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Development of hybrid fir clonal material

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ABSTRACT: There are four testing plots with hybrid clonal material. This material is regularly measured and some characteristics are observed. This paper sumarizes results of measurements from years 1998, 2000 and 2002. Hybrid material is compared with control material *Abies alba* from open pollination. Results show, that hybrid clonal material grow orthotropically and most of them are better in growth characteristics in comparison with control material *Abies alba* from open pollination. Mortality is also very low. All this mean high potential of this material to the future not only for further breeding, but also for use in special plantations.

Keywords: hybridization; mutation breeding; *Abies* spp.

European silver fir (*Abies alba* Mill.) was one of our most important forest tree species. Its occurence extremely decreased because of changing ecological conditions as so as vitality of trees in surviving local populations. Fir is important tree species because of higher production ability than Norway spruce (*Picea abies* [L.] Karsten) and it has high ecological importance as well.

European silver fir is one of those domestic tree species maintenance of which is done especially by its resistance. It is possible to observe some cases of fir regeneration in Europe but increase of its resistance is still very important question. Fir cannot be bred for specific resistance like elms. That is why breeding has to be directed to general resistance increasing.

One of important approaches for increasing fir resistance by breeding is interspecific hybridization because it is known that hybrids within genus *Abies* often reach effect of heterosis not only in growth but also in increased vitality in comparison with parental species. Higher tolerance to various stress factors including air polution pressure is connected with (and possibly higher tolerance to expected climate warming).

Adaptive reaction of domestic forest tree populations cannot be only one solution from viewpoint of population genetics because of very fast ecological changes. After fast ecological change genetic adaptation of domestic populations continues on basis of selection and mutation process minimally 10 generations or much more. Introduction of exotic tree species adapted to similar conditions and intensive forest tree breeding utilizing resources of domestic and exotic tree species including their hybridization can play important role.

Rohmeder and Schönbach (1959) have refered that interspecific hybrids of *Abies alba* Mill., *Abies veitchii* Carr., *Abies concolor* Lindl. et Cord., *Abies procera* Rehd., *Abies nordmanniana* Spach. grow faster than intraspecific hybrids. Mergen and Gregoire (1988) also found out that progenies from interspecific hybridization grow better than from intraspecific. They found this on basis of diameter, height and crown profile measurement of 17 years old hybrids which were obtained during various fir species hybridization in Connecticut, USA in spring 1960 and 1962. Hybrids from crossing of species with near areals grow better than those from crossing of species with distant areals. Kantor and Chira (1971) were interested in fir hybridization in former Czechoslovakia with using *Abies alba* Mill., *Abies cephalonica* Loud., *Abies nordman-*

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niana Spach., *Abies pinsapo* Boiss., *Abies cilicica* Carr., *Abies concolor* Lindl. et Cord., *Abies grandis* Lindl. trees for interspecific and also intraspecific hybridization.

Kobliha (1988, 1989) was concerned with testing of some these hybrids. *Abies cephalonica* progenies from intraspecific crossing and also with using of γ rayed pollen showed excellent results. All hybrid combinations with genetic participation of *Abies alba* had faster growth in comparison with control *Abies alba* from open pollination. Kobliha and Pokorný (1990), Kobliha et al. (1991), Kobliha and Králík (1992) were interested in various methods of vegetative propagation of these hybrids.

Greguss (1988, 1992) and Kormuťák (1985, 1992) were concerned with fir hybridization in Slovakia. Greguss (1986) mentioned that European silver fir grow slower than hybrids and that differentiation among hybrid progenies and progenies of individual species from open pollination is increasing. Similar results were achieved by Kormuťák (1986). He recommended especially combinations *Abies alba* × *Abies cephalonica*, *Abies cephalonica* × *Abies numidica*, *Abies nordmanniana* × *Abies alba*, *Abies pinsapo* × *Abies cephalonica*, *Abies pinsapo* × *Abies alba*, *Abies numidica* × *Abies nordmanniana*, *Abies numidica* × *Abies cephalonica*, *Abies concolor* × *Abies grandis* for silviculture in our conditions. He recommended mainly using *Abies alba* and Mediterranean fir species. The same species already played the most important role in experiments of Kantor, Chira and Greguss mentioned earlier.

