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# HYBRIDIZATION RESULTS USING THE HYBRID *Abies cilicica* × *Abies cephalonica*

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**Abstract.** *Abies fraseri* (FF) is a North American conifer which is crucial for growers of Christmas trees. This species suffers 100% mortality after infection of root rot caused by *Phytophthora cinnamomi*. The hybrid material *A. cilicica* × *A. cephalonica* (CZ) and *A. koreana* × (*A. cilicica* × *A. cephalonica*) was utilized as mother trees for applications of *Abies fraseri* pollen. In addition to resistance to fungal disease, a long-term monitoring of growth of young seedlings along with the impact of artificial crossing on morphology of the progeny is covered. Except for increased resistance, some offspring has also exhibited a remarkable heterosis effect at younger age and different growth habit and different shape of their needles. All this may offer in the future a wide range of applications, both for crops under strong anthropogenic pressure with changing climatic conditions and for crops grown for production of Christmas trees and outstanding ornamental cultivars. Annual crossing success was determined in present study using X-ray imaging. The most successful combinations in 2012 were those of CZ1 × FF25 and CZ1 × FF76 with 7% of full seeds, followed by CZ2 × FF25 with 6% and CZ2 × FF31 with 4% of the full seeds. The evaluation of young seedlings from control crossing in 2007 highlighted significant accented height growth of complicated hybrid (*A. koreana* × (*A. cilicica* × *A. cephalonica*)) × FF PC. Morphology of needles showed variability in their length as compared with the needle width which was found to be less appropriate characteristic for distinguishing between different combinations.

**Key words:** *Abies*, hybridization, needle proportions and morphology

## INTRODUCTION

Interspecific hybridization is one of the ways how to influence the genetic basis of stands, increase their production, survival and resistance to ecosystem changes. Department of Dendrology and Forest Tree Breeding of Faculty of Forestry and Wood Sciences at the Czech University of Life Sciences in Prague has been dealing with the issue of breeding for resistance in the genus *Abies* for many years. This paper summa-

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rizes the results of artificial crossings made between the hybrid *A. cilicica* × *A. cephalonica* and some other Euro-American fir species in 2012. The interspecific hybrids often exhibit conspicuous heterosis effect in the first decades of their growth, and to some extent increased tolerance to selected climatic factors and some pathogens [Kobliha et al 2013]. They also exhibit intermediate characteristics in many traits [Klaehn and Winieski 1962]. In order to use these features and increase resistance to plantations of Christmas trees, North Carolina State University started Christmas Tree Genetics Program in year 1996 with the aim to improve Christmas tree species. Emphasis was put on Fraser fir (*Abies fraseri* (Pursh) Poir.) – growing in the western N.C. Mountains, USA [Frampton 2005]. Genetic resistance is widely used to combat diseases caused by *Phytophthora* spp. in agriculture and horticulture [Erwin and Ribeiro 1996]. Earlier research efforts of NCSU focused on identifying resistant Fraser fir material in greenhouse inoculation trials [Frampton and Benson 2012]. These trials have confirmed experiences obtained in highly infested Christmas tree plantations, namely that Fraser fir is extremely susceptible to *P. cinnamomi*.

The aim of this paper is to present results of 2012 control crossings using the same methods and describing similar material as in the past years. Hybridization results from the years 2006, 2007, 2008, 2009, 2010, 2011 were published earlier by Kobliha and Stejskal [2009] Stejskal et al. [2011] and Kobliha et al. [2013]. It also presents the first results of height growth and needles morphology evaluation of hybrid seedlings from control crossing in 2007.

