shoots that govern the potential for differentiating seed and pollen cones.

Finally, the operational use of GA$_{4/7}$ to enhance Douglas-fir seed orchard yields bears special mention. Heretofore, practical difficulties of applying the growth regulator to especially larger trees have restricted the use of this highly effective treatment (Ross and PHARIS, 1982; BONNET-MASIMBERT, 1987). The preferred method of application for Douglas-fir — continuous stem injection by the "hanging bottle" technique used here — is very labor intensive. A much simplified method was recently described by PHILIPSON (1985) for treating pole-size Picea sitchensis (BONG.) CARR. grafts. A concentrated ehanolic solution of GA$_{4/7}$ is injected into shallow holes drilled on opposite sides of the main stem, followed by retreatment two weeks later. Ross and BOWER (1989) have since shown that a single injection of GA$_{4/7}$ at the proper dose and time can be a highly cost-effective treatment for promoting flowering in Douglas-fir seed orchards, especially when applied in conjunction with stem girdles.

Acknowledgements

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Outcrossing Rates and Seed Characteristics in Damaged Natural Populations of Abies alba Mill.

By S. SCHROEDER

Forstliche Versuchs- und Forschungsanstalt
Baden-Württemberg,
Abteilung Botanik und Standortskunde,
Wonnhalde 4, D-7800 Freiburg, FRG

Abstract

Open pollinated seeds were collected of 251 single trees originating from 9 natural Abies alba populations located in the southwest of Western Germany. On an average the weight per 1000 seeds was 47 g and the amount of empty seeds 29%. Results of isozyme analyses are reported for the enzyme systems IDH and 6-PGD. Heterozygote frequencies of the progeny in general were found to cor-

respond to the panmictic expectation. Outcrossing rates were estimated according to single locus as well as to multilocus methods. They varied among populations but especially the intrapopulational variation was high. A mean outcrossing value t_m of 0.89, and mean inbreeding coefficients of 0.05 (IDH) and 0.02 (6-PGD) were found. No significant correlations were found between the characteristics outcrossing rate, extent of needle loss of the tree, seed weight and percentage empty seeds.

Key words: Abies alba, isozymes, outcrossing, seed.

Zusammenfassung

In 9 natürlichen Weißtannenbeständen Südwestdeutschlands wurde an insgesamt 251 Einzelbäumen Saatgut geerntet. Im Mittel betrug des Tausendkorngewicht 47 g und der Hohlkornanteil 29%. Isoenzymanalysen wurden für die Enzymsysteme IDH und 6-PGD durchgeführt. Die Häufigkeiten der heterozygoten Nachkommen weichen in der Regel nicht von den im panmiktischen Gleichgewicht erwarteten ab. Die Fremdbefruchtungsraten wurden nach zwei unterschiedlichen Methoden ermittelt. Sie variieren stark zwischen den einzelnen Beständen, besonders jedoch zwischen Einzelindividuen. Eine mittlere Fremdbefruchtungsrate t_m von 0,89 sowie mittlere Inzuchtkoeffizienten von 0,05 (IDH) und 0,02 (6-PGD) wurden gefunden. Zwischen den Merkmalen Fremdbefruchtungsrate, Nadelverlust, Samengewicht und Hohlkornanteil wurden keine signifikanten Korrelationen festgestellt.

Introduction

In Germany *Abies alba* is of great economic and ecological importance. It's dieback has been going on for a long time and nowadays even the survival of the species is severely threatened within certain areas. Compared to other conifer species silver fir has a very low germination percent (normally found to be 50%; MAYER, 1977) and a high percentage of empty seeds as well. High pollen and seed weights seem to restrict the gene flow. Silver fir is supposed to have relatively low genetic variation and high levels of inbreeding and of homozygotes (VINCENT and KANTOR, 1971; LARSEN, 1986). The finding of a level of self-fertility of 0.72 (MOULALIS, 1986), which is very high compared to that found in *Picea abies* (0.32; DITCKERT, 1964a), would support this inbreeding theory to a certain extent. On the other hand, in a study comprising about 100 plant species, HAMRICK *et al.* (1979) found that there is no strong association between genetic variation and seed dispersal distance; however, a positive association between the amount of outcrossing and genetic variation was found.