Kormuťák et al. (1997) found rather than general compatibility among individual fir species existing specific compatibility among selected parental trees on basis of wide experiments with artifical hybridization of species within *Abies* genus. These results limited widely accepted postulate of high crossability within *Abies* genus. Unsuccessfull hybridization of *Abies concolor* and *Abies grandis* with *Abies alba, Abies numidica, Abies nordmanniana* and *Abies cephalonica* showed on barrier among North American and European members of genus. Comparative cytological research of pollinated eggs in period from pollination to young embryo stage showed damaged archegonia like proof of general desintegration in incompatible combinations. Archegonia degeneration can be direct impact of unability for growth of pollen grains on egg nucellus.

French research (Arbez et al. 1990) was intensively interested in Mediterranean fir species and their hybridization. French researchers also recommended using of fir progenies from self-pollination because of their higher tolerance against inbreeding. Mediterranean fir species, their hybrids and their hybrids with European silver fir can be important for solving actual problem connected with global warming. Fady (1993) described details about geographical, altitudional and bioclimatic distribution of *Abies cephalonica* and *Abies borisii-regis* in Greece as so as about their soil demands, forest types, colonizing ability, natural regeneration, silviculture, pests and diseases, wood production and quality. He also introduced here results from observation of various Greek provenances of these firs in series of experimental plantations in South of France. Results indicate that these provenances are generally well adapted on relatively low precipitation (less than 650 mm) and summer aridity and they are mostly undemanding on substrate.

Kobliha (1994) obtained F2 generation hybrids. He used like mother tree hybrid *Abies cilicica* × *Abies cephalonica*, its flowering graftings respectively. He realized successfully self-pollination, pollination by *Abies alba, Abies pinsapo, Abies homoleppis, Abies concolor*. Pollen of mentioned hybrid was used for pollination of *Abies pinsapo, Abies koreana, Abies grandis* and *Abies lowiana*. Kobliha and Janeček (2001, 2003) tested various hybrid progenies and also hybrid clones. Most of hybrid progenies and clones were better than control *Abies alba* from open pollination.

MATERIAL AND METHODS

Kantor and Chira (1971) obtained 16 progenies from inter- and intraspecific hybridization. There were combinations of *Abies alba*, *A. cephalonica*, *A. cilicica* and *A. nordmanniana*. As a control progeny *A. alba* from open pollination was used. In some combinations pollen irradiated γ rays in various doses was used. All 17 progenies were tested by Kobliha in 1988.

Five most viable individuals from every progeny were selected (see Fig. 1). Choosen individuals were used for autovegetative propagation (Kobliha, Pokorný 1990; Kobliha et al. 1991). In September 1993 plantation with this rooted cuttings was established in Tree Breeding Station Truba near Kostelec nad Černými lesy (Table 1, Figs. 2–4).

Growing form of cuttings was observed, what is important especially with regard to known problem with fir cuttings plagiotropy. Mortality is observed too. In 1998, 2000 and 2002 height (accurate to 1 cm) and diameter (accurate to 1 mm) of rooted cuttings were measured. Results from 2000 and 2002 were statistically processed by analysis of variance and, where possible, Duncan's test was made.

Fig. 1. Fir hybrids in progeny test (Middle Moravia)

This plot is in following text marked as Plantation 93 (see Fig. 5).

The second plot was established in 1995 (marked in following text as Plantation 95). It has similar origin like the first plot. The difference is in propagationing of individual trees selected for very high viability. There are 10 hybrid clones from intraspecific hybridization of *A. alba*, *A. cephalonica* and interspecific hybridization *A. alba* × *A. cilicica* (see Table 2). There are three individuals (designated F2) on this plot too. Their origin will be explained later. As a control material K3 from Plantation 93 was used.

Propagation by cuttings was done in years 1990 to 1992. The same characteristics like in first plantation are measured and observed here.

Multiclonal mixture of hybrids from the genus *Abies* (generation F2) grows in the third plot (marked in following text as Plantation 96) and three more individuals are on second plot (Plantation 95). In 80's experiment with control self pollination of hybrid *A. cilicica* × *A. cephalonica* was realized (Kobliha 1994). This material was propagated by cuttings in 90's – rooted cuttings were planted in autumn 1996 (208 individuals). In autumn 1998, 2000 and 2002 height (accurate to one cm) and diameter (accurate to one mm) were measured.

In spring 2000 another plot with mixture of clones was established (270 individuals). Origin of this material is the same, cuttings were used for propagation in years 1993 and 1994. Height was measured here in 2002. This plantation is in following text marked as Plantation 2000.

