[1]POLLINATION EFFECTS IN A LARCH HYBRID SEED ORCHARD[1]

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ABSTRACT

Seed from a hybrid seed orchard, consisting of 4 clones of *Larix decidua* Mill. and 1 clone of *Larix kaempferi* [Lamb.] Carr. was investigated by isoenzyme analysis. The gene locus *Skdh–A* was used as a genetic marker for the identification of hybrid seed and the partly differentiation between the clones of *L. decidua*. From each clone, seed was harvested from 9 ramets, separately for the southern and the northern crown part. In this material, the proportion of full seeds, the proportion of hybrids and the frequency of the alleles were determined. The results allow the use of the seed from the *L. decidua* clones as hybrid seed. The use of seed from the *L. kaempferi* clone cannot be recommended for the investigated crop. The necessity of testing of the seed from hybrid seed orchards before use is shown. Beside that the results refer to some important aspects of the orchard design.

Key words: *Larix decidua*, *Larix kaempferi*, hybrid seed orchard, isoenzyme analyses

INTRODUCTION

European larch (*Larix decidua* Mill.) is not a natural component of the forest communities in Saxony. Nevertheless, this species has been planted in forestation and finds suitable growing conditions especially in the lower and middle elevations of the mountainous regions of Saxony.

In the beginning of the fifties, intensive breeding activities have been started in the genus *Larix* by means of interspecific hybridisation. It was well known, that hybrids mainly between the species *Larix decidua* and *Larix kaempferi* [Lamb.] Carr. show a heterotic growth and a high vitality (DENGLER 1941, LANGNER 1952).

Of high importance was the fact, that the combining ability of the crossing parents is a decisive factor for the success of the crossings (SCHÖNBACH 1967). Beside wood production, stem form, vitality and disease resistance, the aims of breeding in Saxony were a high tolerance in respect to air pollution and to the special climatic conditions in the mountain regions. In 1968, breeding results led to the first proposal for approval of a tested hybrid variety (SCHÖNBACH & HAASEMANN 1968). In 1969, a hybrid seed orchard consisting of 5 tested clones of *Larix decidua* and *Larix kaempferi* was established.

In the following years, a considerable number of hybrid combinations have been produced and tested (HAASEMANN 1972, HERING *et al.* 1989, HERING 1994). For mass production of hybrid material with desirable traits, several hybrid seed orchards have been established.

An essential question for the meaningful use of crops from seed orchards is to estimate the composition of the seed lots. In the genus *Larix*, isozyme markers were applied in several studies to identify the hybrids and to determine their proportion in the seed (BERGMANN & RUETZ 1987, BRAUN *et al.* 1990, HÄCKER & BERGMANN 1991, BURCZYK *et al.* 1997). They were also used to describe the mating system in intraspecific seed orchards and in stands as well as for the estimation of outcrossing rates (LEWANDOWSKI *et al.* 1991, GÖMÖRY & PAULE 1992). Áýžťčšľltťddčč

Seed of the hybrid seed orchard investigated in this study was analysed by BRAUN *et al.* (1990) from an earlier crop. The aim of the present investigation is the estimation of the proportion of hybrids in the seed in relation to the spatial distribution of the mother trees in the orchard and in comparison to the results of BRAUN *et al.* (1990).

MATERIAL AND METHODS

The seed orchard investigated was established in 1969 in Graupa, forest district Lohmen, on an area of 2.4 ha. It is located on podsolic brown soil at an elevation of

[1)] This paper was presented at the International Conference held at the occassion of 100[th] anniversary of the foundation of forestry research in Slovakia on October 5, 1998.

