

Crossing Success in *Abies nordmanniana* Following Artificial Pollination With a Pollen Mixture of *A. nordmanniana* and *A. alba*

By O. K. HANSEN^{*} and U. B. NIELSEN

Danish Centre for Forest, Landscape and Planning, University of Copenhagen, Hørsholm Kongevej 11, 2970 Hørsholm, Denmark

(Received 15th November 2006)

Abstract

Controlled crossings were conducted with three *Abies nordmanniana* genotypes acting as mothers and a pollen mixture of three *Abies nordmanniana* genotypes and one *Abies alba* genotype acting as potential fathers. The aim was to investigate hybridization success under circumstances where pollen from both species are present, which is a potential risk in Danish clonal seed orchards of *Abies nordmanniana*. The number of seeds sired by each father was determined through SSRs and compared to the expected numbers based on the pollen mixture composition. All three mother genotypes of *Abies nordmanniana* had more progenies with the *Abies alba* as father (hybrids) than expected, based on proportions in the pollen mix. This indicates that no reproductive barriers between the two species exist, and that seed orchard managers should take precautions to avoid hybrids in seed crops. Furthermore, the experiments revealed quite different siring success of the three *Abies nordmanniana* genotypes, depending on which clone was the mother. *Abies nordmanniana* seed orchards should therefore not be established in the vicinity of *Abies alba* in the flowering age, and if a few-clone set up is chosen, the mating interaction should be investigated beforehand through pollen mix experiments.

Key words: *Abies nordmanniana*, *Abies alba*, hybridization, pollen mixture, seed orchard contamination.

Introduction

Interspecific hybridization is an important issue in many research disciplines such as taxonomy, ecology and conservation genetics (RHYMER and SIMBERLOFF, 1996; ARNOLD, 1997; RIESEBERG, 1997). In forest tree breeding, interspecific hybridization has traditionally been seen as a means to obtain desirable genotypes which could be useful in forestry (e.g. KLAHN and WINIESKI, 1962; WRIGHT, 1976; FOWLER, 1978), and several successful examples exist for conifers (FOWLER, 1978). However, in other forest tree improvement cases interspecific hybridization is highly undesirable. The present study deals with such a situation.

Abies nordmanniana (Stev.) Spach, originating from the Caucasian region in Southern Russia, Eastern Georgia and North-eastern Turkey, and *Abies alba* Mill., originally distributed in central and southern Europe (LIU, 1971), are widely used exotic conifers in Denmark. *A. nordmanniana* is used mainly for Christmas tree and greenery production on farmland and, to a lesser extent,

on forest sites while *A. alba* is used for timber production in forests. The use of *A. nordmanniana* for Christmas tree production in Denmark has increased considerably in the last 10–15 years peaking around 2004 with 10–11 mill. trees sold per year (Kaj Østergaard pers. comm.). The industry is mainly based on imported material (DITLEVSEN and NIELSEN, 1997), but seeds for Christmas tree production have been harvested in Danish stands of *A. Nordmanniana* for years as well. Furthermore, a Danish breeding programme for *A. nordmanniana* was started in the early 1990s with the aim of producing improved planting material for the production of Christmas trees (NIELSEN, 1994). Seed orchards made of progeny tested clones were established in this programme; they are expected to produce seeds in commercial amounts in a few years (NIELSEN, 2000).

Danish Christmas tree growers have been aware of hybridization between the two species for a long time, based on phenotypic observations (JACOBSEN, 1988). The hybrids are considered less suitable as Christmas trees, having tendencies to give 'open' trees because of vigorous growth, flatter foliage structure, poorer post harvest needle retention and earlier bud break (JACOBSEN, 1988; NIELSEN and CHASTAGNER, 2005). The latter gives increased risk of frost damage which is unacceptable in Christmas trees. Artificial hybridization experiments have confirmed this ability of *A. nordmanniana* and *A. alba* to hybridize (KORMUTAK, 1997). Thus, *A. alba* is present in forest stands at variable distances from the orchards, and at the same time it is well known that some species show long distance transportation of pollen and contribute substantially to background pollination in seed orchards (PAKKANEN et al., 2000; PARANTAINEN and PULKKINEN, 2003). Consequently a closer investigation of hybridization between the two species seemed important for *A. nordmanniana* seed orchard management.