## MATERIAL AND METHODS

All of the Czech seed orchards were founded as biclonal-grafts originated from 2 interspecific hybrids of the first generation *Abies cilicica* × *Abies cephalonica*, clones CZ1 and CZ2. These seed orchards with regular coning were suitable for control pollination experiments. Owing to good experiences with flowering and fertility of this material and also outstanding growth and vitality characteristics that suggested great potential for hybridizations, it was decided to further utilize this material. At first, $F_2$ material and new interspecific hybrids were obtained. Part of this material is cultivated within the Breeding Station Truba near Kostelec nad Černými lesy. Secondary grafts were taken to establish above mentioned hybridization seed orchards. Hybridization seed orchards with presence of female strobili before 2006 had been utilized mainly for production of $F_2$ hybrids. A list of plantations below outlines their historical and present state.

**Hybridization seed orchard No. 1** was established in 1994 directly at the Breeding Station Truba from the material grafted in 1991 and 1992. Original number of 217 grafts with 4 × 2 m spacing was reduced due to secondary water logging to current number of 154. Clone CZ2 is represented to a lesser extend – 30 grafts. Female flowering has been observed since 2004.

**Hybridization seed orchard No. 2** was established in 1996 within School Forest Enterprise Kostelec nad Černými lesy in forest stand 20A2 by planting of 70 grafts (35 clone CZ1 and the same count of clone CZ2). The orchard was designed in two

lines, each line account for 35 grafts from the same clone in spacing 2 × 2 m. First flowering was detected in 2008.

**Hybridization seed orchard No. 3** was established in 1997 from the material grafted in 1993 within a nursery in the village Seč near Prostějov. In total 200 grafts were planted in a row along a fence (100 grafts per clone). Clone CZ1 is alternated by clone CZ2 in a spacing of 3 m. This out planting is generally in a very good shape with mortality being rather exceptional here. Female coning registered since 2003.

**Hybridization seed orchard No. 4** was established in May 1999 within School Forest Enterprise Kostelec n. Č. l. in forest stand 20A9 by planting of 298 grafts (139 grafts CZ1, 159 grafts CZ2) in 3 × 3 m spacing. Covered area has around 0.31 ha. Grafts were planted in 20 rows; about 15 trees on each row. This plantation began to cone in 2008.

**Hybrid progeny test** was established in 1996 beside the seed orchard No. 4. There is a recent use of the complicated hybrid *Abies koreana* × (*Abies cilicica* × *Abies cephalonica*) for control pollinations. This hybrid material shows three overlapping introgression forms. Each individual tree is genetically unique.

In 2012 at the turn of April and May control pollination in seed orchards in Kostelec n. Č. l. and Seč was carried out. Fresh pollen of *A. fraseri* was supplied by NCSU. For seed orchards in Kostelec was used the pollen of clones FF31, FF61, FF64 and FF76. It was applied to individuals of clones CZ1 and CZ2 ($F_1$ generation hybrid *A. cilicica* × *A. cephalonica*) in orchards No. 1, No. 2, No. 4. Also, a 3-way hybrid *A. koreana* × (*A. cilicica* × *A. cephalonica*) was pollinated with *A. fraseri* clone FF64. There were in total 60 macrostrobili isolated and pollinated along with 17 macrostrobili of the 3-way hybrid individuals.

In the seed orchard No. 3 in Seč by Prostějov the pollen of *A. fraseri* (FF25, FF31, FF64 and FF76) was applied to 161 female flowers of clones CZ1 and CZ2 clones. During autumn, mature cones from open pollination were also collected in number of 12 pieces.

In 2013, the pollen of *A. fraseri* (clone FF61 collected in 2011 and FF11 collected in 2010) was applied only to ramets of clone CZ1 in seed orchards No. 1, No. 2 and No 4. Total number of pollinated cones was 66. Four macrostrobili of the clone CZ1 were left to open pollination. Three-way hybrid *A. koreana* × (*A. cilicica* × *A. cephalonica*) was pollinated with fresh pollen of *Abies numidica* collected in Arboretum Kostelec nad Černými lesy.