RESULTS

Plantation 93

Mortality was observed in this plantation. First year mortality was only 4%, second year 38%. In 2002, 9 years after planting mortality was 53%.

Fig. 2. Hybrid fir clonal material (Tree Breeding Station Truba)

Fig. 3. Hybrid fir clonal material (Tree Breeding Station Truba)

Fig. 4. Establishment of hybrid fir clonal test (Tree Breeding Station Truba)

Height

In autumn 1998 the mean height of material ranged between 86 cm (combination No. 16) and 127 cm (combination S2). Control material *A. alba* from open pollination (K3) reached the mean height 104 cm. The smallest combination on average – combination No. 16 – reached 83% of mean height of control *A. alba* (K3). The highest material on average – S2 – reached 122% of mean height of *A. alba* (K3). Combinations 1, 2, 3, 4, 6, 9, 16, 19 and W. N. (Without Number) has not reached mean height of *A. alba* (K3). Their height reached value between 83% and 96% of mean height of *A. alba* (K3). Clone material coming from combination K1 and 17 had the same mean height like *A. alba* (K3). Combinations S2, 10, 11, 12, 18 were on average higher than *A. alba* (K3). They reached from 103 to 122% of mean height of *A. alba* (K3).

The mean height in autumn 2000 was 181 cm. The mean height reached from 157 cm (combination 3) to 238 cm (combination 11). Control material *A. alba* (K3) reached 174 cm on average. Combinations 1, 3 and 16 did not reach mean height of *A. alba* (K3). They reached from 90 to 93% of mean height of *A. alba* (K3). Combinations 4 and 9 had the same

Fig. 5. Hybrid fir clonal test (Tree Breeding Station Truba)

Table 1. Origin – numbers behind family show individual trees and letter ž shows locality – Žarnovica (Slovakia)

Number	Origin	RTG doses or comment
1	*A. alba* 2 × *A. alba* ž	500–1,000
2	*A. alba* 3 × *A. nordmanniana*	
3	*A. nordmanniana* × *A. nordmanniana*	self pollination
4	*A. alba* 3 × *A. alba* ž	
6	*A. cephalonica* 2 × *A. cephalonica* 2	self pollination
9	*A. cephalonica* 1 × *A. cephalonica* 3	300
10	*A. cephalonica* 1 × *A. cephalonica* 3	1,500
11	*A. cephalonica* 1 × *A. cephalonica* 3	1,000
12	*A. cephalonica* × *A. cephalonica* 3	500
16	*A. cephalonica* 2 × *A. cephalonica* 3	1,500
17	*A. cephalonica* 2 × *A. cephalonica* 3	3,000
18	*A. cephalonica* 2 × *A. cephalonica* 3	500
19	*A. cephalonica* 2 × *A. cephalonica* 3	1,000
K1	*A. alba* 1 × *A. cilicica*	
K3	*A. alba* × *A. alba*	control from open pollination
S2	*A. cephalonica* 3 × *A. cephalonica* 3	self pollination
W.N.	(Without number) – various	mixture of rooted cuttings

height like control material. Combinations 2, 6, 10, 11, 12, 17, 18, 19, K1, S2, W.N. were higher than control material. Their height was between 102 and 137% of mean height of *A. alba* (K3).

In the year 2002 was the mean height 285 cm. It reached from 254 cm (combination 16) up to 341 cm (S2). Control material (K3) reached the mean diameter 264 cm. Only combinations 16 (96% of mean diameter of control material) and 9 (262 cm – 99% of control) did not reach the mean diameter of K3. The mean height of other material was from 100% up to 129% of mean height *Abies alba* from open pollination.

Analysis of variance showed there is not statistical significance of influence of combination on plant height.

Table 3 shows mean height (in cm) of every combination from this plantation. Fig. 6 shows mean height of every combination in percentage of mean height of control *Abies alba*.