Normally, hybridization among species is investigated through the use of controlled crossings with one species used as mother and the other as father (pollen donor). These settings, however, are quite far from the reality the seed producing trees experience in the seed orchards. In *A. nordmanniana* orchards, the air is expected to contain mostly pollen of this species and perhaps some *A. alba* pollen from surrounding forests. Therefore, it seems to be more relevant to look at how easily hybridization takes place when pollens from both species are present than to carry out pure interspecific crossings. Our protocol was inspired by LINDGREN and

^{*} Correspondence and reprints: Tel. +45 35 33 16 47, Fax: +45 35 33 15 17, E-mail: okh@life.ku.dk.

YAZDANI (1988) and others (e.g. NAKAMURA and WHEELER, 1992a), who used pollen mixtures with known proportions of pollen from different genotypes and afterwards studied the resulting progeny. However, besides mixing pollen from different genotypes of the same species, we also included pollen from another hybridizing species. The composition of the offspring will allow us to estimate the rate of hybridization between *A. nordmanniana* and *A. alba* under circumstances which are likely to be found in the operational seed orchards of *A. nordmanniana*.

Material and Methods

In general, in order to be sure of adequate sample sizes (constrained by limited resources) only a few genotypes were selected for the study. Also in the preparation of the pollen mix (see below), statistical considerations supported by power analysis led to the chosen composition with 50% of *A. alba* pollen, even though this is not likely to be found in the pollen cloud of an *A. nordmanniana* seed orchard.

Collection, extraction and storage of pollen

Branches with male strobili were collected from three *A. nordmanniana* ortets (selected plus-trees included in the breeding programme) in the approved Danish seed stands F.526 and F.527 and a second generation stand originating from F.525 (located respectively in Tversted Dune forest, departments 599 and 623 and Lilleheden dept. 852c). The branches were collected on May 13, 2003, and pollen extraction started the day after by placing twigs with male strobili in terylene bags at 25°C and letting them shed pollen through a combined filter and funnel into glass ampoules. Shedding normally takes place within few days. *A. alba* pollen originated from a tree standing in the Arboretum in Hørsholm (Accession no. 1959.4023 – originating from Delnice in Croatia) and was treated in the same way as the *A. nordmanniana* pollen. After extraction the pollen lots were freeze dried in order to improve storage and homogenize pollen moisture content. The pollen was then stored at minus 20°C until May 2004, when the pollen mixtures were prepared by the use of a balance.

Pollen viability was tested using a modified Brewbaker and Kwack's solution (BREWBAKER and KWACK, 1963; WEBBER and PAINTER, 1996). After 48 hours at 26°C the numbers of swollen and germinating pollen grains were counted and the percentage of viable pollen was calculated for each potential father. Furthermore, the size of the pollen grains of the different sources was measured under a microscope with 400x magnification. Pollen testing and measurements were carried out a year after pollination, on the same pollen lots as those used in pollination.

Seed orchard and pollination procedure

The pollinations were conducted in the clonal seed orchard (CSO) FP259, established in 1993–94 in Silkeborg State forest District (Jutland). It consists of clones selected in the approved Danish seed stands F.526 and F.527 (1st generation from directly imported seeds) and

in the seed stand Lilleheden dept. 852c (2nd generation). All the clones have been selected for foliage appearance. The possibility of having hybrids in the 2nd generation material (Lilleheden) is therefore highly unlikely since hybrids have flatter foliage structure with an unattractive appearance.

Female flowers were isolated on 27–29th April 2004, using terylene bags and water repellent cotton wool. Flowers were isolated on clones of three different genotypes (one ramet per clone), chosen for their high number of female flowers. Two genotypes originated from Lilleheden dept. 852c and one from F.526 (Tversted). Ten terylene bags, typically isolating two or three female flowers, were put on each of the three clones. Female flowers were pollinated twice on May 4 and 6 with a pollen mixture. The mixture was made of 50% of *A. alba* pollen and 15.7% of pollen from each of the three *A. nordmanniana* plus-trees – based on weight. One single glass ampoule containing the pollen mixture was used on each of the pollination days meaning that all three mother clones were exposed to exactly the same pollen mixture. The bags were removed on June 6 and the cones were harvested on September 28. The cones collected from each clone were pooled together and the three cone crops were kept separate. Then, the seeds were extracted manually, dried, cleaned and kept at 5°C until stratification and germination.

