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MATING SYSTEM AND EMPTY SEEDS IN SILVER FIR (*ABIES ALBA* MILL.)

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ABSTRACT

Sixteen trees of silver fir (*Abies alba* Mill.) were artificially selfed and outcrossed. Wind-pollinated seeds from the same trees were also analyzed. The average yield of filled seeds following selfing was 58 % of that following artificial outcrossing. This could be interpreted as the effect of 2–3 recessive lethals. Investigations on empty seeds following selfing in conifers were compiled. Average wind pollination filled seed yield was 90 % of that following outcrossing, but the difference from artificial outcrossing was not significant.

Key words: *Abies alba*, filled seeds, pollination, selfing, inbreeding depression, embryonic lethals

INTRODUCTION

Conifers have not developed efficient mechanisms preventing self-pollination, although many selfed zygotes never develop because of a recessive genetic load resulting in a high frequency of empty seeds (KOSKI 1973, PLYM FORSHELL 1974, KOSIŃSKI 1981, BURCZYK *et al.* 1991, KÄRKKÄINEN & SAVOLAINEN 1993, WILLIAMS & SAVOLAINEN 1996). Conifers are monoecious and anemophilous, and seeds from open pollination will be a mixture of selfed and outcrossed zygotes. The proportion of seeds which originates from selfing varies widely among individual trees and among species.

There are two investigations in the genus Abies concerning the effects of self-pollination. SORENSON *et al* (1976) studied *Abies pocera* and MOULALIS (1986) studied *Abies alba*. Both reported empty seeds, but for *Abies alba* the progeny following self-pollination were similar to those following cross-pollination.

The objective of the present study was to evaluate the effect of selfing on empty seeds in silver fir (*Abies alba* Mill.).

MATERIALS AND METHODS

Sixteen trees of silver fir growing in different indigenous pure mature stands of the species were artificially self- and cross-pollinated. The pollination program took some years, but self- and cross- pollination of the same tree were made at the same time. Each cross-pollination was made using a single male parent. Cones from open pollination were collected in the same time as those originating from artificial pollination. Only fresh pollen with a high *in vitro* germination was used for artificial pollination. The female strobili were isolated with paper bags soon after the emergence from the bud scales and pollinated at the stage of maximum receptivity. Two weeks after pollination, the isolators were removed from the megastrobili. Mature cones were typically harvested in October of the same year. (Isolated strobili and collected cones were registered for crossings).

Model and calculations

We studied how the rate of self fertilisation may influence the selfing rate of seeds and the frequency of empty seeds. The model, formulae and algorithms used is presented in LINDGREN (1975), and is built on models by BRAMLETT & POPHAM (1971) and KOSKI (1973). Embryonic lethals were assumed to be recessive, independently operating, lethal genes. The parental tree was assumed to be heterozygous at n loci for embryonic lethals. It may be noted that this model is likely to be oversimplified, (cf GRIFFIN & LINDGREN 1985), but as we have access only to selfs and no other types of inbreeding, there are not sufficient grounds to develop a more complex model. Unpollinated ovules can cause empty seeds in silver fir, so for the model to be applied one has to assume that the same frequency of ovules are pollinated for all mating systems. We assume that shared lethal recessives do not cause embryo lethality or empty seeds in outcrossing (thus genetically caused empty seeds can occur only following selfing). We assume that empty seeds caused by factors other than lethal recessives occur in the same proportions following self-pollination, cross-pollination and wind-pollination. Thus we can get information regarding selfing in controlled selfing and wind-pollination by relating the percentage of empty seeds to that following cross-pollination. Each mother tree can be seen as an independent replication for testing the zero hypothesis that there are no differences between mating systems.

Two models were studied. Model 1; a filled seed can be formed only from one embryo, and if that dies because of homozygosity for a recessive lethal, then the seed becomes empty. Model 2; a filled seed can be formed if any of two embryos survive, which have the same maternal gamete but different paternal gametes. If both are killed by homozygosity for recessive lethals, then we get an empty seed. If both survive, the remaining one is decided at random.

RESULTS

Of 307 cross-pollinated strobili, 76.2% developed into cones, while after selfing of 267 strobili only 71.9% developed into cones. As selfing resulted in lower cone yield than controlled outcrossing for only 6 out of 16 mother trees, these differences are inconclusive.

The filled-seed yield for individual trees are presented in Table 1. Pooling the values of all 16 trees, the average percentage of filled seeds was 26.1% following selfing, 40.6% following wind-pollination and 45.0% following artificial cross-pollination. A *t*-test for significance was made for the 16 individual tree ratios of selfing versus cross-pollination, the zero-hypothesis that there was no difference could be safely rejected ($P < 0.001$). The same test was done for the ratios of wind-pollination versus cross-pollination. In this case no significance was obtained ($P > 0.05$). Selfing produced 58% and wind-pollination 90% of that following artificial outcrossing.

