Results of Czech-American cooperation in interspecific fir hybridization in 2008 and 2009

J. Stejskal[1], J. Kobliha[1], J. Frampton[2]

[1]*Department of Dendrology and Forest Tree Breeding, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences in Prague, Czech Republic*

[2]*Christmas Tree Genetics Program, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, North Carolina*

ABSTRACT: This Czech-American research collaboration is investigating interspecific hybridization among various fir species produced via control pollination. Its aim is the development of newly bred material for specific needs of Christmas tree production. The specific target of the breeding is increased growth rate, development of resistance to diseases, insect pests, and limiting environmental conditions (e.g. drought). Experimentation follows a traditional hybridization program of the Czech department focusing on the genus *Abies* and a long-term breeding program of the American department aimed at Christmas tree production. For hybridization, mainly Mediterranean fir species are used together with American species (especially *Abies fraseri*) and other species (e.g. *Abies koreana*). Generally overcoming 5% of viable seeds in the sample can be considered a success. Only few of our hybrid combinations have complied with this condition so far. In 2008 the hybrid combination CZ1 × NC73 brought 16% of viable seeds. In 2009 the most successful hybrid combination CZ1 × FF81 brought 6% of viable seeds. These crossing experiments will initially be followed by *Phytophthora cinnamomi* resistance screening trials.

Keywords: *Abies*; *Abies* fraseri; Christmas tree production; hybridization; *Phytophthora cinnamomi*

In North Carolina a major limiting factor for the culture of true fir Christmas trees is their susceptibility to water moulds of the *Phytophthora* genus. In the local Christmas tree industry alone, over $1.5 million is lost annually due to Phytophthora root rot disease (mainly caused by *Phytophthora cinnamomi* Rands). While chemical methods are available for controlling this disease in seedling and transplant beds, chemical control in plantations is stop-gap at best. Severely infested sites must be abandoned, perhaps permanently, for Fraser fir (*A. fraseri* [Pursh] Poir.) cultivation, threatening the sustainability of Christmas tree production in the region (Frampton 2007).

Fraser fir is the only *Abies* species native to the Southeastern U.S. The systematic research has been supported by extreme economic importance of this fir species. Its utilization as a major Christmas tree species brings over $100 million annually to the industry in North Carolina. North Carolina has recently been the second-leading Christmas tree producing state within the U.S. According to Moody (2007), Agriculture Ext. Agent of Avery County Cooperative Extension Service, Fraser fir production represents 67% of total agricultural income of the county with over 1 million Fraser firs harvested annually. In addition to that, the native forest stands of Fraser fir are located along the Blue Ridge Parkway and Great Smoky Mountains National Park.

Christmas Tree Genetics Program at N.C. State University started July 1, 1996 with the charge to improve Christmas tree species important to the state. Emphasis of the program is on Fraser fir

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. ME914, and by the Program "Fir breeding for forestry and Christmas tree production", Project No. KONTAKT ME 914.

(*Abies fraseri* [Pursh] Poir.) which is grown at elevations above 1,000 m in the western N.C. Mountains (Frampton 2006).

Since genetic resistance is widely used to combat diseases caused by *Phytophthora* spp. in agriculture and horticulture (Erwin, Ribeiro 1996), earlier research efforts focused on identifying resistant Fraser fir material in greenhouse inoculations trials. These trials have confirmed experiences in highly infested Christmas tree plantations that Fraser fir is extremely susceptible to *P. cinnamomi*.

A previous trial that formed the basis for this collaboration was conducted at NCSU. Seedlings of 32 *Abies* species were inoculated with *P. cinnamomi* and showed that North American species are almost completely susceptible while many Mediterranean and Asian species have some trees with resistance. Toros fir (*Abies cilicica* Carr.) from southern Turkey and Greek fir (*A. cephalonica* Loud.) were ranked fourth and eighth, respectively, for the frequency of resistant seedlings (Frampton 2007).