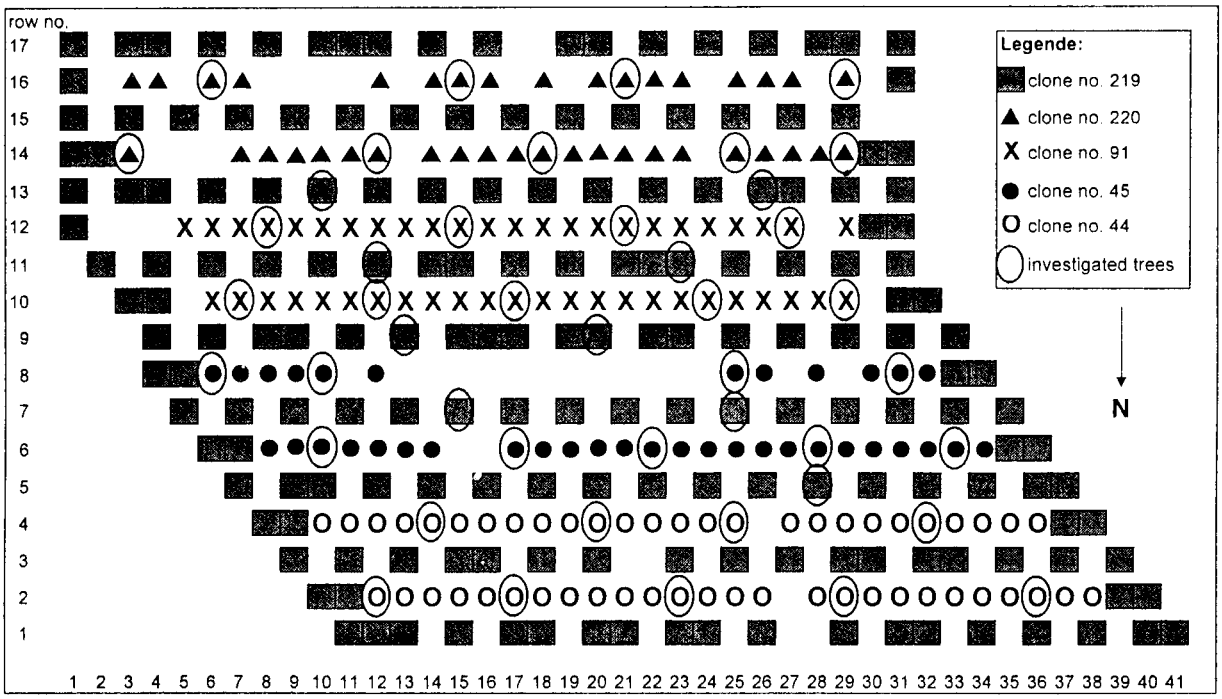

Figure 1. Design scheme of the larch hybrid seed orchard Graupa.

130 m a. s. l., with an annual mean temperature of 9.1°C and annual mean precipitation of 682 mm.

The orchard consists of 5 clones, 4 of them belonging to the species *Larix decidua* (no. 220, 91, 45, 44) and one to *Larix kaempferi* (no. 219). The clones were chosen according to their general and specific combining ability. Their progenies with different partners in general, as well as with this specific Japanese larch clone achieved results above average in progeny tests.

Another aspect for the selection of the clones were phenological observations. The clones of *L. decidua* no. 44, 91 and 220 are flowering almost in the same time. The culmination point of clone no. 45 occurs a bit later, while the clone no. 219 (*L. kaempferi*) is slightly earlier in male as well as in female flowering. In general, for normal years the flowering periods were found to overlap sufficiently.

The grafts of each European larch clone are planted in two rows, which alternate with one row of the grafts of the *L. kaempferi* clone (219) (Figure 1). The whole orchard is surrounded by grafts of the clone no. 219. The spacing in the orchard is 4 × 7 m, the rows are directed from east to west.

In 1996, after abundant flowering a abundant seed crop was obtained from this orchard. From each clone of *L. decidua*, 9 regular distributed grafts were chosen. From the clone no. 219, 9 grafts were chosen in the central part of the orchard. From all of these selected trees, 20 cones from the northern and 20 from the southern part of the crowns were harvested separately. Seeds were extracted of the cones by hand. The number of full and empty seeds were registered during the preparation of the embryos for the analyses. From each tree, 30 to 34 embryos of both crown sides were analysed.

The first step for the identification of hybrids had to be the search for suitable genetic markers for the distinction of the clones, or at least of the species. The orchard clones were tested at 16 enzyme systems by starch gel electrophoresis of buds and endosperms. The extraction of the isoenzymes, their separation by electrophoresis and staining of the gels were carried out by methods for beech, as are described in MÜLLER-STARCK & STARKE (1993).

In accordance to BRAUN *et al.* (1990) the distinction of the species principally was possible at the systems Aspartate-amino-transpherase (AAT, EC 2.6.1.1) and Diaphorase (DIA, EC 1.6.4.3). But in contrast to the experiences of BRAUN *et al.* (1990), who used polyacrylamide gels, the embryos showed very weakly stained zymogramms in the starch gels at these loci. Due to this fact they could not be applied in this investigation.