DNA extraction

Seed stratification started in the beginning of February 2005. The seeds from each lot were wrapped in wet paper, placed in plastic bags and kept refrigerated at 5°C. Normally *A. nordmanniana* seeds need 3–6 weeks of stratification before they start to germinate (NYHOLM, 1986), but our seeds did not start to germinate before the 9th week. The germinating seeds were then removed successively from the refrigerator and kept for ten days at 15°C, allowing the embryos to elongate faster. The elongated embryos were cut out of the seeds and DNA was extracted using the DNeasy Plant Mini Kit from QIAGEN (Germany). Mother and father clones were characterized using DNA extracted from needles.

Parent and progeny genotyping

The three mother clones, the four pollen donors and all viable progeny in the three seed lots were genotyped with microsatellites (SSRs) recently developed in *A. nordmanniana* (HANSEN et al., 2005). On the whole, 225 seeds were genotyped, including 67, 80 and 78 seeds from the three mother clones C26, C91 and C94 respectively. Among the five SSRs available, two were sufficient (NFH3 and NFH15) to identify the pollen donors of the progenies. The microsatellites were multiplexed using the QIAGEN[®] Multiplex kit (catalogue no. 206143). PCR conditions followed the standard multiplex PCR protocol given in the instructions of the QIAGEN multiplex Kit and amplifications were carried out in a Perkin Elmer thermal cycler (model 9700). Fragment sizes of the amplified labelled SSRs were determined using the CEQ 2000XL Fragment analysis system from Beckman Coulter.

Statistics

For each mother tree a chi-square goodness of fit test was performed (SNEDECOR and COCHRAN, 1980), to compare actual and expected contributions of the pollen donors ($df = 3$). In order to test for differences in viable seed production ability between *A. nordmanniana* and *A. alba* pollen, the seeds sired by the three clones of *A. nordmanniana* were pooled and a chi-square test ($df = 1$) was performed on the number of progenies obtained from each species. This analysis was carried out at two levels: i) for each mother tree separately and ii) globally after pooling the data obtained in the three mother trees. Differences in pollen viability and size were tested using a standard ANOVA.

Results

The genotypes of the potential parents are shown in *Table 1*. The exceptionally high power of discrimination of the applied markers was emphasized by the fact that only one parent (*A. nordmanniana* C94) was homozygous – and only for one of the two microsatellites. Only alleles which exist in the potential parents were found in the seeds analysed, giving strong evidence that no pollen-contamination had occurred, and confirming that female flowers had been isolated in proper time.

The results of the paternity analysis are indicated in *Table 2* and illustrated in *Figure 1*. The *A. alba* pollen contributor in each seed lot has produced more than 50% of the offspring, even though its pollen only consti-

Table 1. – Parental genotypes given in base pairs for each allele in the two microsatellites and pollen size and viability of the four potential fathers. Pollen sizes and viability percentages followed by different letters are significantly different (5% level).

	Origin	Microsatellite: NFH15		NFH3		Pollen size / mm			Pollen viability
		Allele1	Allele2	Allele1	Allele2	Mean	Std	CV	Mean
Fathers									
<i>A. alba</i> Delnice	Croatia	107	122	105	120	0.0959 ^a	0.0039	0.04	58.6 % ^{ab}
<i>A. nord.</i> C19	F.526	103	105	127	161	0.0964 ^a	0.0049	0.05	48.6 % ^a
<i>A. nord.</i> C46	F.527	103	113	125	137	0.0975 ^a	0.0050	0.05	62.3 % ^b
<i>A. nord.</i> C81	F.525	99	105	118	124	0.1041 ^b	0.0057	0.05	78.9 % ^c
Mothers									
<i>A. nord.</i> C26	F.526	101	113	120	140				
<i>A. nord.</i> C91	F.525	103	105	118	131				
<i>A. nord.</i> C94	F.525	103	105	125	125				

Table 2. – Numbers of seeds sired by each father clone in three clones of *Abies nordmanniana* (C26, C91 and C94). (a) Expected proportion of seeds from each pollen donor. (b) Observed number and percentage of seeds from each pollen donor in each of the three seed lots. (c) Observed and expected number of seeds – pooled results from all three seed lots – observed also given in percentage. (d) Expected number of seeds for each pollen donor, adjusted for differences in pollen size. (e) Expected number of seeds for each pollen donor, adjusted for differences in pollen viability. (f) Expected number of seeds for each pollen donor, adjusted for differences in both pollen size and viability.