Determination of the number of full seeds was performed by means of X-ray imaging, which is a nondestructive method. Methodology was in compliance with ČSN 48 1211 standard (forest reproductive material-collection, quality and quality test of fruit and forest tree seeds). In accordance with the aforementioned standard, samples of 4 × 100 seeds of each hybrid combination were X-rayed with the exposure time of 2 seconds.

In addition, a height growth of seedlings from control crossing in 2007 was measured. They represent the hybrid combinations of CZ1 × PC (polycross or polymix is a mixture of Fraser fir pollen from various clones), CZ2 × PC, $F_2$ Kostelec/$F_2$ Prostějov (stands for open pollinated CZ1 and CZ2 clones), CZ2 × FF52, CZ1 × FF73, CZ2 × FF73, CZ2 × FF84 including a complicated 4-way hybrid (*A. koreana* ×

(*A. cilicica* × *A. cephalonica*) labeled 4/4) × FF PC. Three simplified groups were created for subsequent analysis – $F_2$, CZ × FF and (*A. koreana* × (*A. cilicica* × *A. cephalonica*)) × *A. fraseri*. The reason for such segregation was a large variability within the $F_2$ progeny and a close relationship of hybrid combinations of clones CZ1 and CZ2.

The needles of individual hybrid combinations were subjected to morphometric analysis. In-laboratory, the needle length and its width in the middle part of a needle were measured. The obtained data were statistically processed using Statistica program version 10. ANOVA test was applied, followed by the method of multiple regression analysis and post – hoc Duncan's test-method of multiple comparisons.

## RESULTS

Cone crop in 2012 was below average compared to previous years. The results of the test roentgenograms revealed a large seed infestation by insect pests in orchards No. 1 and No. 4 (Kostelec). The most successful combinations with 7% of full seeds were CZ1 × FF25 and CZ1 × FF76 of seed orchard No. 3, followed by CZ2 × FF25 with 6% and CZ2 × FF31 with 4%. For other samples from seed orchard No. 3 a significant part of the seed was very poorly developed. Samples of seed orchards in Kostelec were heavily infested by insects and the prediction based on roentgenograms was less than one percent of full seeds in average. Samples from plantation of complicated hybrids *A. koreana* × (*A. cilicica* × *A. cephalonica*) yielded the following results. The combination of 4/5 × FF64 and 1/2 × FF64, the share of full seeds reached to 3%, the combination of 1/4 × FF64 offered 2% of full seeds.

Table 1. Most successful mating combinations in seed orchard No. 3, Seč u Prostějova, 2012

| | Combination | | | |
|---|---|---|---|---|
| | CZ1 × FF 25 | CZ1 × FF 76 | CZ2 × FF 25 | CZ2 × FF 31 |
| Number of cones | 6 | 13 | 17 | 18 |
| Average cones leght (cm) | 12 | 16 | 16 | 17 |
| Total cones weight (g) | 493 | 1480 | 1879 | 1852 |
| Average weight of 1 cone (g) | 82 | 114 | 111 | 103 |
| Total weight of seeds (g) | 47 | 157 | 200 | 280 |
| Average weight of seeds in 1 cone (g) | 8 | 12 | 12 | 16 |
| Absolute weight of 1000 seeds (g) | 55 | 70 | 76 | 67 |
| Total number of seeds | 855 | 2243 | 2632 | 4179 |
| Average number of seeds in 1 cone | 142 | 173 | 155 | 232 |
| Full seeds fraction in sample (%) | **7** | **7** | **6** | **4** |
| Expected full seeds number | 60 | 157 | 158 | 167 |

In bold – highlighted full seed fraction in a sample, which illustrates a crossing success
FF – clones of *Abies fraseri*

Seed crop in 2013 in all seed orchards was rather poor. Substantial flowering was observed only within plantation of complicated hybrids of *A. koreana* × *(A. cilicica* × *A. cephalonica*). As the most successful combination has proven $F_2$ generation of seed orchard No. 4. with 8% of full seeds. The largest quantity of seed was obtained from complicated hybrid *A. koreana* × (A. *cilicica* × *A. cephalonica*) pollinated with *A. numidica* pollen. Despite the fact that X-ray images showed adequate quality, it was very difficult to distinguish between full and empty seeds. In this case it will be necessary to establish a germination experiment to verify the amount of full seed.