Additionally, at the system shikimate dehydrogenase (SKDH, EC 1.1.1.14) the clones had completely different banding patterns between the species, and the zymogramms were of good quality. The genetic control of the only zone in this system (*Skdh–A*) was proved by HÄCKER & BERGMANN (1991), so it could serve as a genetic marker. The other tested isoenzyme systems

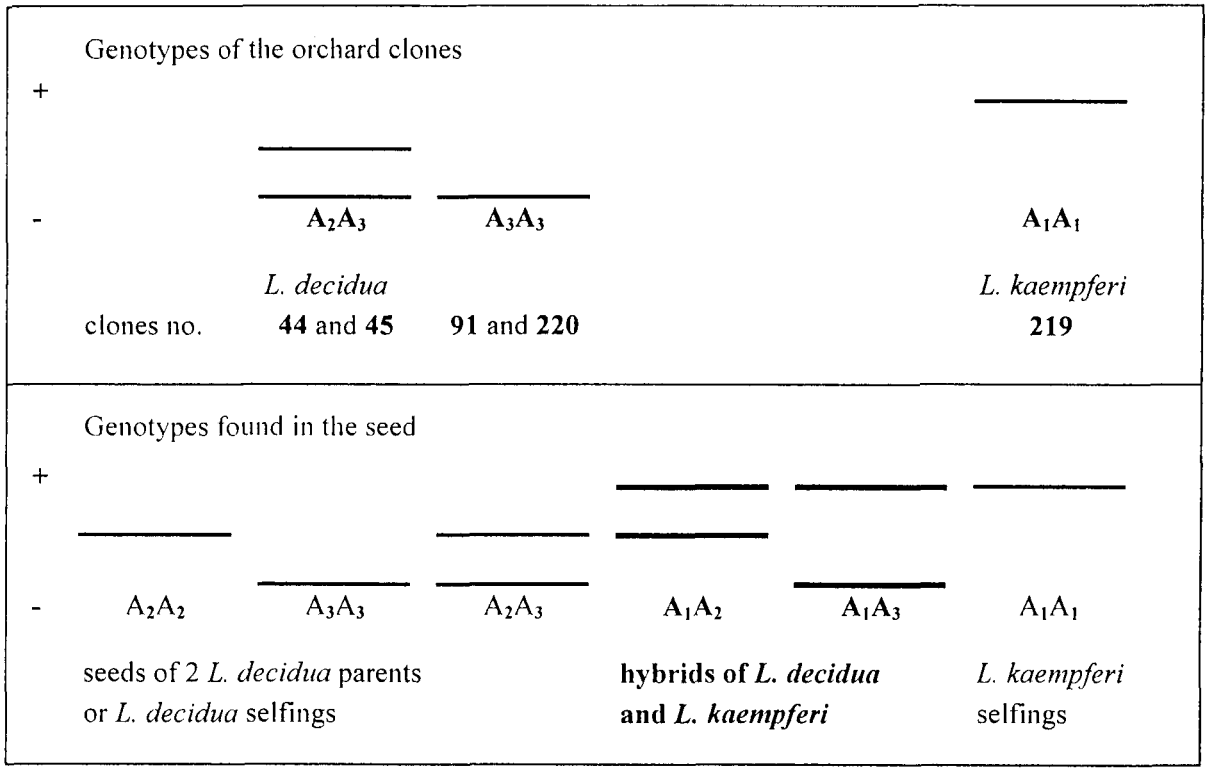

Figure 2. Genotypic variants occurring in the orchard clones and their progenies at the gene locus *Skdh–A*.

Table 1. Proportion of full seeds and hybrids on average of the clones and separately for the southern and northern crown parts.

Clone No.	Exposition	Proportion of full seed [%]	Amount of analysed seed	Proportion of hybrids [%]
44 *L. decidua*	totally	**62**	**601**	**64**
	north	58	300	61
	south	67	301	67
45 *L. decidua*	totally	**51**	**604**	**45**
	north	44	298	46
	south	63	306	44
91 *L. decidua*	totally	**41**	**589**	**75**
	north	38	294	72
	south	48	295	79
220 *L. decidua*	totally	**48**	**582**	**75**
	north	45	290	69
	south	51	292	81
219 *L. kaempferi*	totally	**19**	**594**	**38**
	north	18	297	34
	south	20	297	41

were not suitable for the present study. Therefore the separation of the clones within the species *L. decidua* was only partly possible.

RESULTS AND DISCUSSION

The clones of the orchard investigated show 3 genotypic variants (Figure 2). Therefore, 6 genotypes are expected totally in the progenies, 5 in the embryos originating from the clones of *L. decidua* and 3 in the progenies of the *L. kaempferi* clone. The genotypes *Skdh*–A_1A_2 and *Skdh*–A_1A_3 identify the hybrid seeds. The occurrence of the allele A_2 in the seed from the clones 91, 220 and 219 indicates the influence of pollen from either clone no. 44 or no. 45.

