JOURNAL OF FOREST SCIENCE, *59*, 2013 (2): 64–71

Recent results of Czech-American fir hybridization research

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ABSTRACT: Fraser fir (*Abies fraseri)* Christmas tree plantations in North Carolina are infested by root rot caused by *Phytophthora cinnamomi*. This disease kills almost 100% of Fraser fir material and leaves the soil permanently infested. Control crossings of Mediterranean fir hybrids *Abies cilicica* × *Abies cephalonica* with *Abies fraseri* were performed to ensure possibly resistant hybrid material of desired Christmas tree parameters. Pollen of various clones of *A. fraseri* was shipped to the Czech Republic by NCSU. Control pollinations were performed in April/May 2010 and 2011 in our hybridization seed orchards. Female strobili were isolated in the period of their highest receptibility. Cones were collected during September. Cones were dried and completely disintegrated. Basic parameters of cones and seeds were assessed for each seed lot. Seed samples of the individual seed lots were X-rayed for assessment of the final share of full seeds. In 2010, the most successful combination CZ2 × NC81 brought 7% of viable seeds. In 2011 the most successful combination was CZ2 × NC26 with 18% of viable seeds.

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

The Phytophthora root rot disease is a limiting factor in the North Carolina Christmas tree plantations. In the local fir Christmas tree industry, over 1.5 million USD is annually lost (mainly caused by *Phytophthora cinnamomi* Rands). 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 cultivation, threatening the sustainability of Christmas tree production in the region (Frampton 2005).

Fraser fir is the only *Abies* species native to the southeastern U.S. Its utilization as a major Christmas tree species brings over 100 million USD 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 the personal communication by Jerry Moody (2007), Agriculture Extension 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.

Christmas Tree Genetics Program at North Carolina 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 (*Abies fraseri* [Pursh] Poir.), which is grown in the western N.C. Mountains (Frampton 2006). Genetic resistance is widely used to combat diseases caused by *Phytophthora* spp. in agriculture

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. ME 914, by the Ministry of Agriculture of the Czech Republic, Project No.QH81160, and by the Czech University of Life Sciences Prague, Project No. IGA 20124329.

and horticulture (Erwin, Ribeiro 1996). Earlier research efforts of NCSU focused on identifying resistant Fraser fir material in greenhouse inoculation trials. These trials have confirmed experiences in highly infested Christmas tree plantations that Fraser fir is extremely susceptible to *P. cinnamomi.*

American species are almost completely susceptible to *P. cinnamomi,* while many Mediterranean and Asian species possess some degree of resistance. Toros fir (*Abies cilicica* Carr.) from southern Turkey and Greek fir (*A. cephalonica* Loud.) took the front places for the frequency of resistant seedlings (Frampton 2007). 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 (*Abies procera* Rehd.) Christmas trees (Chastagner 2009), 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 (*Abies balsamea* var. *phanerolepis* Fern.). Fraser fir (mortality of 23%) and white fir (*Abies concolor* [Gord.] Hopes.) were the next most susceptible species after Shasta fir (*Abies magnifica* var. *shastensis*) (70%) and noble fir (*Abies procera* Rehd.) (60%). For instance, less than 5% of the Turkish (*A. bornmuelleriana*) and Nordmann (*Abies nordmanniana* [Steven] Spach.) 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.

Momi fir (*Abies firma* [Siebold] Zucc.) from Japan was the most resistant fir within NCSU test (Frampton 2007). 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 (Hinesley, Frampton 2002). Grafting Fraser Fir onto rootstocks of selected *Abies* species may offer a potential solution according to the study of Hinesley and Frampton (2002) and Hibbert-Frey et al. (2010). Differences in survival appear to reflect interspecific variation in resistance to Phytophthora root rot. Grafting is biologically feasible, but the real economic feasibility remains to be determined.

The Czech University of Life Sciences Prague (CULS) has utilized Toros and Greek fir in a long-term hybrid breeding effort aimed at developing a faster growing fir that is 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, 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).

The aim of this paper is to present results of 2010–2011 control crossings following the same methods and describing similar material as in past years. Hybridizations of previous years 2006, 2007, 2008, 2009, 2010 were published by Kobliha and Stejskal (2009) and Stejskal et al. (2011).

