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RESEARCH ARTICLE

Conjugate Variability of Signs of the Female Generative System of Scots Pine (*Pinus sylvestris* L.) in the Conditions of Magnesite Pollution

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ABSTRACT

In the conditions of environmental pollution by emissions of magnesite production (Combined Magnesite, Satka, Chelyabinsk region), a study of the conjugate variability of signs of the female generative system of the Scots pine (*Pinus sylvestris* L.) was carried out. Signs with a stable connection have been identified, as well as signs whose direction of interconnection changes under the influence of aerotechnogenic pollution. Thus, the relationship of the signs characterizing the size and mass of cones is rigidly genetically determined and is not violated in conditions of a strong level of pollution. The seed productivity of the pine stands, regardless of the growing conditions, is more closely related to the survival rates of ovules in the gametophytic period than in the period of embryonic development. However, in conditions of heavy pollution, the relationship between the survival of ovules in the 1st vegetative and embryonic periods is negative, which may indicate the effective elimination of abnormal gametophytes and the release of the stand from them in conditions of pollution. The strong weakening of trees and the low availability of mineral resources in conditions of high pH of the growing medium in the zone of heavy pollution cause an increase in the negative relationship between seed productivity and seed weight to a significant level. Also, the conditions of heavy pollution “contributed” to the development of a strategy of trees for the formation of small seeds with high quality and quality indicators, seedlings developed. Only in these growing conditions, with a decrease in seed weight, the indicators of germination energy, germination percentage, and morphometric indicators of seedlings significantly increased.

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1. Introduction

The study of the variability of quantitative and functional characteristics in natural populations is one of the main directions of population-biological research. The fundamental basis for the adaptation of animals and plants is the initial heterogeneity and functional heterogeneity of individuals in the population (Chetverikov, 1926; Bezel et al., 2001; Zhujkova & Bezel, 2009). It is known that in living organisms, the level of variability of traits is closely related to habitat conditions (Mamaev, 1973; Bezel et al., 2001) and, therefore, can be used to assess these conditions. The structure of interrelations of many quantitative traits reflects the internal mechanisms underlying the sustainable development of the organism (Berg, 1959; Yablokov, 1978; Zhivotovskij, 1984; Batygin, 1986). The influence of external factors, including aerotechnogenic pollution, can lead to a change in these relationships. The effect of aeropollutants on the reproductive system can lead to a

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change in the structure of the connections of the whole complex of its features (Mamaev, 1973; Anikeev et al., 2000; Mahneva et al., 2003; Tihonova, 2005; Udval et al., 2020).

According to literature data, the maternal tree habitat greatly influences seed quality and seed reproduction (Makhniova et al., 2019). A negative effect on generative system development could be caused by *(i)* overwetting and drought (Velisevich, 2017), *(ii)* weather conditions in years when the generative structures were initiated and developed (Pukkala et al., 2010), *(iii)* the sum of positive temperatures during summer in the North of Eurasia (Fedorkov, 2007), *(iv)* species biological features (Lyanguzova, 2011), and *(v)* genetic traits of the populations (Hisamoto & Goto, 2017). In addition, many researchers have reported increases in the disturbance of male and female pine generative systems in areas of technogenic pollution (Tretyakova & Noskova, 2004; Kalashnik et al., 2008; Vasilevskaya & Petrova, 2014; Korshikov et al., 2015; Chropenova et al., 2016; Makhniova, 2016; Mohnachev et al., 2016; Makhniova et al., 2017). High levels of heavy metals and other elements might also be a factor in increasing abortive pollen frequency in plants (Reshetova et al., 2015; Chropenova et al., 2016). Pollutants have also been found in plant seeds (Lyanguzova, 2011), although these authors specify that the degree of accumulation is significantly lower than that in other plant parts (Makhniova et al., 2019).

The aim of the work is to study the conjugate variability of the characteristics of the female generative system of Scots pine (*Pinus sylvestris* L.) in the conditions of emissions of magnesite production.