Momi fir (*Abies firma*) from Japan was the most resistant fir within this trial. However, momi fir does not make a desirable Christmas tree due to its coarse branching habit, wide needles, and prickly foliage. Further, it breaks bud 3–4 weeks before Fraser fir making it extremely susceptible to spring frost damage. However, many North Carolina growers have been purchasing greenhouse-produced momi fir to use as rootstock to graft Fraser fir onto and planting the grafts in known Phytophthora-infested areas (Frampton 2009).

Grafting Fraser Fir onto rootstocks of selected *Abies* species may offer a potential solution according to the study of Hinesley (2002) and Frey et al. (2010). Differences in survival appear to reflect interspecific variation in resistance to Phytophthora root rot. Grafting may offer the potential to grow *Abies* Christmas trees on previously unsuitable sites, or to reclaim or continue using sites already seriously impacted by root rot (Hinesley 2002). Grafting is biologically feasible, but the economic feasibility remains to be determined.

Czech University of Life Sciences (CULS) has utilized Toros and Greek fir in a long-term hybrid breeding effort aimed at developing a faster growing fir that are hardier to changing ecological conditions than the native European silver fir (*A. alba* Mill.). As a result of these efforts seeds of F_1, F_2, and complex hybrids with additional fir species are available. Due in parts to collaborative breeding efforts, some of these complex hybrids include Fraser fir, the primary Christmas tree species in North Carolina which is completely susceptible to *P. cinnamomi*. Screening this material for resistance to root rot may progress toward the development of resistant Christmas tree planting stock and also provide insight into the genetic control of resistance (Frampton 2007).

Asexual propagation by stem cuttings could help meet the future demand for elite Fraser fir Christmas trees. Desirable genotypes could be propagated by stem cuttings for preservation and archival purposes as well as for commercial use. The influence of growth stage, auxin type and concentration on the rooting of stem cuttings of Fraser fir was studied by Rosier et al. (2004).

Potter et al. (2005) studied impacts of balsam woolly adelgid on the southern Appalachian spruce-fir ecosystem and the North Carolina Christmas tree industry. Attacking mostly Fraser fir natural stands it can considerably endanger its important seed sources. The balsam woolly adelgid, an exotic aphid-like insect from Europe, has brought a considerable ecological load on the boreal red spruce-Fraser fir ecosystem endemic to the Southern Appalachians. During the last 50 years, the adelgid has decimated the Fraser fir stands that exist on a few of island-like high-elevation ridge systems, and has imposed significant economic costs on the regionally important Christmas tree industry. The virtual elimination of mature fir trees from their natural stands has altered the plant and animal communities unique to the red spruce-Fraser fir forest type.

Apart from its domination in North Carolina, Fraser fir is being utilized in other major Christmas tree growing states such as Washington, Michigan and Oregon. Christmas tree growers encounter different problems in their specific conditions. Four trials are currently underway at Puyallup, WA, to determine the susceptibility of various true firs to Phytophthora root rot. This disease is a common problem encountered in the production of noble fir Christmas trees, particularly at sites with high soil moisture. Eight species of *Phytophthora* have been associated with root rot development on noble fir in Oregon and Washington Christmas tree plantations. The most aggressive species include *P. cactorum*, *P. cambivora*, *P. cinnamomi* and *P. cryptogea* (Chastagner 2009). Altogether 12 fir species were examined in this trial including Fraser fir and its close relative Canaan fir. Fraser fir (mortality of 23%) and white fir were the next most susceptible species after Shasta fir (70%) and noble fir (60%). For instance, less than 5% of the Turkish and Nordmann fir had evidence of root rot (Chastagner 2009). This trial was developed from Oregon and Washington Christmas tree growers' perspective, but brings interesting results and insight into *Phytophthora* resistance, even though these results are preliminary.

This paper is a second of our department's efforts on this topic in this Journal. Its aim is to present results of 2008 and 2009 control crossings following the same methods and describing similar material. Hybridizations of previous years 2006, 2007 were published by Kobliha and Stejskal (2009).

MATERIAL AND METHODS

Experimental plots

All of the Czech seed orchards were founded as biclonal – grafts originated from 2 interspecific hybrids of the first generation F_1 *Abies cilicica* × *Abies cephalonica*. 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, Kostelec nad Černými lesy. Secondary grafts were taken to establish the above-mentioned hybridization seed orchards.