MATERIAL AND METHODS

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*, 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 a 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 at the Truba Breeding Station near Kostelec nad Černými lesy. Secondary grafts were taken to establish mentioned hybridization seed orchards. Before 2006 hybridization seed orchards with the presence of female strobili were used 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 Truba Breeding Sta-

tion from the material grafted in 1991 and 1992. The original number of 217 grafts with 4 × 2 m spacing was reduced due to secondary waterlogging to the current number of 154. Clone CZ2 is represented to a lesser extent – 30 grafts. Female flowering has been observed since 2004.

Hybridization seed orchard No. 2 was established in 1996 in the demonstration forest enterprise Kostelec nad Černými lesy in forest stand 20A2 by the planting of 70 grafts (35 clones CZ1 and the same number of clone CZ2). The orchard was designed in two lines, each line accounts for 35 grafts from the same clone at a spacing of 2 × 2 m. First flowering was detected in 2008.

Hybridization seed orchard No. 3 was established in 1997 from the material grafted in 1993 in a nursery near the village of Seč near Prostějov. In total 200 grafts were planted in a row along the 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 registered since 2003.

Hybridization seed orchard No. 4 was established in May 1999 in the demonstration forest enterprise Kostelec nad Černými lesy in forest stand 20A9 by the planting of 298 grafts (139 clones CZ1, 159 clones CZ2) 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.

Hybrid progeny test was established in 1996 beside seed orchard No. 4. The complicated hybrid *Abies koreana* (*Abies cilicica* × *Abies cephalonica*) has recently been used for control pollinations. This hybrid material shows three overlapping introgression forms. Each individual tree is genetically unique.

In March 2010 pollen of *Abies fraseri* was obtained from North Carolina State University. More specifically, we obtained frozen pollen of clones NC23, NC24, NC41, NC42, NC46, NC53, NC81, NC85, NC95, NC96, NC125, NC184 collected in 2008 and pollen of clones NC136, NC25, NC72, NC55, NC54, NC52, NC51, NC43 collected in 2006. In Czech seed orchard No. 1 pollen of *Abies cilicica* × *Abies cephalonica* hybrid (clones CZ1 and CZ2) has been collected annually since 2006 (Kobliha, Stejskal 2009; Stejskal et al. 2011). This pollen has been frozen and spared for the control pollinations in the USA. Control pollination was performed in spring 2010 (beginning on April 25) in seed orchard No. 1, No. 3 and also in seed orchard No. 4. Applied was pollen of *A. fraseri* (NC81). In seed orchard No. 1 pollen was applied to ramets of clone CZ1. In seed orchard No. 4 pollen was applied to ramets of clone CZ2. One week later during pollination in seed orchard No. 3 there was a similar situation – pollen of *Abies fraseri* was used (NC125, NC23, NC184) with the occasional open pollination (F_2 Prostějov). In seed orchard No. 3 *A. fraseri* pollen was applied to 9 CZ1 and 7 CZ2 ramets. Detailed description of all pollination combinations is given in Tables 1–3.

In 2011 pollen of *A. fraseri* clones NC11, NC26, NC42, NC44, NC46, NC51, NC53, NC54, NC97, NC113, NC142, NC153 was obtained from the USA. Control pollination was performed on April 26–27 in seed orchard No. 1 (pollen of *A. fraseri* NC51 and NC113). The pollen was applied to 11 ra-

Table 1. Mating in seed orchard No. 1, Kostelec nad Černými lesy – Truba, 2010/2011

	2010	2011	
Combination	CZ1 × NC81	CZ 1 × NC113	CZ 1 × NC51
Number of cones	2	6	12
Average cone length (cm)	16	12	11
Total cone weight (g)	250	410	860
Average weight of 1 cone (g)	125	68	72
Total weight of seeds (g)	25	38	67
Average weight of seeds in 1 cone (g)	13	6	6
Absolute weight of 1,000 seeds (g)	49	62	63
Total number of seeds	510	616	1,062
Average number of seeds in 1 cone	255	103	88
Full seed fraction in a sample (%)	5	**2**	**0**
Expected full seed number	26	1	3