Diameter

Mean diameter of clones (grouped by hybrid combination) reached from 26 mm (combination 16) to 50 mm (combination 12) in 1998. The highest com-

Table 2. Numbers in third column are combination's numbers (see Table 1)

4/5	*A. cephalonica* × *A. cephalonica*	17
5/2	*A. cephalonica* × *A. cephalonica*	19
6/1	*A. alba* × *A. alba*	4
13/1	*A. cephalonica* × *A. cephalonica*	18
13/2	*A. cephalonica* × *A. cephalonica*	18
18/8	*A. cephalonica* × *A. cephalonica*	16
21/23	*A. alba* × *A. cilicica*	K1
21/24	*A. alba* × *A. cilicica*	K1
23/10	*A. cephalonica* – self pollination	S2
F2	*A. cilicica* × *A. cephalonica*	F2 generation

Table 3. Height in Plantation 93 (cm)

Combination	1998	2000	2002
1	94	163	267
2	97	196	295
3	96	157	269
4	95	174	303
6	99	184	264
9	100	173	262
10	107	177	306
11	115	238	320
12	118	219	321
16	86	157	254
17	104	197	312
18	116	198	294
19	97	192	302
K1	104	185	289
K3	**104**	**174**	**264**
S2	127	231	341
W. N.	89	180	273

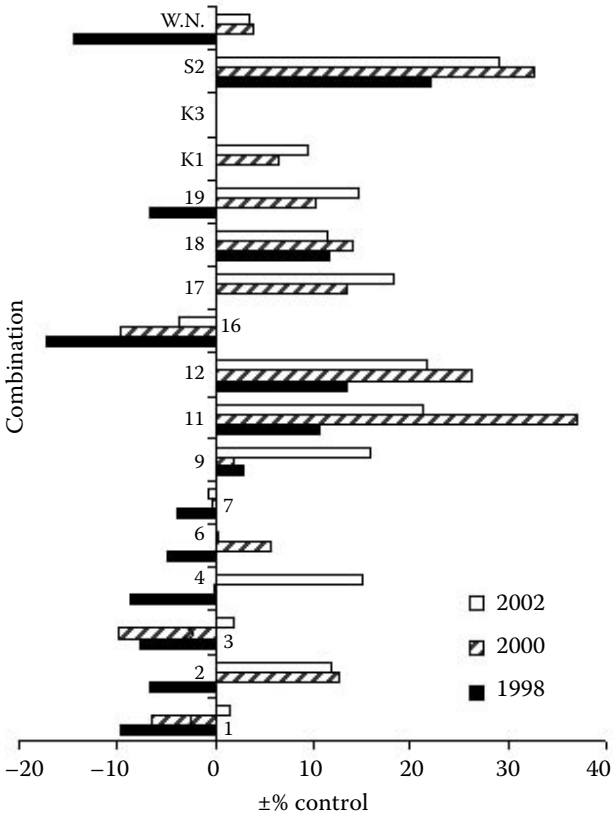

Fig. 6. Height in Plantation 93 (difference in %, 0 is control material K3)

bination S2 reached the 2nd place in mean diameter (49 mm) with combination 11. Combination 11 was the 3rd with mean height 115 cm. Control material *A. alba* (K3) reached the diameter 30 mm. The thinnest combination – 16 – reached 87% of mean diameter of *A. alba* (K3). The thickest combination – 12 – reached 167% of mean diameter of *A. alba* (K3). Combinations 16 and W.N. did not reach mean diameter of *A. alba* (K3) (87 and 93%). Combination 1 had the same mean diameter like control material. Combinations 2, 3, 4, 6, 9, 10, 11, 12, 17, 18, 19, K1 and S2 were thicker than *A. alba* (K3). They reached from 103% to 167% of mean diameter of *A. alba* (K3).

Mean diameter of this material in autumn 2000 was 55 mm. According to combination the diameter was from 44 mm (control *A. alba* – K3) to 80 mm (combination 11). All combinations overtook control material K3. Combinations reached from 107 to 182% of diameter of control material. Analysis of variance showed that there is not statistical significance of influence of combination on plant diameter again, but results are much closer to significant limit than height results.

In the year 2002 the mean diameter was 71 mm. Control material (diameter 62 mm) has been overtoken by all combinations again. Combination 11 reached 169% of control mean height.

Table 4 and Fig. 7 show mean diameter for every combination from this plantation in years 1998, 2000 and 2002 and the difference between combinations and control material.

Plantation 95

Mortality was regularly observed too in this plantation. First year, mortality was only 1.5%. In 2002, 7 years after planting, mortality was still very low, only 4%.