This investigation was started to test the validity of the inbreeding theory by experimental data. The method of isozyme analysis of single tree seed lots was used to estimate outcrossing rates in silver fir.

Materials and Methods

The seed material was collected in 1986. That year the fructification rate of *Abies alba* was very high (estimated 75% of full crop). Eight indigenous stands located in the southwest of Germany were harvested (*table 1*). Seeds of on an average 29 single trees per stand were collected (open pollinated families in *table 2*). All stands show symptoms of silver fir dying. When the trees were harvested for each single tree the percentage of needle loss was registered.

In this study data are included of an additional population harvested in 1982 (population no. 11, with 16 single trees harvested). This material had already been studied.

The seeds were stored at -5^0 C before using them for electrophoresis.

100 seeds per single tree were weighed to determine the weight per 1000 seeds. These were cut open to determine the percentage of empty seeds.

For enzyme analysis 10 seeds per single tree were assayed for IDH (E.C. 1.1.1.42) and 6-PGD (E.C. 1.1.1.44). However, populations no. 11 and 14b were characterized by analyzing 40 seeds per single tree. Endosperm as well as embryo tissue was analyzed. Details of electrophoretic procedures and genetic interpretation are given elsewhere (SCHROEDER, 1989).

Estimates of outcrossing rates were obtained by two different methods. Single locus estimations (t_s) were based on the method of SHAW and ALLARD (1982b), using data of megagametophyte-embryo pairs within progeny arrays of homozygote maternal trees. Multilocus estimates (t_m) followed the method described by SHAW *et al.* (1981).

Chi-square analysis (k $\times$ m contingency table) was used to detect any existing heterogeneity between subsets. Concerning the outcrossing estimates for each group of homozygote maternal trees the individual proportions homozygote to heterozygote progenies were compared. Single trees that caused heterogeneity within their group were omitted for estimating outcrossing rates.

Table 1. — Data characterizing the stands of *Abies alba* and their seed samples.

Stand No.	Provenance	Latitude	Longitude	Elevation (m)	Average stand age	Species % Abies alba	Weight per 1000 seeds(g)	% empty seeds
11, 11a	Ellwangen-Ost	49°00'	10°11'	520	130	35	45 (7)	33 (12)
2a	Calw-Hirsau	48°40'	8°48'	540	135	49	51 (5)	33 (16)
2b	Bad Teinach	48°42'	8°34'	740	114	25	44 (7)	28 (9)
1b	Ottenhöfen	48°33'	8°10'	630	100	45	45 (7)	29 (12)
1a	Oberkirch	48°31'	8°12'	710	110	41	46 (7)	28 (8)
1c	Gengenbach	48°27'	8°07'	680	125	60	45 (8)	28 (12)
14a	Ravensburg	47°49'	9°29'	670	125	25	48 (7)	22 (10)
14b	Überlingen	47°44'	9°25'	630	115	30	49 (8)	31 (13)

Standard deviations are given in parentheses.

Table 2. — Allele and heterozygote frequencies, WRIGHTS fixation index F, single locus (t_s) and multilocus (t_m) estimates of outcrossing rate in 9 populations of *Abies alba*.

Stand No.	Open pollinated families/ Seeds analyzed	Frequency of the less common allele IDH	6-PGD	Frequency of heterozygote offspring IDH obs.	exp.	6-PGD obs.	exp.	Wright's fixation index F IDH	6-PGD	Single locus outcrossing rate t_s IDH	6-PGD	Multilocus outcrossing rate t_m
11	16/640	0.24	0.36	0.34	0.36	0.37^{xx}	0.46	0.06	0.20	0.53(0.27)	0.81(0.15)	0.76(0.23)
11a	26/260	0.25	0.46	0.35	0.37	0.50	0.50	0.05	0	0.87(0.41)	1.03(0.39)	0.97(0.39)
2a	20/200	0.28	0.29	0.36	0.40	0.45	0.41	0.10	-0.10	0.81(0.41)	1.04(0.60)	0.93(0.43)
2b	26/260	0.34	0.36	0.45	0.45	0.44	0.46	0	0.04	0.83(0.37)	1.09(0.49)	1.00(0.38)
1b	37/370	0.29	0.34	0.39	0.41	0.44	0.45	0.05	0.02	0.84(0.42)	1.06(0.40)	0.97(0.37)
1a	33/330	0.31	0.39	0.35^{x}	0.43	0.42	0.48	0.19	0.13	0.69(0.57)	0.70(0.29)	0.68(0.38)
1c	31/310	0.28	0.30	0.38	0.40	0.45	0.42	0.05	-0.07	0.87(0.49)	1.01(0.44)	0.91(0.46)
14a	38/380	0.42	0.34	0.36	0.34	0.44	0.44	-0.06	0	0.77(0.25)	0.93(0.40)	0.91(0.38)
14b	24/960	0.47	0.22	0.48	0.50	0.33	0.33	0.04	0	0.85(0.20)	0.65(0.29)	0.89(0.26)
							arithmetic mean:	0.05	0.02	0.78	0.92	0.89