In bold – highlighted full seed fraction in a sample, which illustrates a crossing success

Table 2. Mating in seed orchard No. 4, Kostelec nad Černými lesy – Truba, 2010/2011

	2010	2011						
Combination	CZ2 × NC81	CZ1 × NC54	CZ1 × NC44	CZ1 × NC26	CZ2 × NC97	CZ1 × NC42	CZ1 × NC53	CZ1 × NC97
Number of cones	3	20	30	22	10	21	8	5
Average cone length (cm)	13	12	14	13	16	14	12	12
Total cone eight (g)	250	1,380	2,420	1,760	890	1,450	680	630
Average weight of 1 cone (g)	83	69	81	80	89	69	85	126
Total weight of seeds (g)	32	118	325	189	110	213	63	45
Average weight of seeds in 1 cone (g)	11	6	11	9	11	10	8	9
Absolute weight of 1000 seeds (g)	45	54	47	50	53	63	66	61
Total number of seeds	708	2,173	6,900	3,803	2,068	3,392	962	744
Average number of seeds in 1 cone	236	109	230	173	207	162	120	149
Full seed fraction in a sample (%)	**7**	**1**	0	0	**1**	0	**1**	**2**
Expected full seed number	50	22	0	0	21	0	10	15

In bold – highlighted full seed fraction in a sample, which illustrates a crossing success

mets of clone CZ1. In seed orchard No. 4 pollen of clones NC26, NC42, NC44, NC53, NC54, NC97 was used. 24 ramets of clone CZ1 and 3 ramets of clone CZ2 were pollinated.

Recently also seed orchard No. 2 was utilized for control crossings. Due to shade and poor soil conditions its cone yields have been limited so far. Two ramets of clone CZ1 and 3 ramets of clone CZ2 were pollinated. In seed orchard No. 2 pollen of clones NC51 was used.

In seed orchard No. 3 control pollination was done on 28 April 2011. Three ramets of clone CZ1 and 3 ramets of clone CZ2 were pollinated with Fraser fir clones NC11, NC26, NC42, NC53 and NC153.

Hybrid progeny test established in 1996 was included in 2011 experiment. Pollen of Fraser fir clones NC11 and NC51 was applied to the complicated hybrid *Abies koreana* (*Abies cilicica* × *Abies cephalonica*).

Female strobili were isolated with thin paper bags in the period of 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 performed in all plantations. Plastic vials with pollen were transported in styrofoam boxes filled with frozen aggregates.

During the last week of August cones were collected in Kostelec nad Černými lesy and Prostějov. All the cones were stored in Truba greenhouse facilities near Kostelec. During the autumn, cone and seed processing similar to that of 2007, 2008 and 2009 (Kobliha, Stejskal 2009; Stejskal et al. 2011) and in 2011 was performed. Cones were dried in a greenhouse with an average temperature of 18°C. After several weeks cones completely disintegrated. The already dry seeds were processed in our small (single drum) machine. All the seed lots were stored at 5°C before their shipping 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. We ended up with a final sample size of 400 X-rayed seeds for each seed lot. Four samples with 100 seeds were averaged in the results (Tables 1–5).

RESULTS

In 2010, the most successful hybrid combination was CZ2 × NC81 with 7% of viable seeds followed by CZ1 × NC81 with 5%, CZ1 × NC125 with 4% and CZ1 × NC23 with 3%. Other hybrid combinations bore at least 2% of viable seeds. This was a major difference compared to the past seasons of pollina-

Table 3. Mating in seed orchard No. 2, Kostelec nad Černými lesy – Truba, 2011

	Combination	
	CZ1 × NC51	CZ2 × NC51
Number of cones	12	3
Average cone length (cm)	11	11
Total cone weight (g)	940	380
Average weight of 1 cone (g)	78	127
Total weight of seeds (g)	79	15
Average weight of seeds in 1 cone (g)	7	5
Absolute weight of 1,000 seeds (g)	49	45
Total number of seeds	1,629	333
Average number of seeds in 1 cone	136	111
Full seed fraction in a sample (%)	0	**1**
Expected full seed number	0	3

In bold – highlighted full seed fraction in a sample, which illustrates a crossing success

tion experiments. These seed lots will be shipped to the USA accompanying the CZ1, CZ2 pollen (on blue ice) in December 2010. This excludes F_2 *Abies cilicica* × *Abies cephalonica* material. The American research partner has been receiving our F_2 open pollinated material from trials of all previous years and does not demand it any more.

