

In conditions of man-made impact, in comparison with the observed trees, the level of endogenous variability of morphometric characters and structural features of cones in female individuals increases more strongly, and weaker among the individual tree of mixed types. The negative impact of motor vehicle emissions worsens the condition of female and mixed trees. The parameters of cones and condition of male trees (No. 4.9) are the most constant under man-made contamination, which are recommended to be used for obtaining planting material for gardening.

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