Standard deviations are given in parentheses.
Rejection of the null hypothesis that the observed frequency corresponds to the panmictic value
*) at the 5% level
**) at the 1% level

The data were analyzed in a model for multiple linear regressions to determine whether there are correlations among seed and other single tree characteristics.

Results

Expected and observed heterozygote frequencies of the analyzed offspring are given in *table 2*. In general at both loci there is a very good agreement to the expected HARDY-WEINBERG-frequencies. Only two cases of significant deviation were found (deficits in provenances no. 1a and 11 at one locus each).

WRIGHT's fixation indices, also known as inbreeding coefficients, were found to be homogeneous among populations and ranged between −0.10 and 0.20 with mean values of 0.05 for IDH and 0.02 for 6-PGD (*table 2*).

The discernible outcrossed offspring in general gave no significant values for heterogeneity within populations. This is not true for population 14b that showed a higher degree of heterogeneity. To estimate outcrossing values the heterogeneity causing trees were omitted. All outcrossing estimates were found to be heterogeneous over the nine populations. Nevertheless mean values are given (*table 2*).

Single locus estimates of outcrossing rates (t_s) varied considerably between loci and stands (*table 2*) and ranged from 0.53 to 1.09. Moreover, a very wide variation was found between individual trees (see standard deviations). The gene locus 6-PGD used to give higher values for t_s as the IDH locus. The data of the 9 populations resulted in a mean t_s of 0.85 for both loci.

Multilocus estimates of outcrossing rates (t_m) gave less between-stand variation (0.68 to 1.00) but again a considerable variation between single trees. Populations 1a and 11 differ from the other stands in showing markedly lower t_m values. This was also found by single locus estimation. The mean value for t_m is comparable to the t_s mean: based on 184 single trees out of 9 populations a mean t_m of 0.89 is found.

Allele frequencies of ovule and pollen pools are given in *table 3*. With only one exception (provenance 2b) male and female gamete pools did not differ.

The seed characteristics weight per 1000 seeds and percent empty seeds again showed a high tree-to-tree variation that exceed the interpopulational variation (see *table 1*). For the seed crop of stand no. 11 those data were

Table 3. — Male and female allelic contributions to fertilizations monitored at the zygotic stage in *Abies alba*.

Provenance No.	No. seeds analyzed	IDH - B_2 ovules	pollen	x^2(1df)	6 - PGD - A_2 ovules	pollen	x^2(1df)
11	640	479	494	0.96	399	396	0.03
11a	260	196	196	0	144	137	0.38
2a	200	143	145	0.05	146	140	0.44
2b	260	153	188	10.44^{xx}	169	164	0.21
1b	370	266	260	0.24	250	238	0.87
1a	330	225	229	0.11	191	213	3.09
1c	310	228	219	0.65	227	206	3.38
14a	380	216	226	0.54	240	262	2.84
14b	960	434	466	2.14	764	742	1.49

Rejection of the null hypothesis that successful ovule and pollen pools do not differ in allelic frequencies
**) at the 1% level

not available. The mean values for the remaining 8 stands are 47 g/1000 seeds with 29% empty seeds. The observed levels of needle loss range between 0 and 75%. The characteristics seed weight, % empty seeds, t_m and % needle loss of single trees were compared on the populational level as well as for all 165 trees as a whole. No correlation of t_m to % needle loss and of weight per 1000 seeds to t_m were found. The weight per 1000 seeds gave a weak negative correlation to % needle loss ($r \leqslant -0.42$) for three out of eight populations. Yet on the whole (165 individuals) there was found no correlation. The same was true for the comparison % empty seeds to t_m: single populations showed weak correlations but on the whole this could not be confirmed.