		Fathers					
		<i>A. alba</i> - Delnice	<i>A. nord.</i> C19	<i>A. nord.</i> C46	<i>A. nord.</i> C81	Total	
Seedlot	Proportion in pollenmix	½=50%	1/6=16.7%	1/6=16.7%	1/6=16.7%	1=100%	(a)
SLC26	# seeds / seeds in %	39 (58%)	4 (6%)	6 (9%)	18 (27%)	67	(b)
SLC91	# seeds / seeds in %	54 (67%)	8 (10%)	11 (14%)	7 (9%)	80	(b)
SLC94	# seeds / seeds in %	46 (59%)	14 (18%)	17 (22%)	1 (1%)	78	(b)
Overall	# seeds/ seeds in %	139 (62%)	26 (12%)	34 (15%)	26 (11%)	225	(c)
	Expected number (pollen weight)	112.5	37.5	37.5	37.5	225	(c)
	Expected number/ percentage (number of pollen grains)	118.3 (53%)	38.6 (17%)	37.4 (17%)	30.6 (13%)	225	(d)
	Expected number/ percentage (pollen viability)	108.2 (48%)	29.9 (13%)	38.4 (17%)	48.6 (22%)	225	(e)
	Expected number/ percentage (no. of pollen grains + viability)	115.0 (51%)	31.2 (14%)	38.7 (17%)	40.1 (18%)	225	(f)

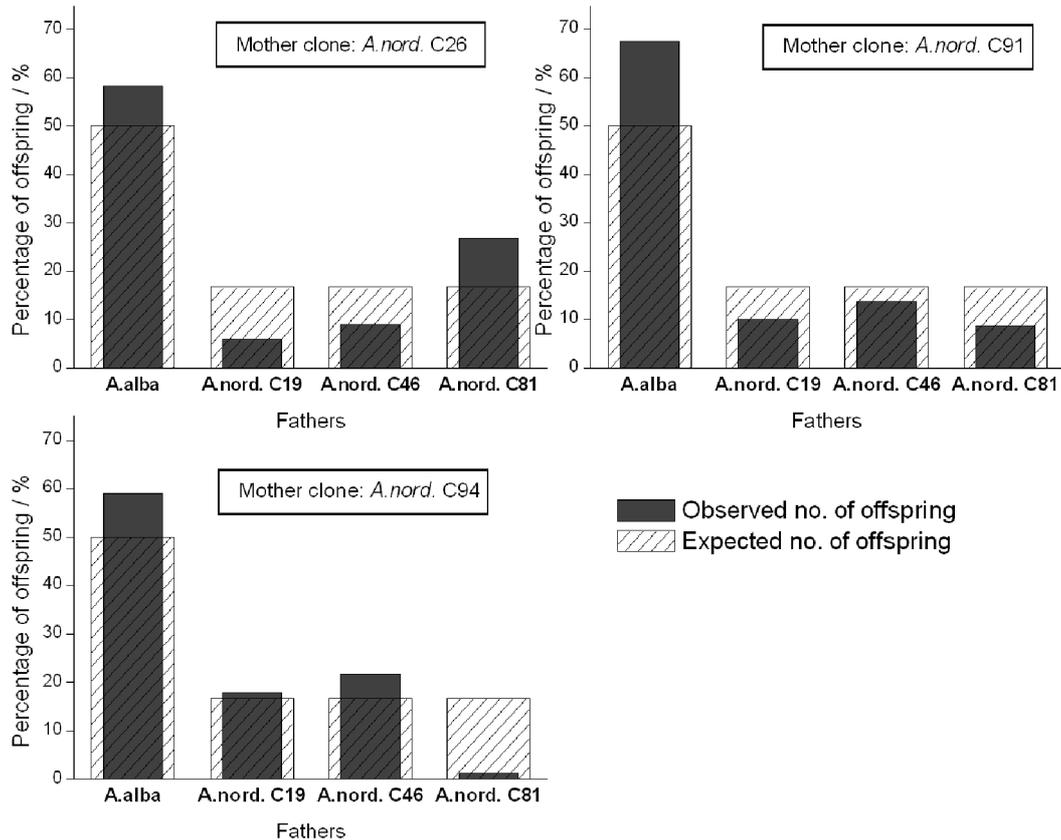


Figure 1. – Observed and expected numbers of seeds sired by different male parents after pollination of three clones of *Abies nordmanniana* (C26, C91 and C94). Expected numbers are equal to the relative proportions (weight:weight) of the fathers in the pollen mixture.