Controlled pollination in 2010 did not show any significantly different results in comparison with the pollinations done in 2007, 2008 and 2009 (Kobliha, Stejskal 2009; Stejskal et al. 2011). It is rather impossible to trace any trend of performance of any hybrid combination. Detailed results of 2010 control pollinations are shown in Tables 1, 2 and 4.

In 2011, the most successful hybrid combination was CZ2 × NC26 with 18% of viable seeds. Next combinations were CZ2 × NC11 with 11%, 1/2 × NC51 8%, CZ2 × NC153 7%, CZ1 × NC26 7% and CZ1 × NC11 with 6% of viable seeds. The other hybrid combinations bore at least 4%. Detailed results of 2011 control pollinations are documented in Tables 1–5.

DISCUSSION

Hybridizing *Abies fraseri* with Mediterranean fir species is a pioneer effort. Our results are rather 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 and the application of *A. cilicica*, *A. alba* and *A. nordmanniana* pollen resulted in 14% germinating seedlings at least. The use of *A. concolor*, *A. grandis* and *A. pinsapo* lowered the germination rate to 0.9 – 3.3%. Also, the use of *A. grandis* as a mother tree appeared to be rather ineffective. Seedlings were obtained (1.9%) only when using *A. concolor* pollen. Other combinations were unsuccessful.

Generally, hybridizations tended to be successful in species with overlapping areas of their distribution (up to 60% field germination). Conversely, hybridizing species with distant natural areas yielded 29% of germinating seedlings at maximum (Mergen et al. 1964).

A high level of cross ability was confirmed only by Mediterranean fir species in works of Greguss (1986, 1988a,b, 1992) and Kormuťák (1984, 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 De Hayes (1985) and Critchfield (1988).

Since the hybridizations of 2007 had shown some promising results (Kobliha, Stejskal 2009), we assumed that the 2008, 2009 (Stejskal et al. 2011) and 2010, 2011 experiments could result in a comparable amount of viable seeds.

However, the results of 2008 (Stejskal et al. 2011) were slightly different in terms of viable seed percentage. A common feature of both seasons is significant differences in performances of different hybrid combinations. It seems that seed orchards No. 1 and No. 3 produced different results each year, but this is only our assumption. The reason for that is unknown and a complex investigation of this incompatibility is beyond the scope of the project.

Table 4. Mating in seed orchard No. 3, Prostějov – Seč, 2010/2011

Combination	2010						2011						
	CZ2 × NC184	CZ2 × NC125	CZ1 × NC23	F_2	CZ2 × NC23	CZ1 × NC125	CZ2 × NC153	CZ1 × NC42	CZ1 × NC26	CZ1 × NC53	CZ1 × NC11	CZ2 × NC11	CZ2 × NC26
Number of cones	4	5	10	21	10	5	5	9	4	13	11	6	1
Average cone length (cm)	14	17	–	15	17	18	15	16	14	17	15	15	16
Total cone weight (g)	360	460	900	1,750	950	510	630	950	240	950	1,300	650	110
Average weight of 1 cone (g)	90	92	90	83	95	102	126	106	60	73	118	108	110
Total weight of seeds (g)	36	77	130	261	125	69	59	110	43	172	140	84	21
Average weight of seeds in 1 cone (g)	9	15	13	12	13	14	12	12	11	13	13	14	21
Absolute weight of 1,000 seeds (g)	42	56	50	50	54	58	57	54	62	63	66	65	61
Total number of seeds	856	1,365	2,610	5,173	2,338	1,191	938	1,831	627	2,472	1,947	1,170	312
Average number of seeds in 1 cone	214	273	261	246	234	238	188	203	157	190	177	195	312
Full seeds fraction in a sample (%)	2	2	3	15	2	4	7	1	7	3	6	11	18
Expected full seeds number	17	27	78	776	47	48	66	18	44	74	117	129	56

