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Genetic load in empty seeds of European larch (*Larix decidua* Mill.)

INTRODUCTION

European larch is a species with a low full seed yield. Messer (1956) reported that the percentage of full seed ranged from 19,9 to 40,2 and Nilsson (1959) found the number of empty seeds to be 50% and more.

The number of full seeds decreases after self-pollination (Nilsson 1959, Dieckert 1964). This is similar to most coniferous trees. In part it is connected with embryo death caused by homozygosity of the so called embryonic lethals. So far there was no report on the number such embryonic lethals in larch.

The aim of this study was to estimate the inbreeding depression in full seed yield after self-pollination and after using self pollen in mixture with different pollen. An attempt to estimate the embryonic genetic load and the influence of the other factors on seed yield in European larch was made.

MATERIALS AND METHODS

The study was conducted on eight clones of European larch (*Larix decidua* Mill.) growing in the Kórnik seed orchard. In spring 1979 controlled pollinations were made. Every mother tree was pollinated with self pollen and also was crossed with four or five male testers. Additionally pollination with a pollen mix from the eight mother clones was carried out. All cones (639) after controlled pollination and also the samples of cones (158) after open-pollination were collected in the autumn. From all these cones 27943 seeds were obtained. The extraction of full seeds was carried out in 70% ethanol (results were checked by the cutting test). Results were presented as the percentage of full seeds after different pollination regimes. The percentage values were treated by the arcsine transformation for the variance analysis. Relative self-fertility (RSF) was calculated for each clone according to the Sorensen (1969):

$$RSF = \frac{\text{proportion good seeds after self-pollination}}{\text{proportion good seeds after cross-pollination}}$$

Number of embryonic lethals was estimated on the basis of the calculation made by K o s k i (1971), using relative self-sterility (RSS = 1 − RSF) as a probability of empty seeds after self-pollination due to genetic background with the assumption of two fertilizations per ovule. The percentage of empty seeds after self-pollination employed by Koski as a measure of the probability of empty seeds due to homozygosity at least one locus of an embryonic lethal was impossible for larch, because of the great probability of empty seeds irrespective of homozygosity. Effectivness of pollination and the number of fertilization per ovule was estimated microscopically after collection of female inflorescences soon after the natural pollen shedding and after the expected time of fertilization.

RESULTS AND DISCUSSION

Analysis of variance has shown signifficant differences in full seed setting depending on clone and the kind of pollination (Table 1).

The interclonal differences are due to the genetic and/or physiological factors. The latter may be due to differences between trees in the optimal time for pollination. The moment of the pollination may have been more favourable for one than for the other clone. This is especially important in control pollination.

The main results of this study was the confirmation of the significant negative effect of self pollen on full seed yield (Table 2). Inbreeding depression was about 50% in relation to the cross-pollination (from 24.0% to 11.3%). Also a very strong negative effect of self pollen in the pollination mixture has been found. The presence of about 12% of self pollen in such a mixture caused a depression of 25% in relation to the cross-pollination (from 24% to 18.1%). It suggests not only that there is no incompatibility systems against self pollen but that such pollen may have caused about 50% of fertilizations and therefore has an advantage over other pollens in the mix. The N e w m a n - K e u l s Test showed that the difference between cross and mixed pollen with self is significant (Table 2).

Table 1

Analysis of variance for the full seed yield after different pollination regimes (after arcsine transformation)

Source of variation	d.f.	M.S.	F
Total	31		
Variables (pollination regime)	3	722.33	26.80**
Replicates (clones)	7	401.81	14.91**
Residual	21	26.95	

** – significant at 0,01 level

Calculated on the proportion of full seed after self- and cross-pollination relative self-fertility ranged from 0.21 to 0.72 (Table 2), and averaged 0.41. It means that on the average, the trees set about 41% as many sound seed after self-pollination as after controlled crossing. I know of no other reports on RFS in larches. In relation to other conifers the RSF of 0.41 is intermediate, but rather high. Sorensen (1971) reported about 0.11 for *Pseudotsuga menziesii* and 0.37 for *Pinus ponderosa* (Sorensen 1970). Bingham and Squillace (1955) estimated the RSF of *Pinus monticola* to be 0,53 and Sorensen et al. (1976) reported about 0.69 in *Abies procera*.

Table 2

Percentage of full seed setting after different kinds of pollination, relative self-fertility, relative self-sterility and number of embryonic lethals in eight clones of European larch

No. clones	Self-poll.	Mix-poll.	Cross-poll.	Wind-poll.	RSF	RSS (1-RSF)	Embryonic lethals
02 - 11	4.8	12.7	22.7	29.1	0.211	0.789	7
02 - 12	4.8	23.0	17.2	27.0	0.279	0.721	6
08 - 02	42.6	57.1	59.4	78.1	0.717	0.283	2
08 - 03	3.2	5.1	13.6	34.0	0.235	0.765	7
10 - 02	7.4	5.3	15.5	27.0	0.477	0.523	4
10 - 25	5.8	7.8	12.7	32.2	0.457	0.543	4
10 - 27	11.1	24.6	18.4	43.9	0.603	0.397	3
15 - 88	10.4	5.0	39.2	61.4	0.265	0.735	6
Mean	11.3	18.1	24.0	41.6	0.406		4.8