2. Materials and Methods

The research was conducted in an aero-technogenic emission zone of Combinat Magnesite (South Ural, Russia). Combinat Magnesite is the largest enterprise in Russia. The study area in the city of Satka is located in the Central part of the subzone of coniferous-broad-leaved forests and southern taiga coniferous forests in the Northern part of the southern Urals. On average, the ridges reach 500-700 m, their slopes are steep and short, watersheds are flat-hilly. Production of magnesite in Satka is characterized by large volumes of emissions into the atmosphere of magnesite dust consisting mainly of magnesium oxide, there is also a lot of sodium and potassium, which, when combined with water, forms alkalis. A particularly high level of environmental contamination was observed in the 1960s and 70s. This significantly increases the pH of the soil. The pH of the soil in the upper root horizons was found ranging from 5.8 to 9.5 (Menshikov et al., 2019).

The female generative system of Scots pine was studied at experimental sites (hereinafter referred to as OU) located in the zone of strong magnesite contamination (OU-2) and in background conditions (OU-K). OU-2 is 1 km away from the pollution source, while OU-K is 25 km away. OU is represented by the pine plantation, established in 1980-1983 by ordinary planting in the gradient of pollution by aeroprom discharges of the Combined Magnesite (Figure 1) and in background conditions. The plantation was established with 2-year-old selected seedlings grown from local seed origin in Satka forest nursery (Menshchikov, 1985).

Figure 1. Plant “Magnesite” in Satka, Chelyabinsk region

OU-K is located from the windward side in a southwesterly direction 25 km from the source of emissions outside the pollution zone (background conditions) on the middle part of the slope of the western exposure, the slope steepness is 3°. Soil type-mountain gray forest medium-podzol, medium-loamy. Type of forest-Berry pine (*Pinus* L.). At the time of the study, Scots pine cultures represent a closed stand (Figure 2). The age of the stand at the time of the research is 35 years (Menshchikov, 1985; Srodnyh, 1986).

Figure 2. Scots pine plantation in background conditions (OU-K)

OU-2 is characterized by a strong level of pollution, located in the north-east direction at a distance of 1 km from the source of pollutants on the slope of the southern exposure in the middle part, the slope steepness is 8-10°. Soil type - mountain gray forest heavily podzolized, easily loamy, rocky. The type of forest is Berry pine (*Pinus* L.) (Figure 3). The age of the trees at the time of the study was 35 years (Menshchikov, 1985; Srodnyh, 1986).

Figure 3. Scots pine plantation in the zone of heavy pollution (OU-2)

The current level of OU-2 pollution is due to high pH, suspended solids, some macronutrients and heavy metals in snow water and in soil compared to OU-K (Menshchikov et al., 2012; Kuzmina & Menshchikov, 2015; Kuzmina et al., 2016; Menshchikov et al., 2020) (Table 1).

Table 1. pH indicator, the content of suspended solids, macronutrients and heavy metals in snow water on the experimental sites (ES).

Indicator	OU-2	OU-K
pH	10.3 ± 0.02	7.4 ± 0.09
Weight of suspended matter (g/m^2)	29.51 ± 1.15	0.96 ± 0.15
Ca^{2+} (mg/m^2)	576.55±62.4	162.29±17.1
Mg^{2+} (mg/m^2)	7202.99±1179.3	208.15±24.5
K^{+} (mg/m^2)	57.01±14.4	4.79±1.4
Na^{+} (mg/m^2)	233.01±37.0	91.74±14.8
Fe (mg/m^2)	2.41±0.56	2.27±0.72
Mn (mg/m^2)	0.21±0.07	0.90±0.17
Zn (mg/m^2)	0.34±0.08	0.80±0.21
Cu (mg/m^2)	0.27±0.07	0.20±0.05
Ni (mg/m^2)	0.12±0.02	0.04±0.015

The relative number of seed trees on which the number of cones was calculated was studied at the OU. Twenty-five seed trees were selected and labeled, from the middle and upper part of the crown of which 50-100 cones were collected, or all the available cones, but not less than 20 pieces. The selected samples of cones (individually for each tree) were thoroughly mixed and, according to the principle of random sampling, 20-40 pieces were selected for analysis. Thirty-five quantitative and functional signs were examined in each bump (Table 2).