tuted 50% of the pollen mixture. What is further striking is the difference in paternal success among the *A. nordmanniana* pollen contributors, when comparing the three seed lots. Thus *A. nordmanniana* C81 only sired one single seed in seed lot SLC94 (instead of the 13 seeds expected), while it sired 18 seeds in seed lot SLC26, where the expected number was 11. In all three seed lots, the number of progenies per father significantly differed from that expected on the basis of the pollen mixture composition (chi-square goodness of fit test with $df=3$, SLC26: $p=0.007$; SLC91: $p=0.015$ and SLC94: $p=0.003$). The causes of these significant deviations vary from one seed lot to another. In SLC26 the biggest contribution to the chi-square test statistic comes from the higher than expected number of *A. nordmanniana* C81 progenies and the fewer than expected progenies from *A. nordmanniana* C19, while the biggest chi-square contribution in SLC91 is due to the high number of *A. alba* progenies (67.5% against the expected 50%). For SLC94 the significant deviation is mostly due to the exceptionally low number of progenies with *A. nordmanniana* C81 as father.

Pooling the offspring from the three *A. nordmanniana* donors and comparing them to the number of *A. alba* progenies showed that despite a higher than expected number (>50%) of *A. alba* progenies in the three seed lots, this higher proportion was only significant in SLC91. ($p=0.179$, $p=0.002$, $p=0.113$ in SLC26, SLC91 and SLC94 respectively). Nevertheless, the overall chi-

square goodness of fit test, comparing the total of 139 *A. alba* progenies in the three seed lots with the 86 having a *A. nordmanniana* father, was highly significant ($p < 0.001$).

Pollen sizes as well as pollen viability of the four potential fathers were measured to test for significant differences and, if any, to be able to adjust the number of expected progenies from each father. Significantly different pollen sizes would imply that e.g. a father with small pollen grains would have relatively more pollen grains in the mixture than indicated by its proportion in weight. Likewise, fathers with significant more viable pollen would have relatively more offspring than indicated by weight proportions in the pollen mixture. In fact, one father (*A. nordmanniana* C81) had significantly larger pollen than the others (Table 1). An adjusted number of expected progenies based on the number of pollen grains of each father in the mixture was calculated assuming that pollen grains were spherical (Table 2 – row d). This adjustment resulted in a reduction of the gap between the observed and expected numbers of progenies, but the difference was still significant ($p=0.005$). The same adjustment used for the data in each cross (results not shown) only led to minor changes in the chi-square tests, and did not change the overall conclusions. Pollen viability also showed a significant variation among potential fathers. *A. nordmanniana* C81 had the highest pollen viability (79% – Table 1) while *A. nordmanniana* C19 had the lowest with 49%

viable pollen. As for pollen size, an adjusted number of expected progenies based on pollen viability of each father in the mixture was calculated (Table 2 – row e). Again, this did not change the overall conclusions much, even though the pollen viability had quite a different impact on expected number of progenies compared to pollen size. For example, *A. nordmanniana* C81 had only an expectation of 13% offspring due to large pollen size, but an expectation of 22% offspring when pollen viability was considered alone. A combined adjusted number, based on both number of pollen grains and viability was also calculated, giving intermediate expectation values compared to the two former adjustments (Table 2 – row f). In all three cases of adjustment, the expected proportion of *A. alba* progenies was close to 50% – the weight proportion in the pollen mix which was the starting point.

Discussion

Overall our study had two key findings: 1) The success of *A. nordmanniana* pollen parents with *A. nordmanniana* females showed considerable variation depending on the male/female combination and 2) A higher mating success of the *A. alba* father (interspecific cross) compared to the *A. nordmanniana* fathers (intraspecific cross).

Mating success variation across different A. nordmanniana male/female combinations

A strong interaction of pollen donors with seed parents are of high practical interest to the Danish *A. nordmanniana* breeding programme. In the production of Christmas trees, using an exotic conifer in short rotation, concern about genetic diversity is not so relevant. A straight forward strategy could therefore be to establish clonal seed orchards with a very limited number of highly selected clones in order to maximize genetic gain (HANSEN and KJÆR, 2006). However, if setting up orchards with e.g. only 3–4 clones, it would be highly desirable to know whether these clones can actually mate and produce viable seeds.