Hybridization seed orchards with the presence of female strobili before 2006 had been utilized mainly for the 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 Truba breeding station near Kostelec n. Č. l. from the material grafted in 1991 and 1992. Original number of 217 grafts with 4 × 2 m spacing was reduced due to secondary waterlogging to current number of 154. Clone CZ2 is represented to a lesser extent – 30 grafts. Female flowering was observed in 2004, 2006–2008.

Hybridization seed orchard No. 2 was established in May 1996 close to the Truba breeding station in a form of two long rows (one clone in each row) by planting material grafted in 1993. There has been no mortality so far, though the locality is rather dry. Flowering has been observed sporadically since 2008.

Hybridization seed orchard No. 3 was established in 1997 from the material grafted in 1993 within a nursery by the village of Seč near Prostějov. Together 200 grafts were planted in a row along a fence (100 grafts per clone). Clone CZ1 is alternated by clone CZ2 at a spacing of 3 m. This outplanting is generally in a very good shape and mortality has been quite exceptional there. Female coning was registered in 2003–2009.

Hybridization seed orchard No. 4 was established in May 1999 within the school enterprise in Kostelec n. Č. l. in forest stand 20 A 9 by planting 298 grafts (159 of clone 2) at a 3 × 3 m spacing. Covered area has around 0.31 ha. Grafts were planted in 20 rows; about 15 trees in each row. This plantation began to cone in 2008 and enormous coning occurred in spring 2009.

One of the experimental plots involved in our recent hybridization trials belongs to a long-term experiment with spontaneous hybrid ancestries established in 1996. After significant mortality in the first year new material *A. koreana* × *(Abies cilicica* × *Abies cephalonica)* hybrids were brought (in 1997) as 5-year old seedlings. Originally 2 plots were established with 25 trees each without significant mortality. These hybrids began to cone in 2004 and female strobili have been observed annually.

2008

In spring 2008 pollination took place in three out of the four seed orchards (1, 3, and 4). The pollen of *Abies fraseri* was obtained from North Carolina State University. More specifically we obtained the frozen pollen of clones NC73, NC52, NC84, NC136 and a polymix of these clones collected in 2006. In Czech seed orchards the pollen of *Abies cilicica* × *Abies cephalonica* hybrid (clones CZ1 and CZ2) was collected. This pollen from seed orchard No. 1 has been frozen.

In addition to that the application of pollen of other species, concretely *Abies balsamea* and *Abies fraseri* originating from Kostelec Arboretum and *Abies koreana* from Průhonice Arboretum, was tried in seed orchard No. 3.

Control pollination was performed in spring 2008 (beginning April 25th) in seed orchard No. 1, No. 3 and for the very first time also in seed orchard No. 4. Applied was the pollen of *A. fraseri* (NC52, NC73) with a negligible part of open pollinated cones (F_2 Kostelec). In seed orchard No. 1 pollen was applied to 11 ramets of the clone CZ1. In seed orchard No. 4 pollen was applied to 7 ramets of the clone CZ1 and 1 ramet of the clone CZ2. One week later during pollination in seed orchard No. 3 there was a similar situation – pollen of *Abies fraseri* was used (NC73, NC84, PC, NC136), plus extra *A. balsamea, A. koreana, A. fraseri* and occasional open pollination (F_2 Prostějov). In seed orchard No. 3 pollen was applied to 41 CZ1 and 29 CZ2 ramets. Detailed description of all hybrid combinations can be found in Table 1.

Female strobili were isolated by thin paper bags in a period of the highest receptibility. For control pollinations we used a set of brushes to utilize the restricted amount of pollen most effectively. The same pollination method was preferred in all plantations. Plastic vials with pollen were transported in styrofoam boxes filled with frozen aggregates.

In the last week of August cones were collected in Kostelec and Prostějov. All the cones were stored in Truba greenhouse facilities near Kostelec. During autumn, cone and seed processing similar to that of 2007 was performed to provide conclusions about pollination results. Cones were dried in the greenhouse environment with average temperature of 18°C. After several weeks cones felt apart completely. The already dry seeds were processed in our small (single drum) machine. All the seed lots were afterwards stored in a refrigerator at 5°C before they were either sown or shipped to the USA. Cones were measured and examined and so were the seeds. Seed samples of the individual seed lots were X-rayed in early October for assessment of the final share of full seeds. Because a relatively small percentage of viable seeds was obtained from most samples, the sample number was multiplied later. We ended up with a final sample size of 300 X-rayed seeds. *Phytophthora* screenings were planned by the American partner for December 2009.