Table 2. Quantitative and functional features of the female generative system of Scots pine.

No.	Signs	OU-2	OU-K
1	Cone length (mm)	3.57±0.12	3.82±0.10
2	The width of the cone (mm)	1.78±0.05	1.90±0.05
3	The shape of the cone (2nd/1st signs)	0.51±0.01	0.50±0.01
4	The mass of the cone (g)	4.90±0.31	5.65±0.42
5	The shape of the apophysis of seed scales [according to Mamaev (1973)]	3.09±0.19	2.60±0.23
6	The length of the seed scales in the middle part of the cone (mm)	1.89±0.05	2.00±0.04
7	The width of the seed scales in the middle part of the cone (mm)	0.80±0.02	0.86±0.03
8	The shape of the seed scales (7th/6th sign)	0.43±0.01	0.43±0.01
9	The height of the shield in the middle part of the cone (mm)	0.83±0.02	0.85±0.01
10	The width of the flap in the middle part of the cone (mm)	0.70±0.01	0.69±0.02
11	Shield shape (10th/9th signs)	0.85±0.02	0.82±0.02
12	The length of the wing in the middle part of the cone (mm)	1.20±0.04	1.23±0.04
13	Width of the wing in the middle part of the cone (mm)	0.37±0.01	0.40±0.02
14	The shape of the lionfish (13th/12th signs)	0.31±0.01	0.33±0.01
15	The number of seed scales (pcs.)	65.35±1.74	64.51±1.95
16	Number of sterile seed scales (pcs.)	48.64±1.37	46.89±1.38
17	The number of potentially fertile ovules (pcs.)	33.43±1.09	35.25±1.40
18	The number of ovules that survived until the beginning of the 2nd growing season (the number of pollinated ovules) (20th+21st sign) (pcs.)	22.16±1.06	25.37±1.69

Table 2. (continued)

No.	Signs	OU-2	OU-K
19	The number of ovules that died in the 1st growing season (the number of unsullied ovules) (17th-18th signs) (pcs.)	11.27±0.45	9.88±0.82
20	The number of ovules that died during the 2nd growing season (the number of unfertilized ovules) (pcs.)	4.52±0.87	5.24±0.97
21	Total number of seeds (22nd+23rd sign) (number of fertilized ovules) (pcs.)	17.64±1.29	20.13±1.67
22	Number of full-grain seeds (pcs.)	15.14±1.1	16.58±1.46
23	Number of empty seeds (pcs.)	2.49±0.42	3.55±0.45
24	Survival of ovules in the 1st growing season (18th/17th signs) (%)	64.61±1.82	70.69±2.94
25	Survival of ovules in the 2nd growing season (21st/18th signs) (%)	78.69±3.8	78.14±3.96
26	Survival of ovules for the entire gametophytic period (21st/17th signs) (%)	51.09±2.96	55.86±3.56
27	Survival of ovules in the embryonic period (22nd/21st sign) (proportion of seeds healthy) (%)	86.56±1.63	81.93±2.07
28	Overall survival of ovules for gametophytic and embryonic periods (22nd/17th signs) (%)	44.58	46.06
29	Weight 1000 pcs. seeds (g)	5.41±0.23	6.31±0.20
30	Seed germination energy (%)	87.29±2.48	85.50±2.68
31	Germination percentage of seeds (%)	91.74±1.91	90.67±1.67
32	Proportion of seedlings with cotyledons at the time of germination determination (%)	75.84±3.73	64.37±6.35
33	Root length of seedlings with cotyledons (mm)	14.44±0.78	15.60±1.08
34	The length of the hypocotelium of seedlings with cotyledons (mm)	28.49±0.56	31.39±0.79
35	Number of cotyledons (pcs.)	5.58±0.07	5.80±0.07