Number of obtained hybrid seeds, number of full seeds and other characteristics of the most successful combinations are given in Table 1.

As to height growth of young seedlings, the hybrid seedlings (*A. koreana* × (*A. cilicica* × *A. cephalonica*) numbered 4/4)) × *Abies fraseri* PC differed significantly from other hybrid combinations. $F_2$ seedlings from seed orchard No. 3 also differed significantly in their heights from other hybrid combinations. The complicated hybrid 4/4 × *Abies fraseri* PC exhibited the fastest growth at this stage, followed by $F_2$ hybrid offspring *A. cilicica* × *A. cephalonica*. Results of statistics are illustrated in Figure 1 and Table 2.

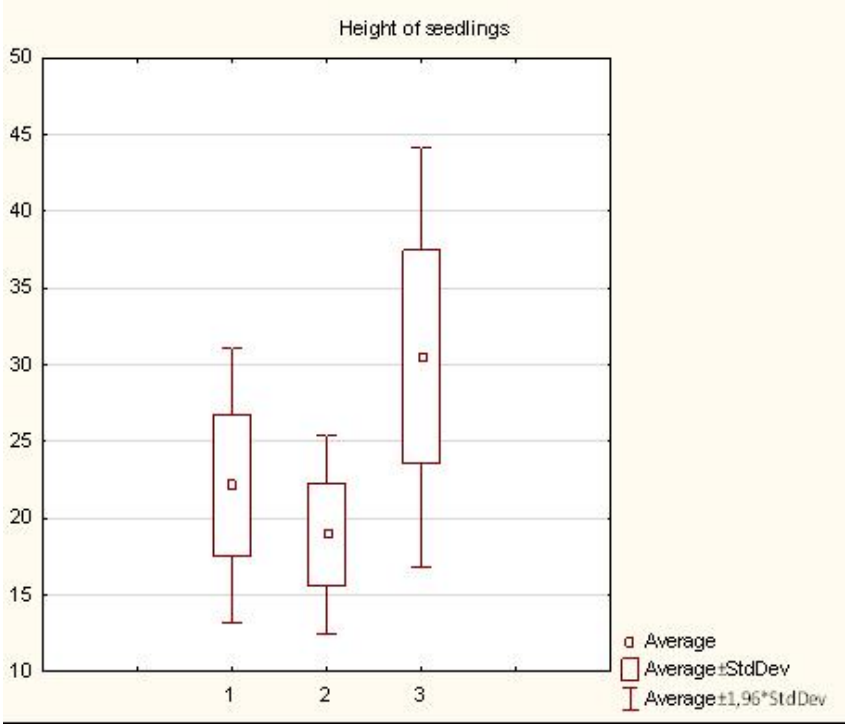


Fig. 1. Differences between mating combinations (cm): 1 – $F_2$ second filial generation of clones CZ1 and CZ2, 2 – CZ × FF (all hybrid combinations), 3 – (*A. koreana* × CZ) × PC

Table 2. Analysis of variance of mating combinations

| Source of variation | Sum of squares | Degrees of freedom | Mean squares | F Ratio | P Value |
|---|---|---|---|---|---|
| Main Effects | 127901.4 | 1 | 127901.4 | 5427.563 | 0.0000 |
| Id | 5082.8 | 2 | 2541.4 | 107.846 | 0.0000 |
| Error | 5467.1 | 232 | 23.6 | | |

Reviews of hybrid progenies according to morphological structure of their needles yielded the following results.