A strong interaction among pollen donors and seed parents was found by APSIT et al. (1989) in crosses with two-parent pollen mixes in a Douglas fir seed orchard. They called this interaction male-female complementarity. Later this male-female complementarity was confirmed in additional studies of Douglas fir (NAKAMURA and WHEELER, 1992ab), but otherwise the literature does not report about this phenomenon in other conifers. A commonly mentioned reason for differential male reproductive success is pollen competition (e.g. ARONEN et al., 2002), which can be influenced by a whole range of factors involved in the striving of pollen grains to fertilize the same ovules (STEINER and GREGORIUS, 1999). This includes pollen viability which we tested, although the assay was conducted one year after pollination. The significant differences in pollen viability found among the four pollen donors, however, can not explain the interaction among the three *A. nordmanniana* and neither the higher mating success of the *A. alba* donor, since the latter only had the third-highest pollen viability.

While pollen competition is a prezygotic event, acting on the haploid male gametophytic level, another factor, often regarded as more important, is postzygotic embryo abortion (MORAN and GRIFFIN, 1985; NAKAMURA and WHEELER, 1992a). This selection process is possible since polyembryony is common in gymnosperm species but only one embryo in each ovule normally develops to maturity (DOGRA, 1967). In conifers this means that several genetically identical archegonia within the same ovule can be sired by pollen from different fathers (HEDRICK et al., 1999). It is quite plausible that polyembryony can be the cause of the strong interaction we observed, simply because the embryos in some mother trees may be more prone to abortion when fertilized by certain pollen donors. However, the approach we used gives no possibility to test this hypothesis, and only few empirical studies have done so. Through controlled pollinations in a seed orchard O'CONNEL and RITLAND (2005) tested whether polyembryony can decrease seed abortion or the proportion of selfed seedlings in *Thuja plicata*. They could not find an increase in seed set attributable to polyembryony, but did actually observe that trees receiving high ratios of self-pollen produced fewer selfed seedlings than expected, which suggest embryo competition.

Finally, one might imagine that the mating success interaction among pollen donors and seed parents in our study could be caused by differences in relatedness among the tested individuals, either at provenance or family level. At provenance level, barriers to crossing between provenances could potentially give interaction. This seems, however, highly unlikely, as our three *A. nordmanniana* pollen donors in the pollen mix came from three different seed sources (Lilleheden, F.526 and F.527) and the three seed parents trees originate from Lilleheden (C91 and C94) and F.526 (C26). Looking at the results (Table 2), it is seen that when the pollen donor and the seed parent were from the same seed source they had the lowest mating success in all three cross combinations. Hypothetically this could be caused by relatedness at family level, where close relationship among pollen donor and seed parent may cause inbreeding and by that lower mating success. Again, this seems not plausible; all three seed sources originally had thousands of trees, and the chance that we have included close relatives in all three of them is minimal.

Higher mating success of the interspecific cross

The ability of *Abies* species to hybridize has not been as extensively investigated as in other genera of Pinaceae such as *Pinus* or *Picea* (CRITCHFIELD, 1988). A few studies were made in the 1960's (KLAHN and WINESKI, 1962; MERGEN et al., 1964) which gave the impression that there is little difficulty in hybridizing fir species and that no reproductive barriers to crossing between individuals of different geographic regions exist (MERGEN et al., 1964). Later studies in the 1980's by HAWLEY and DEHAYES (1985), ST. CLAIR and CRITCHFIELD (1988), CRITCHFIELD (1988) and KORMUTAK (1997) modified this view, and e.g. indicated a strong reproductive isolation between the North American members of the genus and their European counterparts (CRITCHFIELD,

1988; KORMUTAK, 1997). HAWLEY and DEHAYES (1985) studied crossability among seven North American firs, where crossability was estimated by average viable seed yields of intertaxon relative to intrataxon crosses. They found that most crosses among species from the same section (here Balsameae) yielded a percentage of viable seed which was not significantly different from that of intrataxon crosses, and therefore appear to be fully crossable. Most intersectional crossing, however, yielded a low percentage of viable seed and accordingly reflected restricted crossability (HAWLEY and DEHAYES, 1985). This pattern with high crossability within groups but difficult or impossible crosses between groups was also supported by CRITCHFIELD (1988) who studied hybridization of California firs.