Table 1. Number of ramets pollinated for specific hybrid combinations

	2008			2009		
	1	4	3	91	4	3
CZ1 × NC52	11	4	–	5	–	–
CZ1 × NC73	–	3	11	–	–	–
CZ1 × NC136	–	–	6	2	–	–
CZ2 × NC136	–	–	7	–	–	–
CZ1 × NC84	–	–	9	–	4	–
CZ1 × PC	–	–	7	–	5	–
CZ2 × PC	–	–	6	–	3	–
CZ1 × *A. bal.*	–	–	1	–	–	–
CZ1 × *A. fras.*	–	–	1	–	–	–
CZ2 × *A. fras.*	–	–	2	–	–	–
CZ1 × *A. kor.*	–	–	5	1	–	–
CZ2 × *A. kor.*	–	–	5	–	–	–
CZ1 × FF81	–	–	–	4	7	–
CZ1× NC143	–	–	–	4	–	–
CZ1 × NC154	–	–	–	4	–	–
CZ1 × FF24	–	–	–	4	–	–
CZ1 × NC72	–	–	–	4	–	–
CZ1 × *A. umb.*	–	–	–	2	–	–
CZ2 × FF24	–	–	–	1	–	–
CZ2 × NC52	–	1	–	1	–	–
CZ2 × NC55	–	–	–	–	9	–
CZ2 × NC73	–	–	6	–	–	–
CZ2 × NC84	–	–	3	–	2	–

2009

In spring 2009 (beginning May 1^{st}) pollination took place in two out of the four seed orchards (1 and 4). The pollen of *Abies fraseri* was obtained from North Carolina State University. We obtained the frozen pollen of clones NC52, NC55, NC72, FF81, FF24, NC84, NC136, NC143, NC154 and a polymix collected in the Appalachians in 2006 and 2008. In seed orchard No. 1 pollen was applied to 29 ramets of the clone CZ1 and 2 ramets of the clone CZ2. In seed orchard No. 4 pollen was applied to 16 ramets of the clone CZ1 and 14 ramets of the clone CZ2. Detailed description of all hybrid combinations can be found in Table 1.

The same pollination method as in 2008 was preferred in all plantations. We used a set of brushes to utilize the restricted amount of pollen most effectively. Plastic vials with pollen were transported in styrofoam boxes filled with frozen aggregates.

The pollen of *Abies cilicica* × *Abies cephalonica* hybrid (clones CZ1 and CZ2) was collected in seed orchard No. 1 during the pollination period and later dried and stored in sealed vials with CaCl in a refrigerator (–18°C). This collection of pollen was later shipped to the USA under special conditions (sealed vials stored in blue ice).

Our 2009 pollination was restricted only to seed orchards located in Kostelec because of a logistical convenience. Seed orchards 1 and 4 offered enough female strobili, so that *A. fraseri* pollen could have been applied only on these sites.

Applied pollen was *A. fraseri* (the above-mentioned clones) with a negligible part of open pollinated cones (F_2 Kostelec). Available *A. fraseri* clones were split between the two orchards. In addition to that, we applied the freshly collected pollen of *Abies koreana* and *Abies* x *umbellata* from the arboretum in Kostelec.

Later that autumn, cone and seed processing similar to that of 2007, 2008 was managed to make conclusions about pollination results.