All linear features of the elements were measured using a laboratory caliper with an accuracy of 0.01 cm. The shape index of cones, seed scales, scutes and wings was calculated as the ratio of width to length. The structure of the surface of the seminal scales, or the shape of the apophysis, was evaluated according to the scale proposed by Mamaev (1973).

To count seeds, fertile and sterile seed scales, each cone was destroyed mechanically. To do this, the axis of the cone was drilled and disassembled into scales, starting with the basal ones. The beginning of the fertile stage was recorded by the appearance of seed scales with signs of ovule development in the first and second year: empty and full seeds, underdeveloped seeds (separating or not separating from the seed scales) with a size of more than 1 mm. The doubled number of fertile seed scales corresponded to the initial number of potentially fertile ovules (Romanovskij & Hromova, 1992; Abaturova et al., 1997; Romanovskij, 1997). The number of fertilized ovules was determined by the sum of empty and full seeds. The number of empty seeds corresponded to the number of ovules that died during the embryonic period of development. The number of small underdeveloped seeds testified to the number of ovules that died during the second year of development (Romanovskij & Hromova, 1992; Romanovskij, 1997).

The germination percentage and germination energy of seeds were determined in threefold repetition according to GOST (1998) - 13056.6-97.

The correlation matrices of the studied features of the female generative system of pine trees growing in conditions of different levels of magnesite pollution and background conditions were processed by factor analysis using the Statistica 6.0 software package (Halafyan, 2007). During the analysis, factors with eigenvalues greater than 1.0 were taken into account. When studying the composition of factors, signs were discussed whose correlations with the corresponding factor modulo exceeded 0.5.

3. Results and Discussion

Under background conditions, the 1st factor (F1) makes a significantly greater contribution to the variability of indicators (the proportion of the variance explained 34.89%) (Figure 4). In its composition, such signs as the size and weight of cones (signs 1, 2, and 4), the size of seed scales and wings (signs 6, 7 and 12, 13), the number of sterile and the total number of seed scales (signs 15, 16), the number of fertile and pollinated are positively correlated with each other and negatively with the factor ovules (signs 17, 18), total number of seeds (sign 21), number of healthy and empty seeds (signs 22, 23), survival of ovules in the 1st, 2nd and entire gametophytic period (signs 24, 25, and 26), final survival of ovules (sign 28), length the root of the seedling (sign 33). Thus, under these growing conditions, the size and mass of cones (signs 1, 2, and 4) are interrelated with the size of seed scales (signs 6 and 7) and their number (signs 15, 16).

The size of the winglets (signs 12, 13) is related to the size of the seed scales (signs 6, 7). A reliable correlation between potentially fertile (trait 17), pollinated (trait 18) and fertilized (trait 21) ovules, the number of healthy seeds (trait 22) allows us to conclude that the potential of cones to form seeds in the background (OU-K) are fully realized. The yield of seeds (signs 21, 22) is directly proportional to the size of the cones. However, the seed weight (sign 29) and seed quality indicators (signs 30, 31) do not depend on the number of seeds in the cones (signs 21, 22) and the size of the cones (signs 1, 2, and 4). The overall survival of ovules (trait 28) and seed yield (signs 21, 22) are determined to a greater extent by the survival of ovules at the gamete stage (signs 24-26) than at the embryo stage (trait 27). A similar system of connections is partially duplicated in F2 (the proportion of the variance explained 17.53%) and supplemented with signs characterizing the mass of seeds (sign 29) and their germination energy (sign 30): The more intensive the selection at the gamete stage, the larger the seeds and the higher the germination energy. The survival of ovules in the embryonic period of development (sign 27) has a small number of connections with other signs of the female generative system, may depend on the survival of ovules in the 1st growing season (F3-The proportion of the variance explained 12.75%) and determine the length of the hypocotyl of the seedling (F6-The proportion of the variance explained 5.60%). Seed germination (feature 31) is also quite isolated from other features and is interrelated with the germination energy (F4-The proportion of the variance explained 7.63%) or varies independently (F7-The proportion of the variance explained 4.83%). The trait characterizing the number of cotyledons (sign 35) is not reliably associated with any of the studied signs (F5-The proportion of the variance explained 6.27%). In general, highly reliable relationships between the indicators characterizing the development of seedlings and the weight of seeds and their seed quality indicators have not been revealed, because these signs with high values of factor loads are part of different factors, which suggests an imbalance in the postembryonic period of seed development.