Height

The mean height in clonal plantation established in 1995 was between 59 cm (clone 21/24) and 117 cm (clone 5/2) in 1998. Control clone material *A. alba* from open pollination (K3) had been used from previous plantation. Results obtained in 1996 are used for comparison. The mean height of control material was 59 cm. It means, that hybrid clones reached from 100 to 198% of *A. alba* from open pollination (K3) height.

The mean height of 187 rooted cuttings was 134 cm in autumn 2000. The mean height of hybrid clones reached from 107 cm (clone 21/24) to 227 cm (clone 5/2). The smallest and the highest clones are the same like in 1998. Control material

Table 4. Diameter in Plantation 93 (mm)

Combination	1998	2000	2002
1	30	52	68
2	31	53	69
3	32	50	73
4	35	58	78
6	31	51	65
9	42	59	67
10	33	62	77
11	49	80	105
12	50	70	88
16	26	47	72
17	35	57	77
18	40	60	71
19	32	57	85
K1	33	51	68
K3	**30**	**44**	***62***
S2	49	68	64
W. N.	28	50	65

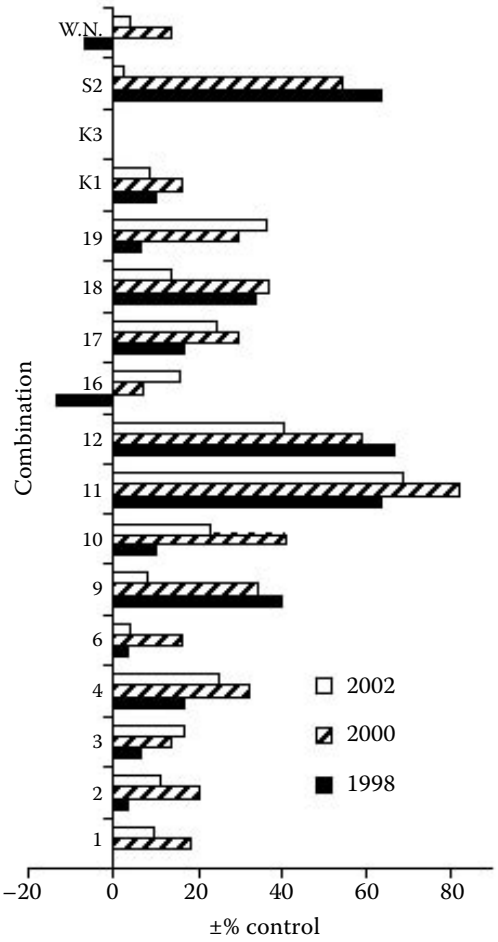

Fig. 7. Diameter in Plantation 93 (difference in %, 0 is control material)

K3 in the year 1998 reached mean height 104 cm. Hybrid clones reached height from 103% to 218% of control material.

In 2002 the mean height was 216 cm, control material mean height was 174 cm. Hybrid clones reached height from 174 cm (23/10 – 100% of control material) to 340 cm (5/2 – 195% of control material).

Mean heights in 1998, 2000 and 2002 are in Table 5. Differences between clones mean heights and control material mean height are in Fig. 8. Analysis of variance shows high statistical significancy of influence of clone on plant height. Duncan's test divided hybrid clones into 3 subgroups.

Diameter

Mean diameter of hybrid clones in 1998 was between 17 mm (clone 21/24) and 34 mm (clone 5/2). These are the same clones as in case of height. Control material K3 reached mean diameter 15 mm in the year 1996. It means that hybrid clones in this plantation reached from 113% to 227% of *A. alba* from open pollination (K3) diameter.

The mean diameter in this plantation reached from 31 mm (clone 23/10) to 62 mm (clone 5/2) in autumn 2000. The lowest clone – 21/24 – reached diameter 33 mm. Control material K3 reached mean diameter 30 mm in the year 1998. That means, that hybrid clones reached in this plantation mean diameter from 103% to 208% of control mean diameter.

The mean diameter in 2002 was 57 mm. All clones overtook control material – from 107% to 216%.

Results of analysis of variance show high level of statistical significancy of influence of clone on plant diameter. Duncan's test divided hybrid clones into three subgroups. Mean diameter of clones in years 1998, 2000 and 2002 are in Table 6. Differences between clones mean diameter and control material mean diameter are in Fig. 9.

Plantation 96

To September 1997 20 trees died (9.5%). Dead trees were compensated from reserve. In autumn 2000 194 individuals survived (mortality after the year 1997 was 14 trees, i.e. 7%).