< Newman – Keuls Test

On the basis of relative self-sterility (RSS=1−RSF) the number of embryonic lethals were calculated assuming two fertilizations per ovule (as judged from microscopic observations). There were from 2 to 7 embryonic lethals per tree, an average of 4.8. In few publications on the above subject, results that have been obtained are of roughly of the same magnitude, though the calculations were made in different ways. The greatest number of embryonic lethals was reported by Fowler (1965) for *Pinus banksiana* — 15, but after treating his results by the method described by Koski (1971), the mean number of embryonic lethals becomes 5.4 (Koski 1971). The number of embryonic lethals for other conifers was considerably greater, for *Pseudotsuga menziesii* 10.0 (Sorensen 1969), for *Pinus sylvestris* 9.4 (8.9), for *Picea abies* 9.6 (Koski 1971 and 1973) and for *Pinus taeda* 8.5 (Franklin 1970). Not calculated averages for *Picea omorika* and *Pinus peuce* were rather lower (Koski 1973), similar to *Abies procera* (Sorensen et al. 1976) — 1.8 for one and 3.4 for two embryos per ovule. A low number of embryonic lethals in larch in relation to other native conifers e.g. *Pinus sylvestris* or *Picea abies* is probably connected with their strong homozygosity in relation to the above species (Mejnartowicz and Bergmann 1975).

The genetic load of European larch estimated on the number of em-

bryonic lethals is rather low in relation to most of the conifers referred to above, and does not explain the great number of unsound seed after cross- and wind-pollination. There are a few possibilities for the explanation of such a phenomenon: lack of pollination, great influence of the external environmental factors, high genetic load of the whole population and for wind-pollination a high proportion of self-pollination. Lack of pollination as a cause of empty seed formation has been reported by Hall and Brown (1977). When sectioned female inflorescences were analysed a lack of pollen in part of the pollen chambers was sometimes found. The influence of external factors (especially temperature) on pollen development has been reported by Ekberg and Eriksson (1967), and on the ovules suggested by Hall and Brown (1977). The susceptibility of larches to changes of temperature during the time of pollination was strongly supported by low full seed settting after any kind of controlled pollination (Table 2). The measurement of the temperature inside the pollination bags showed, that on cloudy days and nights temperatures increased and decreased about 2°C respectively. This permits to suppose, that on sunny days and nights without clouds the temperatures oscilate much more, and may strongly influence the pollen and ovules. Another possible explanation of the great number of unsound seed is a high genetic load of the whole population. It is possible that embryonic lethals are present in a low number for each tree, but with great frequency in the population as a whole. In such a case embryo death my be due to homozygosity of lethals even after cross-pollination. However this explanation is a little doubtfull, because the investigated population was artificialy established by man from selected plus trees.

Finally, the study indicates that preferred selfing is to a considerable degree responsible for the low percentage of full seed. Genetic load expressed as the number of embryonic lethals was not so great, but there probably exist other genetic factors operating before fertilization.

SUMMARY

An experiment carried out on eight clones of European larch showed a significant influence of self pollen on the decrease of full seed setting. Estimated relative self-fertility was on the average 0.41 and the calculated number of embryonic lethals was on average 4.8. It is insufficient to explain the high percentage of empty seeds in larch. In a pollen mix self pollen appears to have an advantage achieving four times more than its share of fertilizations. A strong negative effect of isolation of female inflorescences during pollination time was found and this was probably connected with changes of temperature.

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Obciążenie genetyczne w powstawaniu pustych nasion u modrzewia europejskiego (Larix decidua Mill.)

Streszczenie

Doświadczenie przeprowadzone na 8 klonach modrzewia europejskiego wykazało istotny wpływ samozapylenia na powstawanie mniejszej liczby pełnych nasion.

Oszacowany współczynnik samopłodności wyniósł średnio 0,41, a liczba czynników letalnych powodujących śmierć zarodka wyniosła średnio 4,8 na jedno

drzewo. Jest to zbyt mało aby wyjaśnić wysoki procent pustych nasion u modrzewia. Istnieją więc inne mechanizmy genetyczne i fizjologiczne wpływające na powstawanie pustych nasion.

W mieszaninie pyłków własny pyłek wydaje się posiadać przewagę w stosunku do obcych w ilości zapłodnień. Wydaje się, że nie istnieją systemy niezgodnościowe zapobiegające samozapyleniu. Izolowanie kwiatów żeńskich w czasie zapylenia wywarło silny negatywny wpływ na udział pełnych nasion, co było prawdopodobnie związane ze zmianami temperatury wewnątrz izolatorów.

ГЖЕГОЖ КОСИНЬСКИ

Участие генетической нагрузки в возникновении пустых семян у лиственницы европейской (Larix decidua Mill.)

Резюме

Опыты проведенные на 8 клонах лиственницы европейской выявили существенное влияние самоопыления в возникновении меньшего количества полных семян.

Коэффицент самоопыления был в среднем равным 0,41, а число деталей вызывающих гибель зародыша в среднем 4,8 в пересчете на одно дерево. Этого недостаточно, чтобы выяснить высокий процент пустых семян у лиственницы. Следовательно существуют другие генетические и физиологические механизмы влияющие на возникновение пустых семян.

В смеси пыльцы собственная пыльца по всей вероятности имеет преимущество по сравнению с другой по числу оплодотворений. По всей вероятности не существует системы несоответствия предотвращающей сомоопыление. Отмечено значительное отрицательное влияние изоляторов в период опыления. Оно связано по всей вероятности с температурными изменениями.