The proportions of full seeds at the different clones of *L. decidua* show values between 41 % and 62 %, whereas the clone of *L. kaempferi* has only 19% full seeds (Table 1). A similar tendency is evident in the proportion of hybrids. The progenies of the *L. decidua* clones contain between 45 % and 75 % and the progeny of *L. kaempferi* clone only 38 % of hybrids. At the clone level, the proportion of full seeds as well as of hybrids is in most cases higher from the southern than from the northern part of the crowns. The only exception is the hybrid proportion in the progeny of clone no. 45 (Table 1).

The comparison with the results from the crop of 1987 (BRAUN *et al.* 1990) reflects, that the values of both years are nearly in the same range, but ranking corresponds only in the case of clones no. 45 and no. 219 (Figure 3). This confirms the suggestion of BRAUN *et al.* (1990), that the low proportions of full seeds and hybrids are caused by the early culmination point of flowering of clone no. 219 and the late one of clone no. 45. In 1996, the flowering of all clones in the orchard proceeded in a few days and therefore the differences between clone no. 45 and the other clones of *L. decidua* are not as extreme as in 1987.

On the level of individual trees, the proportions of full seeds and of hybrids were compared between the northern and the southern part of the crowns. The trend already observed on the clone level (see above) was confirmed. In most of the trees, both values were slightly higher on the southern side. But there were some trees, distributed over all clones and all parts of the orchard, that showed higher percentages on the northern part either in one or in both traits. For the proportion of hybrids, the differences were tested by *G*-test.

Only in 7 cases, 5 of them located near the western border of the orchard, were the differences significant. An explanation for this result might be the slightly better climatic conditions for the flower and cone development on the southern side of the crown. The possible earlier flowering on this side should be an advantage for the pollination with the early flowering clone no. 219.

Observations indicating a similar effect were described by Burczyk et al. (1991), who studied the proportion of full seeds and outcrossing rates of L. decidua clones in relation to the crown level. They found, that there were more clones with a higher percentage of full seeds in the upper crown level than in the lower level and that in both groups the higher outcrossing rates were situated in the upper crown part. In this case female flowers also might develop better and may be slightly earlier because of better light and temperature conditions, and they should have contact to the pollen cloud first.

The *G*-test of the allele frequencies between the northern and the southern parts of the crowns showed significant differences only in three cases. All of them are located in row 8 of clone no. 45, which contains a relatively large gap. Beside the special behaviour of the clone no. 45 in the time of flowering these differences

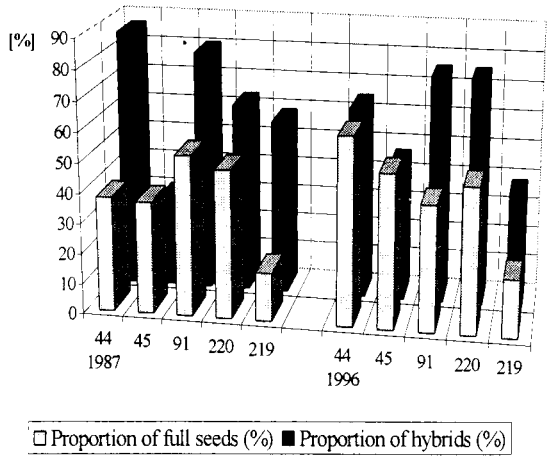

Figure 3. Comparison of the average proportions of full seed and hybrids in the progenies of the clones, determined in seed collected in 1987 (BRAUN *et al.* 1990) and 1996.

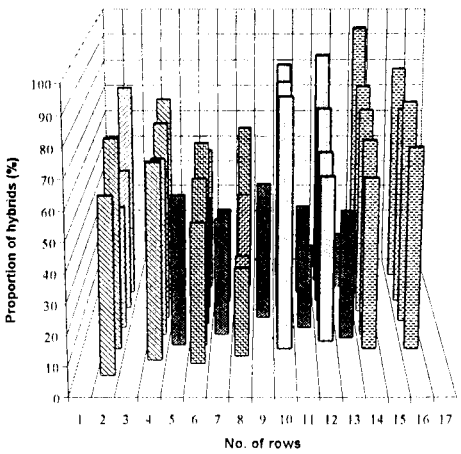

Figure 4. Proportions of hybrids in the progenies of the grafts investigated using the crop of 1996 (with view from west).

Table 2. Percentage of hybrid seed in the eaaastern (E), the middle (M) and western (W) part of the orchard and χ^{-2} test of the differences between them.