Table 2. Mating in seed orchard No. 1and No. 4, Kostelec nad Černými lesy – Truba, 2008

	Combination	
	F_2 (open pollination)	CZ1 × NC52
No.1		
Number of cones	5	37
Average cone length (cm)	15	16
Total cone weight (g)	600	4,200
Average weight of 1 cone (g)	120	114
Total weight of seeds (g)	52	401
Average weight of seeds in 1 cone (g)	10	11
Absolute weight of 1,000 seeds (g)	70	57
Total number of seeds	745	7,030
Average number of seeds in 1 cone	149	190
Full seed fraction in a sample (%)	49	0
Expected number of full seeds	365	0
No. 4	CZ1 × NC73	CZ1 × NC52
Number of cones	4	6
Average cone length (cm)	15	15
Total cone weight (g)	450	700
Average weight of 1 cone (g)	113	117
Total weight of seeds (g)	50	69
Average weight of seeds in 1 cone (g)	13	12
Absolute weight of 1,000 seeds (g)	53	49
Total number of seeds	948	1,416
Average number of seeds in 1 cone	237	236
Full seed fraction in a sample (%)	0	1
Expected number of full seeds	0	14

RESULTS AND DISCUSSION

2008

Most of the seeds were sown within our American partner facilities as opposite to the year 2007. They were shipped to the USA after phytosanitary inspection accompanying the pollen (on blue ice). Sowing and Phytophthora resistance screening tests were in responsibility of our American partner.

The F_2 *Abies cilicica* × *Abies cephalonica* (F_2 Prostějov) seed lot remained in the Czech Republic. It was sown within Truba facilities. According to 2009 observation, this seed lot did not germinate at all. A rather small amount of the open pollinated material was again granted to somatic embryogenesis research in our department.

Individual hybrid combinations brought significantly different results in comparison with the year 2007 (Kobliha, Stejskal 2009). It is rather impossible to trace any trend in performance of any hybrid combination. The most successful hybrid combination CZ1 × NC73 brought 16% of viable seeds. CZ1 × PC (10%), CZ1 × NC136 (7%) and CZ2 × PC (4%) can also be considered successful. Hybridizing *Abies fraseri* with Mediterranean fir species is a pioneer effort. Our results are quite incomparable with other works. However, we mostly compare our results with so called transatlantic hybridizations (ex. *Abies cephalonica* × *Abies grandis*). Kantor and Chira (1971) used *Abies cephalonica* as a mother tree. The application of *A. cilicica*, *A. alba* and *A. nordmanniana* resulted in 14% of germinating seedlings at least. Utilizing *A. concolor*, *A. grandis* and *A. pinsapo* lowered the germination rate to 0.9–3.3%. In addition to that using *A. grandis* as a mother tree was found to be very ineffective. Some seedlings were obtained (1.9%) only when *A. concolor* pollen was used. Other combinations were unsuccessful.

Table 3. Mating in seed orchard No. 3, Prostějov – Seč, 2008

	Combination					
	CZ1 × NC73	CZ1 × NC136	CZ2 × NC136	CZ1 × NC84	CZ1 × PC	CZ2 × PC
Number of cones	21	24	17	49	20	33
Average cone length (cm)	16	17	16	16	17	16
Total cone weight (g)	2,600	2,800	1,600	6,600	2,750	3,950
Average weight of 1 cone (g)	124	117	94	135	138	120
Total weight of seeds (g)	308	390	202	711	282	408
Average weight of seeds in 1 cone (g)	15	16	12	15	14	12
Absolute weight of 1000 seeds (g)	59	61	63	61	65	59
Total number of seeds	5,229	6,360	3,230	11,613	4,340	6,963
Average number of seeds in 1 cone	249	265	190	237	217	211
Full seed fraction in a sample (%)	16	0	7	0	10	4
Expected number of full seeds	837	0	226	0	434	279
	CZ1 × *A. bal.*	CZ1 × *A. fras.*	CZ2 × *A. fras.*	F_2 (open poll.)	CZ1 × *A. kor.*	CZ2 × *A. kor.*
Number of cones	21	1	6	40	3	10
Average cone length (cm)	16	17	17	16	16	16
Total cone weight (g)	2,900	150	800	4,820	330	1,050
Average weight of 1 cone (g)	138	150	133	121	110	105
Total weight of seeds (g)	302	20	102	589	54	164
Average weight of seeds in 1 cone (g)	14	20	17	15	18	16
Absolute weight of 1000 seeds (g)	59	121	65	65	65	64
Total number of seeds	5,145	165	1,560	9,040	834	2,580
Average number of seeds in 1 cone	245	165	260	226	278	258
Full seed fraction in a sample (%)	0	0	0	18	0	0
Expected number of full seeds	0	0	0	1,627	0	0

Generally, hybridizations tend to be successful in species with overlapping areas (up to 60% field germination). On the other hand, hybridizing species with distant natural areas yielded 29% of germinating seedlings at maximum (Mergen et al. 1964).