Multiclonal mixture of hybrids from the genus *Abies* (generation F2) reached mean height 37 cm

Table 5. Height in Plantation 95 (cm)

Clone	1998	2000	2002
4/5	103	166	270
5/2	117	227	340
6/1	108	135	225
13/1	72	161	262
13/2	74	126	273
18/8	81	170	190
21/23	70	136	211
21/24	59	108	188
23/10	60	107	174
F2	99	188	277
K3	**59**	**104**	**174**

Table 6. Diameter in Plantation 95 (mm)

Clone	1998	2000	2002
4/5	32	57	84
5/2	34	62	81
6/1	35	44	66
13/1	23	46	63
13/2	21	40	49
18/8	23	38	47
21/23	20	37	53
21/24	17	33	51
23/10	18	31	51
F2	33	62	95
K3	15	30	44

and diameter 11 mm in autumn 1998. In autumn 2000 194 rooted cuttings reached mean height 64 cm and diameter 21 mm. In autumn 2002 194 rooted cuttings reached mean height 125 cm and diameter 39 mm.

Plantation 2000 was measured in height in autumn 2002 with mean 62 cm.

Growth form of rooted cuttings in all plantations

In the year 1998 most of rooted cuttings from plantations established 1993 and 1995 (i.e. 5, respectively 3 years after planting) grew orthotropically. In autumn 2000 (7, respectively 5 years after planting) there were only few exceptions, which grew plagiotropically. At the same time most of rooted cuttings in plantation established in 1996 started to grow orthotropically.

DISCUSSION

There are no scientific articles on this field, so our results are unique. Knowledge about growth and development of hybrid clonal material of genus *Abies* is very limited, because there is no plantation like this in Europe and possibly in the whole world. Our results show, that nearly all rooted cuttings grow orthotropically, which was earlier assumed impossible. Testing and selection of hybrid fir clonal material is more effective for breeding than testing and selection of hybrid fir progenies.

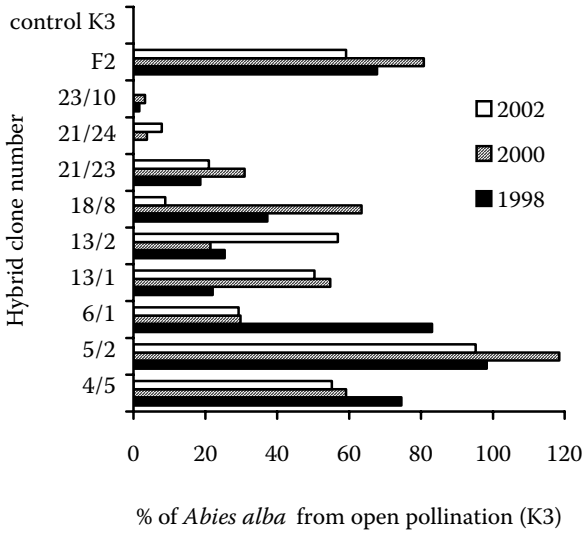

Fig. 8. Height in Plantation 95 (difference in %, 0 is control material)

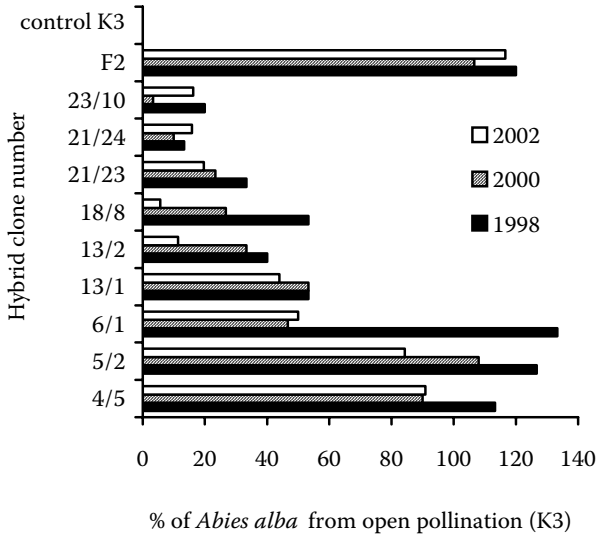

Fig. 9. Diameter in Plantation 95 (difference in %, 0 is control material)

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Vývoj klonového materiálu hybridních jedlí