In terms of needle length, statistically most diverse combinations were those of CZ2 × FF PC with the shortest needles and CZ1 × FF73. They did not differ significantly only between themselves but also with respect to complicated hybrid (*A*. *koreana* × (*A*. *cilicica* × *A*. *cephalonica*) numbered 4/4) × *Abies fraseri* PC. It was also the combination with the shortest length of its needles. The aforementioned complicated hybrid differed from the combination CZ2 × FF73, with the second largest needle length and from $F_2$ progeny (Prostějov) with the longest needles of all examined combinations. Among other hybrid progenies no statistically significant difference in needle length was observed.

Width of needles does not exhibit such a significant variability. The most different from other progenies was hybrid combination CZ2 × FF PC with a minimal width of needles. More significant difference was reflected in a combination CZ2 × FF73 with the widest needles. Hybrid combination CZ2 × FF PC had at the same time the shortest and narrowest needles. Combination CZ2 × FF73 with the widest needles had the second largest needles length of all combinations examined.

## DISCUSSION

Results of control crosses conducted in 2012 confirmed experience with the success of interspecific hybrids CZ1 and CZ2 combined with other Euro-American species of fir. The percentage of full seeds of 7% is a stable value consistent with the results from previous years, which were published by Kobliha and Stejskal [2009], Stejskal et al. [2011] and Kobliha et al. [2013]. In 2013 the number of collected cones was similar as like in 2012. Low amount of full seeds in samples may be possibly due to decreased germination of pollen. Lower number of full seeds is not so much exceptional to the conclusions made by Hawley and Dehayes [1985] who postulate a lower inter-sectional crossability within the genus *Abies*. The success of hybridization also largely depends on the execution of hybridization; hybridization compliance process actually works, also from annually changing external factors, which is consistent with the findings published by Kormuťák et al [2013]. In 2012, seed infestation by insects was particularly large. Also, the increased number of poorly developed seeds was reported in a subset of

seed orchard 3 in Seč at Prostějov where success is traditionally higher. To what extent is this phenomenon influenced by weather cannot be quite pinpointed.

Table 3. Median of needles characteristics in hybrid combinations and pure species

| Hybrid combinations | | | Pure species [Liu 1971] | | |
|---|---|---|---|---|---|
| Clones | length (cm) | width (mm) | length (cm) | width (mm) | Species |
| (*A. koreana* × CZ) × PC | 1.86 | 1.93 | 2.5–4 | 1.5–1.8 | *Abies cilicica* |
| CZ1 × PC | 2.09 | 1.9 | 1.5–3.5 | 2–2.5 | *Abies cephalonica* |
| CZ2 × PC | 1.69 | 1.94 | 1–2 | 2–2.5 | *Abies koreana* |
| CZ2 × FF84 | 2.2 | 1.98 | 1–2 | 2.2 | *Abies fraseri* |
| CZ2 × FF73 | 2.19 | 2.07 | | | |
| CZ2 × FF52 | 2.05 | 1.83 | | | |
| CZ1 × FF73 | 1.84 | 1.92 | | | |
| $F_2$ Prostějov | 2.3 | 1.99 | | | |
| $F_2$ Kostelec | 1.94 | 2 | | | |

CZ1, CZ2 – clones of hybrid *Abies cilicica* × *Abies cephalonica*
F2 – the second filial generation of *Abies cilicica* × *Abies cephalonica*
FF, PC – clones of *Abies fraseri*

On the basis of length and width of the needles it was possible to distinguish certain hybrid combinations, especially those with different needle length where the degree of variation is higher. The observed width of the needles has revealed great variability among different hybrid combinations. In practice, it means that morphology of the needles together with their anatomical structure is an important distinguishing feature for the genus *Abies* [Galgóci 2010] also Kormuťák [2004] which can be considered as an important character in verification of examined hybrids. Table 3 shows comparison of morphological characteristics of needles of hybrid progenies and pure species. The actual morphology is a highly variable character, as confirmed by Liu [1971]. In progenies, it is strongly intermediate character [Klaehn and Winieski 1962].