A high level of crossability was confirmed only by Mediterranean fir species in works of Greguss (1984, 1986, 1988a,b, 1992) and Kormuťák (1984, 1985, 1986, 1992). North American fir species appeared to be reproductively isolated not only from Mediterranean species but also within themselves according to Mergen et al. (1964), Hawley and DeHayes (1985), Critchfield (1988).

Detailed control pollinations results are outlined in Tables 2 and 3.

2009

Most of the seeds were shipped to the USA for *Phytophthora* resistance screenings early in 2010 when just the F_2 *Abies cilicica* × *Abies cephalonica* (F_2 Prostějov) seed lot remained in the Czech Re-

Table 4. Mating in seed orchard No. 1, Kostelec nad Černými lesy – Truba, 2009

	Combination					
	CZ1 × FF81	CZ1 × NC136	CZ1 × NC143	CZ1 × NC154	CZ1 × FF24	CZ1 × NC52
Number of cones	2	28	35	26	32	15
Average cone length (cm)	14	15	14	15	15	15
Total cone weight (g)	250	3,500	3,900	3,000	4,500	1,900
Average weight of 1 cone (g)	125	125	111	115	141	127
Total weight of seeds (g)	24	290	310	230	330	120
Average weight of seeds in 1 cone (g)	12	10	9	9	10	8
Absolute weight of 1,000 seeds (g)	53	57	52	51	55	52
Total number of seeds	450	5,123	5,950	4,490	6,036	2,313
Average number of seeds in 1 cone	225	183	170	173	189	154
Full seed fraction in a sample (%)	1	1	1	2	1	1
Expected number of full seeds	5	51	60	90	60	23
	CZ1 × NC72	CZ1 × *A. umb.*	CZ1 × *A. kor.*	F_2	CZ2 × FF24	CZ2 × NC52
Number of cones	22	30	14	30	3	1
Average cone length (cm)	14	15	16	16	15	12
Total cone weight (g)	3,000	3,900	?	3,600	500	150
Average weight of 1 cone (g)	136	130	?	120	167	150
Total weight of seeds (g)	200	350	150	380	20	9
Average weight of seeds in 1 cone (g)	9	12	11	13	7	9
Absolute weight of 1,000 seeds (g)	50	52	57	64	50	46
Total number of seeds	3,972	6,683	2,625	5,951	402	200
Average number of seeds in 1 cone	181	223	188	198	134	200
Full seed fraction in a sample (%)	1	2	1	56	1	0
Expected number of full seeds	40	134	26	3,332	4	0

public. This specific seed lot came from seed orchard No. 3 located near Prostějov, which has yielded the biggest cone crop to date. A rather small amount of the open pollinated material was again granted to somatic embryogenesis research in our department.

The most successful hybrid combination CZ1 × FF81 brought 6% of viable seeds. Other combinations resulted in hardly any viable seeds spanning from 1 to 2%.

Detailed control pollinations results are outlined in Tables 4–6.

CONCLUSIONS

As the hybridizations of 2007 showed some promising results, we assumed that the 2008 experiment could bring us a similar percentage of viable seeds. Generally overcoming the usual 5% of viable seeds in the sample would be highly surprising (in terms of the interspecific hybrids that we work with).