Assuming that all empty seeds following outcrossing were caused by other factors than embryonic lethals, 42% of selfed seeds were dead because of lethals. If these deaths were caused by independent lethals and one embryo, there were 1.93 lethals. If,

Table 1 Parents and filled seed yield from individual trees for selfing, controlled cross-pollination, open pollination and recessive lethals interpretations involving sixteen silver fir trees

Seed parent				Pollen parent	Pollination year	Filled seed (%)			Lethals/ Embryos	
Tree	Latitude	Longitude	Altitude(m)	Tree		Cross	Wind	Self	One	Two
1	48°55'25"	19°36'55"	1,250	2	1986	46.7	43.0	12.5	4.5	6.4
2				1	1986	57.2	68.7	49.5	0.5	1.0
3	48°32'10"	18°41'00"	475	4	1987	39.0	43.5	23.5	1.8	3.0
4				*	1987	61.2	48.0	13.0	5.4	7.4
5				*	1988	33.5	35.2	15.9	2.6	4.1
6				5	1988	34.6	15.9	14.2	3.1	4.7
7	48°39'50"	19°02'00"	800	8	1989	54.2	53.5	40.2	1.0	1.9
8				7	1989	40.0	30.7	24.5	1.7	2.9
9				8	1989	11.0	11.5	8.2	1.0	1.9
10	48°28'10"	18°34'30"	660	*	1990	19.0	33.0	15.0	0.9	1.6
11	48°31'05"	18°51'20"	580	13	1991	67.5	57.2	20.5	4.2	6.0
12				13	1991	46.0	46.2	54.0	0.0	0.0
13				10	1992	47.5	68.0	42.0	0.5	1.0
14	48°32'20"	18°55'55"	700	10	1992	46.0	37.0	41.0	0.4	0.9
15				10	1992	55.0	27.0	21.5	3.3	4.9
16				10	1992	62.5	30.0	22.5	3.6	5.2
Average						45.1	40.5	26.1		

* – used individuals of silver fir which were not involved in the experiment as mother trees

Table 2 Reported values on yield of filled seeds following selfing in conifers in relation to outcross (artficial outcross or wind pollination)

Species	Reference	Filled seed (% of outcross)
Abies alba	present investigation	58
Larix decidua	KOSIŃSKI 1981	41
Larix laricina	PARK & FOWLER 1982	1
Picea abies	SKRØPPA & THO 1990	27
Picea abies	ANDERSON *ET AL.* 1974	25
Picea abies	KOSKI 1973	16
Picea glauca	FOWLER & PARK 1983	13
Picea omorika	KOSKI 1973	62
Pinus banksiana	FOWLER 1965b	42
Pinus contorta	SORENSON & ADAMS 1993	12
Pinus elliottii	SQUILLACE & KRAUS 1963	22
Pinus monticola	BINGHAM 1973	60
Pinus radiata	GRIFFIN & LINDGREN 1985	43
Pinus radiata	PAWSEY 1964	74
Pinus resinosa	FOWLER 1965a	100
Pinus strobus	FOWLER 1965b	85
Pinus sylvestris	KOSKI 1973	19
Pinus sylvestris	YAZDANI & LINDGREN 1991	43
Pinus virginiana	BRAMLETT & BRIDGWATER 1986	31
Pinus thunbergii	KATSUTA 1966	77
Pseudotsuga menziesii	WOODS & HEAMAN 1989	4
Pseudotsuga menziesii	SORENSON 1971	12
	Unweighted mean	39

however, there were two embryos with shared maternal gametes but different paternal gametes, there would be 3.2 lethals (interpolations in Table 4, LINDGREN 1975). A single tree with a good filled-seed yield following selfing is interpreted as having no recessive alleles.

The relationships between the three seed types can be explained by a model assuming that empty seeds in out-crossing were due to other factors than embryonic lethals. Then, wind-pollination would comprise 24% self-fertilization and 15% of the filled seeds would have embryos from selfing assuming a one embryo model. If there were instead two embroys, the interpretation could be that wind-pollination resulted in 49% self fertilizations and that 27% of the filled seeds would have embryos from selfing.

DISCUSSION

Empty seeds following selfing

Results for conifers reported in other studies are summarised in Table 2. Selfing is compared to the filled seed yield of controlled out-crossing or wind-pollination. Silver fir seems to respond to selfing in a rather typical manner with respect to numbers of filled sound seeds, although the decrease may be slightly below average for the species studied.

Selfing in wind-pollination

Some examples can be cited on the likely incidence of self fertilisation obtained from empty-seed frequencies. The investigations by SARVAS (1962) for *Pinus sylvestris* indicated that 12% of germinable seeds originated from selfing, while those derived by KOSKI (1970) for *Picea abies* varied between 2.4 and 24%. Another source of information are isozyme data that give the yield of germinating selfed seeds, which usually is considerably lower for self fertilizations. Five investigations on selfing among germinable seed-orchard seeds of Scots pine were reviewed by LESTANDER and LINDGREN (1985) indicating values between 2 and 16%, a value of 2.4% was reported for a Finnish seed orchard (Viitaselkä seed orchard, KÄRKKÄINNEN & SAVOLAINEN 1993). ADAMS and BIRKES (1991) reviewed 6 studies for conifer seed orchards resulting in values between 1 and 16%, and 15 studies for natural populations of conifers ranging between 2 and 17%.

SCHROEDER's (1989) estimate of outcrossing rates in 184 single trees of *Abies alba* was based on the enzymatic systems isocitratedehydrogenase and 6-phosphoglukonate dehydrogenase in endosperms and the corresponding embryos and averaged at $T_m = 0.89$, while the mean inbreeding coefficients were found to be 0.05 (IDH) and 0.02 (6-PGD), respectively. Outcrossing rates varied among populations but the intrapopulational variation was high. The author was not able to establish a correlation between outcrossing rate and percentage of empty seeds.

There are many differences between artificial and natural pollination and a large part of the empty seeds have other causes than genetic in silver fir, thus it is uncertain if a difference between wind-pollination and cross-pollination should be given a genetic interpretation. The possible effect of selfing in wind-pollination in this study was not significant, but it does not support that it would be considerable higher than in other conifers.

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