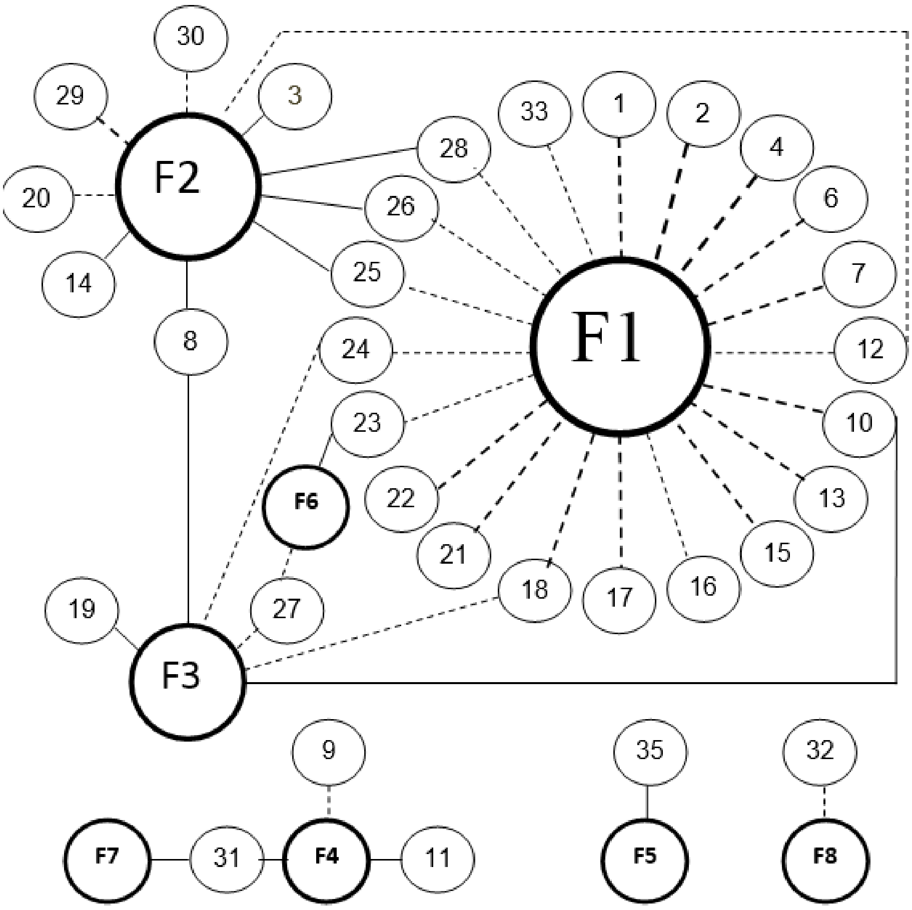

Figure 4. Factor structure of the female generative system of Scots pine in background conditions. For the designation of the signs, see Table 1. — positive connection; ---- negative connection; the level of correlation of signs: ----- 0.7 >r>0.5; ------- 0.9>r>0.7; ----- r>0.9.