Discussion

For several conifer species it is described that the portion of empty seeds increases with increasing levels of selfing (DIECKERT, 1964b; FRANKLIN, 1971 and 1974; KORMUTAK, 1985). In the present investigation both characteristics varied widely with the mean percentage of empty seeds being rather low (29%, range 8% to 76% among 233 single trees). However, we did not find any significant correlation to the corresponding outcrossing rates. MOULALIS (1986) as well found equal amounts of empty seeds after controlled pollinations with se.fing and outcrossing pollen in silver fir.

Severely damaged trees did not differ in their seed weights from more vital ones (criterion needle loss). This is in full agreement with the findings of STUTZ et al. (1987) in Norway spruce. Because only well fructifying trees had been harvested it can be assumed that if the vital and damaged trees allow cone production to a normal extent, then in that event the damage does not reflect in the seed.

Inbreeding is known to be reflected in a higher proportion of homozygotes among the progeny than expected under panmixia. Such deficits are reported for several forest tree species, e.g. for the genera *Fagus*, *Pseudotsuga* and *Pinus* (KIM, 1980; SHAW and ALLARD, 1982a; MUONA et al., 1987). In *Eucalyptus* a high level of homozygotes at the seedling stage was accompanied by a high selfing rate of 37% (PHILLIPS and BROWN, 1977). In the present investigation in general there were no deviations of heterozygosity from the panmictic expectation at the embryo stage. This is also reflected by the finding of low inbreeding coefficients.

Sexual asymmetries may cause an upward bias in heterozygote frequencies relative to panmictic proportions (ROUX, 1977; ZIEHE and GREGORIUS, 1981). The analysis of embryo-endosperm pairs in this investigation allowed the determination of male and female contributions to the successful mating events. The results do not indicate such an asymmetry. Together with mean outcrossing values of 0.85 (t_s) and 0.89 (t_m) the rate of inbreeding therefore does not seem to play an important role in *Abies alba*. However, the outcrossing estimates require further discussion.

Outcrossing values exceeding unity may indicate weak points of the estimation procedures. They have often been obtained and have been discussed by BROWN et al. (1985).

There is found a high tree-to-tree variation resulting in high standard deviations. Finding this we supposed the sample size of 10 seeds per single tree possibly to be too low to give reliable results. In order to test this for two populations (no. 11 and 14b) the sample size was increased to 40 seeds per tree. In fact this seemed to produce more precise results with lower standard deviations (see *table 2*).

However, the remaining individual differences are of considerable extent. In silver fir a wide intrapopulational variation in outcrossing rates obviously exists. For *Picea abies*, Douglas-fir and lodgepole pine this has also been reported (LUNDKVIST, 1979; SHAW and ALLARD, 1982b; EL-KASSABY et al., 1986; PERRY and DANCIK, 1986).

Another reason that may contribute to increasing variation may be spatial and/or temporal aggregations of alleles and genotypes within the population, the single locus estimation method being especially sensitive (SHAW et al., 1981; HAMRICK and SCHNABEL, 1985). In our estimations only insignificant pollen pool heterogeneities are involved. However, minor deviations as well may lead to an unsharpness.

A considerable interlocus heterogeneity of the t_s values has been found. This seems to be a general feature of the method and is also reported by BROWN et al. (1975), MITTON et al. (1981), CHELIAK et al. (1985) and others. Yet there is a good agreement of the t_s and t_m mean values presented here. This demonstrates that the sensitivity of the single locus method may be compensated to some extent by a sufficiently large sample size.

Estimates for t_m should be based on the analysis of numerous loci. Therefore further enzyme systems will have to be studied.