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ABSTRAKT: Ve čtyřech pokusných výsadbách hybridních klonů jedle byly měřeny růstové charakteristiky. Měření pocházejí z let 1998, 2000 a 2002. Hybridní materiál byl srovnáván s kontrolním klonem druhu *Abies alba* z volného opylení. Výsledky ukazují, že materiál namnožený řízkováním nejenže roste ortotropně, ale ještě většinou předstihuje v růstových charakteristikách kontrolní materiál *Abies alba* z volného opylení. Také mortalita tohoto materiálu je velmi nízká. To vše předznamenává vysoký potenciál těchto klonů nejen pro další šlechtění, ale také pro jejich využití při zakládání speciálních lesnických výsadeb.

Klíčová slova: hybridizace; mutační šlechtění; *Abies* spp.

Zastoupení jedle bělokoré (*Abies alba* Mill.) v našich lesích pokleslo pod 1 % v důsledku působení více faktorů. Tuto dřevinu je třeba v našich lesích udržet, protože na některých stanovištích má dokonce vyšší produkční schopnost než smrk ztepilý (*Picea abies* [L.] Karsten) a nelze opominout ani její vliv ekologický. Její udržení v našich porostech závisí na celkovém zvýšení její odolnosti. To lze provést buď výběrem a dalším množením vůči stresu tolerantních jedinců, nebo hybridizací. Hybridizace má význam zvláště v souvislosti s měnícím se klimatem a s ním spojeným zvýšením intenzity působení některých stresových faktorů – snížení srážkového úhrnu a zvýšení teploty.

Mnoho autorů uvádí lepší růst mezidruhových hybridů v rámci rodu *Abies* v porovnání s druhy rodičovskými a s tím spojenou zvýšenou vitalitu na základě heterozního efektu. Těchto zahraničních zkušeností bylo využito i v České republice. Ze 16 potomstev získaných Kantorem a Chirou (kombinace *Abies alba, A. cephalonica, A. cilicica* a *A. nordmanniana*, v některých kombinacích byl použit pyl ozářený gama paprsky) bylo z každé kombinace vybráno pět nejvitálnějších jedinců a ti byli rozmnoženi vegetativně. V roce 1993 byli vysazeni na plochu označenou jako Výsadba 93.

Z materiálu vysazeného ve Výsadbě 93 byli vybráni jedinci s velmi vysokou vitalitou a ti byli dále množeni vegetativně. Vysazeni byli vedle předchozí plochy v roce 1995 a označeni jako Výsadba 95.

Kontrolní materiál je v obou případech označen jako K3 a jedná se o *Abies alba* z volného opylení.

V roce 1996 (označení Výsadba 96) byla založena třetí plocha, na které se nachází potomstvo generace F2. Jedná se o klonovou směs hybridů, která vznikla z experimentů se samoopylováním hybridu *Abies cilicica* × *Abies cephalonica*. Opět to jsou řízkovance.

V těchto výsadbách byly v letech 1998, 2000 a 2002 měřeny výšky, tloušťky a sledovala se mortalita a přechod k ortotropní formě růstu.

Čtvrtá výsadba (Výsadba 2000) byla založena ze stejného materiálu jako výsadba předchozí. Zde byla v roce 2002 měřena výška.

Na všech plochách naprostá většina řízkovanců roste ortotropně, což je jeden z nejvýznamnějších poznatků, který umožňuje použití tohoto materiálu v praxi. V nejstarší výsadbě sice analýza rozptylu neprokázala statistickou významnost vlivu klonu na výšku a tloušťku, ale je patrné předstižení kontrolního materiálu většinou klonů a prohlubování rozdílů mezi hybridy a *Abies alba*.

Ve Výsadbě 95 je po sedmi letech po založení mortalita pouze 4%. Výsledky ukazují vysokou statistickou významnost vlivu klonu na výšku a tloušťku materiálu a všechen hybridní materiál předstihl kontrolu.

Ve Výsadbě 96 a 2000 je opět velice nízká mortalita. Změřené charakteristiky prozatím nemohou být s ničím porovnávány.

Tyto výsledky jsou prakticky unikátní, podobná výsadba se podle dostupných informací nenachází nikde ve světě. Jak již bylo konstatováno, většina řízkovanců roste ortotropně a postupně odrůstá kontrolnímu materiálu. V měření a šetření se bude samozřejmě nadále pokračovat.

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