In conditions of heavy pollution (OU-2), as part of the 1st factor (28.99% of the total dispersion), such signs as the size and mass of cones (signs 1, 2, and 4), the size of seed scales, shields and wings (signs 6, 7, 9, 10, 12, 13), the number of sterile and total number of seed scales (signs 15, 16), as well as the number of fertile and pollinated ovules (signs 17, 18), the total number of seeds (sign 21), the number of healthy seeds (sign 22), the survival of ovules for the entire gametophytic period (sign 26), the final survival rate of ovules (sign 28) (Figure 5).

In conditions of heavy pollution (OU-2), as well as in background conditions (OU-K), the size of cones and their mass (signs 1, 2, and 4) are interrelated both with the size of seed scales (signs 6, 7) and with their number (signs 15, 16) (Figure 5).

The size of the wings (signs 12, 13) are related to the size of seed scales (signs 6, 7). The number of pollinated, fertilized (the total number of seeds), as well as the number of healthy seeds are related to the number of fertile ovules, i.e., the potential of trees for seed formation in these conditions are fully realized.

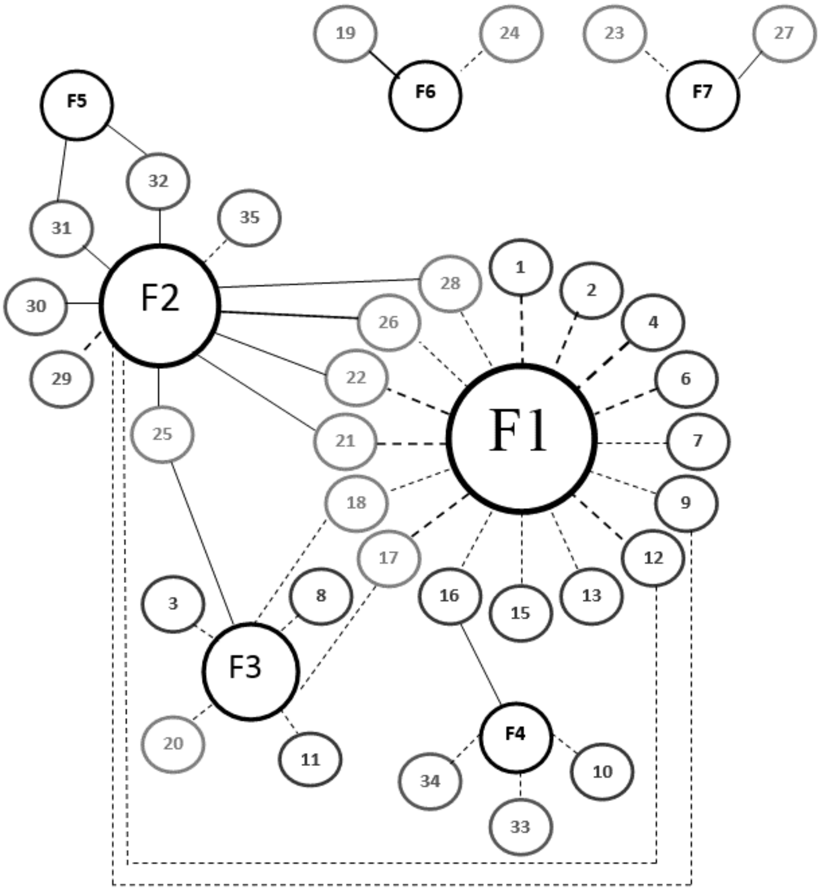

Figure 5. Factor structure of the signs of the female generative system of Scots pine in conditions of severe pollution (OU-2). For the designation of the signs, see Table 1. — positive connection; ---- negative connection; the level of correlation of signs: ----- 0.7 >r>0.5; ------- 0.9>r>0.7; ----- r>0.9.