To our knowledge this is the first estimation of outcrossing rates in silver fir. Possibly the estimation procedures for t may not always produce totally correct absolute values. Yet they allow very well the comparison among tree species with respect to their outcrossing rates. With a mean t_m rate of 0.89 obviously *Abies alba* does not differ from other conifer species. The values reported are for *Pinus* 0.84 to 0.98 (MITTON et al., 1981, and others), for *Pseudotsuga* 0.90 to 0.93 (SHAW and ALLARD, 1982; EL-KASSABY et al., 1986), for *Picea abies* 0.85 (MÜLLER, 1976) and for *Picea mariana* 0.93 (BOYLE and MORGENSTERN, 1986). The high tree-to-tree variation may be caused by: 1) genetically (high inbreeders versus high outbreeders), 2) environmentally (microhabitats, microclimates), 3) genetically and environmentally and 4) artificially through experimental conditions (already discussed). In order to test if possibilities 1 to 3 are true it may be helpful to use the same trees for seed collections in several successive years.

At least we would like to emphasize a further aspect of this investigation: Outcrossing estimates varied widely between individuals as well as between populations. According to BROWN et al. (1985) one and the same population was found to show differing outcrossing rates in the seed crops of different years. Populations no. 11 and 11a of this investigation are in direct neighbourhood, partially overlapping. However, the outcrossing values of the seed harvests 1982 (no. 11) and 1986 (no. 11a) are very different (0.76 to 0.97 respectively). Stand no. 2b with high significance shows genetically different ovule and pollen pools, whereas all other stands have equal gamete allele frequencies. Therefore generalizations of estimates of mating system parameters to the species level should be done with great care.

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Cost-Effective Promotion of Flowering in a Douglas-Fir Seed Orchard by Girdling and Pulsed Stem Injection of Gibberellin A$_{4/7}$

By S. D. Ross*) and R. C. Bower**)

Abstract

Douglas-fir (Pseudotsuga menziesii) grafts of 5 cm to 14 cm diameter received different combinations of partial saw-cut stem girdles and ethanolic solutions of GA$_{4/7}$ injected into shallow holes drilled around the main stem. Grafts averaged 79 seed-cone buds and 4500 pollen-cone buds each without treatment. Girdling alone increased the tree production of seed- and pollen-cone buds to 325 and 9300, respectively. GA$_{4/7}$ alone was nearly as effective as girdling alone, the response being marginally greater at the high than low dosage of GA$_{4/7}$ (3.82 vs. 1.27 mg cm^{-2} of stem cross sectional area), but independent of whether the total dose was applied all at once or over two or three injections at 2-week intervals. Together, girdling and GA$_{4/7}$ had an additive effect on flowering, increasing the tree production of seed- and pollen-cone buds to 585 and 18,250. The combined treatment was particularly effective on smaller trees that flowered poorly or not at all without treatment, while also enhancing production significantly on larger trees. The combined treatment was safe and highly cost effective. It cost $ 63.75 per year to maintain each tree in the orchard, so that without any treatment the cost per seed-cone bud initiated was $ 0.91. Girdling (at $ 2.07 per tree) reduced this cost to $ 0.20, and girdling + GA$_{4/7}$ (at $ 7.87 per tree) to only $ 0.13.

Key words: Cone induction, Economics, Gibberellins, Girdling, Seed orchard, Pseudotsuga menziesii.

Introduction

Numerous studies have established the efficacy of exogenously applied gibberellin A$_4$ and A$_7$ mixture (GA$_{4/7}$) for promoting flowering in Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) (see Pharis et al., 1987). The treatment is particularly effective in conjunction with such cultural practices as girdling and rootpruning (Ross et al., 1985; Pharis and Ross, 1986; Pharis et al., 1987). However, owing to practical problems of applying the growth regulator, GA$_{4/7}$ may be occassionally used to accelerate breeding but seldom operationally to enhance cone production in seed

*) British Columbia Ministry of Forests, Research Laboratory, 1320 Glyn Road, Victoria, B. C., Canada V8Z 3A6

**) MacMillan Bloedel Ltd., Woodlands Services Division, 65 Front Street, Nanaimo, B.C., Canada V9R 5H9
Present address: MacMillan Bloedel Inc., P. O. Box 336. Pine Hill, AL 36769, USA