Our study with *A. nordmanniana* and *A. alba*, both in the *Abies* section (LIU, 1971), is not fully comparable with the above cited studies. We used pollen mix containing several genotypes and two species, in contrast to the earlier reported crosses with no possibility for pollen competition etc. Nevertheless, our results, where the intertaxon combination of seed parent and pollen donor had higher competitiveness in relation to mating success, strongly suggest full crossability between *A. nordmanniana* and *A. alba*, even though only one *A. alba* genotype was tested. This general trend in all three crosses could hypothetically be explained by different effects of storage on the pollen viability; say the *A. alba* pollen had lost more viability during the year from the crossing experiment until the pollen viability was measured. We have, though, no reason to believe so. Also, a few other reports about higher intertaxon success in *Abies* exist. KORMUTAK (1992) found higher efficiency of interspecific hybridization between *A. concolor* and *A. grandis* compared to intrataxon crosses, resulting in a 170% degree of crossability. Likewise, HAWLEY and DEHAYES (1985) observed that intrasectional crosses involving *Abies fraseri* pollen and female parents of *Abies balsamea* var. *phanerolepis* almost always produced more viable seed than the intrataxon controls.

In relation to management of seed sources of *A. nordmanniana* the present study makes it possible to draw the following conclusions: 1) The earlier reported crossing compatibility between *A. nordmanniana* and *A. alba* has been verified, and extended to conditions where pollens of both species are present. 2) Although only one specimen of *A. alba* has been tested, our results are in line with other studies which have reported high within section crossability in *Abies* species and 3) There seems to be large interaction among pollen donors and seed parents of *A. nordmanniana*, which may have importance in relation to the establishment of few-clone seed orchards.

A. alba generally has earlier vegetative bud break than *A. nordmanniana* material of central Georgian origin (LØFTING, 1973). However, this difference in timing might not hold true for reproductive buds, and a substantial overlap in flowering period is a potential risk. Seed source managers could have hoped, that reproductive barriers would make interspecific crosses relatively less frequent than intraspecific crosses, but our results do not support that hope. On the contrary, the present

study shows that an *A. alba* tree can be just as competitive during pollination (and even more so). Therefore, managers have to take the risk seriously, e.g. by removing *A. alba* trees in the flowering age standing in the vicinity of the seed source, or by establishment of clonal seed orchards on agricultural lands, away from forested areas.

The issue about hybridization between *A. alba* and *A. nordmanniana*, together with the strong interaction in siring success observed among fathers of *A. nordmanniana*, should be further explored in future crossing experiments.

Acknowledgments

We thank ERIK D. KJÆR for helpful comments and inspiring discussions in relation to the pollination work. We also thank an anonymous reviewer for constructive suggestions to the manuscript, the Arboretum in Hørsholm for free use of the collection, KRISTIAN STOUSGAARD JACOBSEN for help with seed handling and JERRY W. LEVERENZ for valuable corrections of the language. Finally we are grateful to "Foreningen Plan-Danmark" who supported the presented work financially.

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Spontaneous Hybridization between *Pinus sylvestris* L. and *P. mugo* Turra in Slovakia

By A. KORMUTAK^{1,*}, B. DEMANKOVA¹ and D. GÖMÖRY²

(Received 28th November 2006)

Abstract

Molecular evidence for spontaneous hybridization between *Pinus sylvestris* L. and *P. mugo* Turra in the putative hybrid swarm populations of the species in Slo-

vakia was provided based on PCR-RFLP analysis of the cpDNA *trnV-trnH* region. Species-specific restriction profiles generated by *Hinf* I digests of the cpDNA products reliably identified *P. sylvestris* and *P. mugo* haplotypes of the embryos from open pollination. Simultaneous analysis of the respective cpDNA region in megagametophytes and embryos of individual seeds along with needles of a given maternal tree has enabled to score either the *P. sylvestris* or *P. mugo* haplotypes in the embryos illustrating hybridization patterns between the two species. Data obtained in this way indicate a

¹) Institute of Plant Genetics and Biotechnology, Slovak Academy of Sciences, Akademická 2, P.O.Box 39A, SK-950 07 Nitra, Slovak Republic.

²) Technical University in Zvolen, Faculty of Forestry, T. G. Masaryka 24, SK-960 53 Zvolen, Slovak Republic.

*) Corresponding author: E-mail: nrgrkorm@savba.sk