The indicator characterizing the number of fertile ovules is part of two factors (F1-The proportion of the variance explained 28.99% and F3-The proportion of the variance explained 12.87%) and in both correlates directly with the number of pollinated ovules, i.e., all trees of the OU have a unidirectional relationship of these indicators. However, with an increase in the number of pollinated ovules, both the number of fertilized (survival in the 2nd growing season increases) (F1) and unfertilized ovules (survival in the 2nd growing season decreases) (F3) can increase. The connection of pollinated and fertilized ovules is stronger (F1), probably in most trees of the ovules that have successfully passed the pollination process, most of them will be fertilized. However, in both cases, the selection result is uncertain-there is no connection with the quality of seeds. For part of the indicators characterizing the number of seeds and the survival of ovules, the composition of F1 (the proportion of the variance explained 28.99%) and F2 (the proportion of the variance explained 20.61%) is duplicated. The system of connections does not contradict the above, but the set of indicators is supplemented by indicators characterizing the weight of seeds and their quality. Thus, in the zone of heavy pollution, the negative relationship between seed productivity (the number of full-grained seeds and the total number of seeds) and seed weight reaches a significantly significant level (F2). This can be explained by a lack of nutrients for trees, since in these growing conditions they are greatly weakened, the crown has a high degree of defoliation, the life span of needles is shortened (Zavyalov et al., 2016, 2018). A significant negative relationship of seed mass with germination energy and germination was revealed (Figure 5). Seeds of low mass (it can be 2.38 g/1000 pcs., which is 2.65 times less than the average for OU-K) have high sowing qualities of seeds and form in laboratory conditions by day 15 developed seedlings with a high proportion of “cotyledon” plants and a small number of cotyledons. The relationship of seed mass indicators with the number of seeds and seed quality indicators is not repeated as part of other factors, which suggests a unidirectional reaction of trees according to these indicators to growing conditions. The length of the formed seedlings varies; however, the ratio of the length of the root and hypocotyl is predictable: The longer the length of the root, the longer the length of the hypocotyl, which follows from the positive correlation of these indicators in the composition of F4 (the proportion of the variance explained 7.99%). Regardless of the weight, seeds with high sowing qualities of seeds form seedlings of longer length than seeds with small values of seed quality indicators (F5-The proportion of the variance explained 6.86%).

Under these growing conditions, the survival of ovules in the 1st growing season is not associated with any parameters of the female generative system and is part of the 6th factor (the proportion of the variance explained 5.21%), which is negatively associated with. The survival of ovules in the embryonic period is also isolated from other signs and is part of factor 7 (the proportion of the variance explained 4.49%).

4. Conclusion

(i) The relationship of the features characterizing the size and mass of pine cones is rigidly genetically determined, because it is not violated in conditions of a strong level of pollution.

(ii) The seed productivity of pine stands is more closely correlated with the survival rates of ovules in the gametophytic period than in the period of embryonic development. It should be noted that under background conditions, the survival of ovules in the 1st vegetative and embryonic periods are positively associated, and under pollution conditions, the relationship of these signs is negative, which suggests the existence of effective elimination of abnormal gametophytes and the release of the stand from them under pollution conditions.

(iii) In conditions of heavy pollution, a negative relationship between seed productivity and the mass of pine seeds was revealed. The reason for this may be a strong weakening of trees and low availability of mineral resources in conditions of high pH of the growing medium.

(iv) The structure of the relationship between the indicators of seed mass and their sowing qualities is changing. The relationship of seed mass with germination energy and germination in background conditions is positive, and in conditions of heavy pollution, a significant negative relationship of these signs was revealed.

(v) In seedlings formed under pollution conditions, the structure of the relationships between their morphometric indicators and indicators of seed mass and quality changes. Thus, the length of the root and hypocotyl of seedlings formed under background conditions are not related to their germination energy, germination and mass of seeds, and in conditions of heavy pollution are positively related to the main quality indicators and negatively to their mass.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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