Clone No.	Hybrid proportions [%]			χ^2-values of differences between the crown parts		
	E	M	W	E – M	E – W	M – W
44	67.5	58.7	66.5	3.329	0.045	2.601
45	48.2	44.4	45.1	0.575	0.383	0.019
91	81.6	71.9	72.1	**5.184***	**5.023***	0.003
220	84.1	74.9	65.8	**5.054***	**17.403*****	3.796
All clones	**70.2**	**62.3**	**62.5**	**11.189*****	**10.652*****	0.008

*** = $P < 0.005$; * = $P < 0.05$.

might be influenced by other thermic turbulences because of this gap. Within the rows, an increase of the proportion of hybrids could be observed in easterly direction (Figure 4).

In order to prove this observation, the orchard was divided into three parts: eastern, middle and western part. In each part three analysed grafts of each clone of *L. decidua* were located. The clone of *L. kaempferi* could not be included in this test because of the unequal distribution of its investigated grafts in the orchard. The collectives formed in that way were tested by χ^2-test within the clones and for all clones together (Table 2). The results of this test show, that in the eastern part of the orchard the proportion of hybrids in the progenies is significantly higher than in the middle and the western part.

Although the χ^2-values of the clones no. 44 and no. 45 do not reach the significance level, they also confirm this trend.

The increase of the proportion of hybrids in easterly direction is assumed to be essentially caused by the main wind direction from the west, during the pollination period.

The results of BURCZYK *et al.* (1997) show, that a higher proportion of hybrid seed can be resolved by a place-by-place mixing of the grafts of the different species.

The occurrence of the allele *Skdh–*A_2 in the progenies within the rows is shown in Table 3. A strong decrease is evident in the rows not including the clones no. 44 and no. 45. In this rows, the allele *Skdh–*A_2 was mainly found on the northern part of the crowns. On the southern parts it appeared only in 3 seeds. This confirms the suggestion that the pollination process was influenced by wind direction and air movement within the rows.

These results implicate, that the influence of the clones of *L. decidua* on each other is rather small.

Table 3. Occurence of the allele *Skdh–*A_2 within the rows.

Row No.	Clone No.	Frequency of allele A_2	
		Absolute	Relative
2	44	249	37.39
4	44	196	36.57
5	**219**	**18**	**13.43**
6	45	211	32.07
7	**219**	**9**	**3.46**
8	45	178	33.84
9	**219**	**2**	**0.77**
10	**91**	**9**	**1.37**
11	**219**	**0**	**0.00**
12	**91**	**4**	**0.77**
13	**219**	**0**	**0.00**
14	**220**	**3**	**0.47**
16	**220**	**0**	**0.00**

Therefore one of the aims of the design of the orchard – minimal production of intraspecific crossed seed – is nearly achieved. The other consequence has to be taken into account, that a relatively high proportion of the non-hybrid seed is selfed material.

CONCLUSIONS

For the year of crop 1996, the following recommendations for the use of the seed gained from this orchard can be given: The seed from all clones of *L. decidua* in this orchard can be used as hybrid seed. The utilisation of the seed of the clone of *L. kaempferi* has to be refused, at least for this year of the crop, due to its low proportion of full seed and of hybrids in the progeny. A short overview on characteristic growth and quality traits of larch hybrids as well as recommendations on their cultivation and silvicultural treatment are given by HAASEMANN (1997).

The differences in the results of the seed collections 1987 (BRAUN et *al. 1990*) and 1996 make evident, that it is necessary to determine the proportion of hybrids in the seed of hybrid seed orchards from each collection year and separately for the different clones before use. Especially in the cases of clone no. 45 and clone no. 219, it was shown, that the values can vary between different crop years to a considerable degree. Therefore, the recommendations for the application of the seed from different clones can differ from year to year, also.

In general, the results allow the following conclusion in relation to the design of seed orchards established with the aim of producing proved hybrid seed from two or a few hybridisation partners:

The proportion of full seeds, of desirable and undesirable crossing combinations as well as of selfings is essentially affected by the spatial design of the

orchard. Three main characteristics for a successful establishment of this type of hybrid seed orchard have to be considered:

- The spatial distribution of the different clones (structure of the clone mixture).
- The basic geometric arrangement of the grafts (e.g. in rows or in homogeneous square spacing).
- The orientation of the rows in relation to the prevailing wind direction during the flowering period.

In case of the orchard investigated in the present study, the proportion of hybrids probably could have been increased by mixing of some single grafts of the *L. kaempferi* clone into the rows of the *L. decidua* clones. The separation of the *L. decidua* clones from each other would have achieved a higher degree by planting two rows of *L. kaempferi* between them instead of only one row. But the basic idea of the production of different proved hybrid combinations separately in one orchard has been realised.

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