However, the results of 2008 were slightly different in terms of the viable seed percentage. A common trait of both seasons may be significantly different performance of different hybrid combina-

Table 5. Mating in seed orchard No. 4, Kostelec nad Černými lesy – Truba, 2009

Combination	CZ1 × PC	CZ2 × PC	CZ2 × NC55	CZ1 × NC84	CZ1 × FF81	F_2
Number of cones	15	9	18	13	14	5
Average cone length (cm)	16	16	14	14	12	16
Total cone weight (g)	2,300	1,400	2,100	1,300	1,150	650
Average weight of 1 cone (g)	153	156	117	100	82	130
Total weight of seeds (g)	190	90	130	130	80	50
Average weight of seeds in 1 cone (g)	13	10	7	10	6	10
Absolute weight of 1,000 seeds (g)	56	61	46	47	42	54
Total number of seeds	3,410	1,469	2,824	2,781	1,894	921
Average number of seeds in 1 cone	227	163	157	214	135	184
Full seed fraction in a sample (%)	0	0	0	1	6	46
Expected number of full seeds	0	0	0	28	114	424

Table 6. Mating in seed orchard No. 3, Prostějov – Seč, 2009

Combination	F_2
Number of cones	30
Average cone length (cm)	16
Total cone weight (g)	3,750
Average weight of 1 cone (g)	125
Total weight of seeds (g)	360
Average weight of seeds in 1 cone (g)	12
Absolute weight of 1,000 seeds (g)	55
Total number of seeds	6,511
Average number of seeds in 1 cone	217
Full seed fraction in a sample (%)	23
Expected number of full seeds	1,498

tions. It seems that seed orchards 1 and 3 brought different results each year, but this can be only an assumption. The cause for that is unknown and a complex investigation of this incompatibility is beyond the scope of the project.

In 2008, we excluded *A. koreana* × (*Abies cilicica* × *Abies cephalonica*) from the hybridization in favour of the more promising F1 *Abies cilicica* × *Abies cephalonica*. Also one new taxon was included – *Abies balsamea*. This idea was based on its close relationship to *Abies fraseri*, so it can work as a related substitute when running out of *A. fraseri* pollen.

In 2009 *A. koreana* × (*Abies cilicica* × *Abies cephalonica*) hybrids were again skipped from the pollinations. Seed orchards located in Kostelec n.Č.l. fructified sufficiently, which resulted in control pollinations being restricted to Kostelec. After all available *A. fraseri* pollen was applied, we tested the pollen of *Abies koreana* and *Abies numidica* from a local source.

Most of the hybrid combinations did not yield more than 1% of viable seeds according to X-rays. Only the combination CZ1 × FF81 (Kostelec 4) resulted in 6% of viable seeds. All seed lots with any chance of future germination were sent to the USA. 2009 brought an enormous cone harvest in general. Seed orchard No. 3 located in Prostějov yielded almost 700 kg of open pollinated cones, which resulted in almost 70 kg of seeds. Control X-rays showed nearly 25% of viable seeds in this concrete material. Later in October this open pollinated F_2 material was sown at the facility of Military Forests of the Czech Republic.

However, as the transport of most seeds from that year's harvest to the USA was organized, their sowing in our facilities was not planned. At this point *Phytophthora* resistance screenings performed at NCSU are strongly preferred by both sides, for they will provide the most important results and a needed feedback to us. After a completion of these tests, it will be much easier to pick the most promising hybrid combinations for our future work.

References

Critchfield W. B. (1988): Hybridization of the California firs. Forest Science, ***34***: 139–151.

Erwin D.C., Ribeiro O.K. (1996): *Phytophthora* Diseases Worldwide. St. Paul, APS Press: 562.

Frampton J. (2004): Exotic fir research in North Carolina. Exotic Conifer News, 14: 5–8.

Frampton J. (2009): The search for fir resistant to phytophthora root rot. In: Proceedings of the Great Lakes Fir

Conference II: Growing True Fir in the Great Lakes Region. Grand Rapids, 21.–22. September 2009. Grand Rapids, Michigan State University: 14–15.

Frampton J., Isik F. (2006): Conservation and breeding of mediterranean conifers. In: Proceedings of the IUFRO Division 2 Joint Conference: Low Input Breeding and Conservation of Forest Genetic Resources. Antalya, 9.–13. October 2006. Antalya, IUFRO: 60.