## CONCLUSIONS

Number of full seeds in tested seed orchards has fluctuated in recent years, which is a long-term trend whose causes are not fully clarified. When hybridizing clones *A. cilicica* × *A. cephalonica* and *A. fraseri*, the best clonal combination achieved around 18%. Normally, however, it falls below 7%. Regularly, a higher percentage of full seeds occurs in the $F_2$ generation *A. cilicica* × *A. cephalonica*. Achieved percentage of its cross-ability with *A. fraseri*, despite considerable evolutionary isolation of the species involved, can be considered to be high. Growth of young seedlings is very good,

as confirmed by the results of complicated hybrid *A. koreana* × (*A. cilicica* × *A. cephalonica*).

Interspecific hybridization is a perspective way of forest tree breeding mainly due to increased vitality and resistance of hybrids and because of their rapid growth due to heterozygosity [Kobliha et al. 2013]. A certain disadvantage is the increased cost of hybrid seeds, which can reach three times the price of conventional seed orchard. The knowledge gained during hybridization experiments is used in genetic conservation programs, traditional tree breeding for forestry and fir breeding for resistance especially in close connection with the production of Christmas trees.

Large degree of variability in crossings between Mediterranean, Asian and American species of fir reflects the very complex relationship between the species and in this respect the author confirms the findings published by Stejskal et al. [2011]. At the same time, however, it gives clear evidence that it is possible to produce viable offspring from these very distant species. Increased resistance of hybrid individuals against root rot caused by fungi of the genus *Phytophthora* should also be stressed. On the basis of results published by Frampton et al. [2013], we can state constantly increasing tendency of hybridization in the genus *Abies*, as fully justified and targeted action with a direct positive impact on forestry.

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## WYNIKI HYBRYDYZACJI PRZY UŻYCIU HYBRYDY *Abies cilicica* × *Abies cephalonica*

**Streszczenie.** *Abies fraseri* (FF) jest północnoamerykańskim iglakiem ważnym z punktu widzenia hodowców choinek świątecznych. Gatunek ten wykazuje 100% śmiertelność po porażeniu zgorzelą korzenia spowodowaną przez *Phytophthora cinnamomi*. Materiał hybrydowy *A. cilicica* × *A. cephalonica* (CZ) oraz *A. koreana* × (*A. cilicica* × *A. cephalonica*) został wykorzystany jako drzewa matczyne do aplikacji pyłku *Abies fraseri.* Poza odpornością na chorobę odgrzybową, badania dotyczyły długoterminowego monitorowania wzrostu młodych siewek oraz wpływu sztucznego krzyżowania na morfologię potomstwa. Oprócz zwiększonej odporności, niektóre młode rośliny wykazały znaczny efekt heterozy, różny wzrost i różny kształt igieł. Wszystko to może w przyszłości dać wiele różnorodnych zastosowań, zarówno w przypadku roślin pod silną presją antropogeniczną przy zmiennych warunkach klimatycznych jak i dla roślin hodowanych do produkcji choinek świątecznych oraz na wybitne odmiany ozdobne. Powodzenie corocznego krzyżowania określono w niniejszym badaniu pryz użyciu promieniowania rentgenowskiego. Najbardziej udanymi kombinacjami w 2012 r. były CZ1 × FF25 i CZ1 × FF76 z 7% pełnych nasion, a następnie CZ2 × FF25 z 6% i CZ2 × FF31 z 4% pełnych nasion. Ocena młodych sadzonek pochodzących z kontrolnego krzyżowania w 2007 r. wskazała na istotnie zaznaczony wzrost złożonej hybrydy (*A. koreana* × (*A. cilicica* × *A. cephalonica*)) × FF PC. Morfologia igieł wykazała różnorodność ich długości w porównaniu z szerokością igieł, która była mniej istotną cechą do odróżnienia kombinacji.

**Słowa kluczowe**: *Abies,* hybrydyzacja, proporcje i morfologia igieł

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