Greguss L. (1984): Program of interspecific fir hybridization and its realization. In: Proceedings of the National Seminar Hybridization and Variability of Forest Tree Species. Zvolen, 22.–23. May 1984. Zvolen, Vysoká škola lesnícka a drevárska: 117–122. (in Slovak)

Greguss L. (1986): Breeding program for increasing a hardiness of fir by hybridization. In: Proceedings of the 7th National Breeding Conference. Spišská Nová Ves, 14.–16. October 1986. Žilina, Dom techniky ČSVTS: 34–41. (in Slovak)

Greguss L. (1988a): Permanent experimental Plot with Hybrid Firs Drieňová. Excursion Guidebook. Banská Štiavnica, VÚLH Zvolen: 9. (in Slovak)

Greguss L. (1988b): Interspecific hybridization – substitute for fading European Silver fir. Lesnictví, ***34***: 797–808. (in Slovak)

Greguss L. (1992): Evaluation of initial growth of interspecific fir hybrids on an example of permanent experimental plot Drieňová Lesnícky časopis – Forestry Journal, ***38***: 223–238. (in Slovak)

Hawley G.J., de Hayes D.H. (1985): Hybridization among several North American firs. I. Crossability. Canadian Journal of Forest Research, ***15***: 42–49.

Hibbert-Frey H., Frampton J., Balzich F.A., Hinesley E. (2010): Grafting Fraser fir (*Abies fraseri*): Effect of grafting date, shade, and irrigation. HortScience, ***45***: 617–620.

Hinesley E., Frampton J. (2002): Grafting fraser fir onto rootstocks of selected *Abies* species. HortScience, ***37*** (5): 1–4.

Chastagner G. (2009): Susceptibility of true firs to phytophthora root rot. In: 9th International Christmas Tree Research and Extension Conference Field Tour Guide. Puyallup, 18. September 2009. Pullman, Washington State University: 24–28.

Kantor J., Chira E. (1971): On the possibility of crossing certain species of the genus *Abies*. Acta Universitatis Agriculturae, ***40***: 15–27.

Kobliha J., Stejskal J. (2009): Recent fir hybridization research in the light of Czech-American cooperation. Journal of Forest Science, ***56***: 162–170.

Kormuťák A. (1984): Hybridization as a mean of forest tree breeding. In: Proceedings of the National Seminar Hybridization and Variability of Forest Tree Species. Zvolen, 22.–23. May 1984. Zvolen, Vysoká škola lesnícka a drevárska: 45–52. (in Slovak)

Kormuťák A. (1985): Study on species hybridization within the genus *Abies*. Acta Dendrobiologica: 127.

Kormuťák A. (1986):Height growth of selected species of exotic firs and their hybrids. In: Proceedings of the 7th National Breeding Conference, Spišská Nová Ves, 14.–16. October 1986. Žilina, Dom techniky ČSVTS: 123–131. (in Slovak)

Kormuťák A. (1992): Hybridization of *Abies concolor* (Gord. et Glend./Lindl.) and *Abies grandis* (Dougl./Lindl.) in Slovakia. Lesnictví – Forestry, ***38***: 759–769. (in Slovak)

Liu T.S. (1971): A Monograph of the Genus *Abies*. Taipei, Taiwan University: 608.

Mergen F., Burley J., Simpson B.A. (1964): Artificial hybridization in *Abies*. Der Zuchter, ***34***: 242–251.

Potter K.M. (2006): Evolutionary History and Genetic Conservation of Fraser Fir (*Abies fraseri* [Pursh] Poir.). [Ph.D Thesis.] Raleigh, NC State University.

Potter K., Frampton J., Sidebottom J. (2005): Impacts of Balsam Woolly Adelgid in Southern Appalachia and North Carolina. In: Proceedings of Third Symposium on Hemlock Woolly Adelgid. Asheville, 1.–3. February 2005. Morgantown, Forest Health Technology Enterprise Team: 25–41

Rosier C., Frampton J., Goldfarb B., Blazich F., Wise F. (2004): Growth stage, auxin type, and concentration influence rooting of stem cuttings of fraser fir. HortScience, ***39***: 1397–1402.

Received for publication August 11, 2010
Accepted after corrections November 19, 2010

Corresponding author:
Ing. Jan Stejskal, Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences,
Kamýcká 1176, 165 21 Praha 6-Suchdol, Czech Republic
e-mail: stejskalj@fld.czu.cz