In bold – highlighted full seed fraction in a sample, which illustrates a crossing success

The most successful hybrid combination in 2008 CZ1 × NC73 resulted in 16% of viable seeds. Combinations CZ1 × PC (10% of viable seeds), CZ1 × NC136 (7%) and CZ2 × PC (4%) were also considered successful (Stejskal et al. 2011). In 2009 the most successful hybrid combination CZ1 × NC81 resulted in 6% of viable seeds while other combinations gave hardly any viable seeds, ranging from 1 to 2% (Stejskal et al. 2011). In 2010, the most successful was the hybrid combination CZ2 × NC81 with 7% of viable seeds. Other three hybrid combinations oscillated around 5% and not a single one yielded less than 2% of viable seeds. That was the largest difference in comparison with the last three years of field trials. In 2010, the most successful hybrid combination was CZ2 × NC26 with 18% of viable seeds. In 2009 seed orchards located at Kostelec nad Černými lesy fruited sufficiently, which resulted in the control pollinations being restricted to Kostelec. After all available *A. fraseri* pollen was applied, we tested pollen of *Abies koreana* and *Abies numidica* from a local source (Stejskal et al. 2011).

In 2010 all our hybridization seed orchards yielded dramatically lesser amounts of cones. In Kostelec our pollination work was restricted to two ramets only. *A. fraseri* pollen of clone NC81 was chosen deliberately according to its results within 2009 pollination (Stejskal et al. 2011). Also seed orchard No. 3 located near Prostějov had a low cone year. In comparison with 2009 only 16 ramets (both CZ1 and CZ2) to 30 ramets of 2009 were pollinated, which is a 50% decline. In 2009 there was an enormous cone harvest in general. Seed orchard 3 located at Prostějov yielded almost 700 kg of open pollinated cones, which resulted in almost 70 kg of seeds (Stejskal et al. 2011).

In the year 2011 more cones were pollinated within seed orchards near Kostelec nad Černými lesy in comparison with 2010. However, the number of viable seeds decreased remarkably. Best resulting hybrid combinations of the year 2011 offered only 2% of viable hybrid seeds, which is relatively similar to rather limited results of 2009 (Stejskal et al. 2011).

In seed orchard No. 3 near Prostějov practically the same amount of cones as in the year 2010 was pollinated. In this plantation we recorded a moderate increase in viable seed number: the most successful combination CZ2 × NC26 provided 18% of viable seeds; CZ2 × NC11 with 11% of viable seeds was the second best combination. Interesting results in terms of viable seed number were recorded by 3-way hybrids *A. koreana (Abies cilicica × Abies cephalonica).* Hybrid combination 1/2 × NC51

Table 5. Mating in hybrid progeny test – Kostelec nad Černými lesy, 2011

	Combination	
	3/4 × NC 11	1/2 × NC 51
Number of cones	1	2
Average cone length (cm)	–	6
Total cone weight (g)	50	70
Average weight of 1 cone (g)	50	35
Total weight of seeds (g)	7	8
Average weight of seeds in 1 cone (g)	7	4
Absolute weight of 1000 seeds (g)	7	11
Total number of seeds	986	727
Average number of seeds in 1 cone	986	364
Full seed fraction in a sample (%)	4	8
Expected full seed number	39	58

In bold – highlighted full seed fraction in a sample, which illustrates a crossing success

reached 8%, 3/4 × NC11 reached 4% – these results rank above the average of CZ1/CZ2 clones.

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. Results of post *Phytophthora cinnamomi* inoculation survival of hybrid seedlings obtained by NCSU revealed dramatic differences in resistance within *Abies* species and in different interspecific hybrid combinations. Mortality of open pollinated Fraser fir seedlings ranged from 90 to 100% already 17 days after germination. On the other hand, hybrid seedlings originating from interspecific crosses of CZ1/CZ2 clones with Fraser fir show about 50% mortality. *A. cephalonica* as a pure species showed around 50% mortality after inoculation and *Abies cilicica* even significantly lower mortality (14%). Open pollinated F_2 hybrids of CZ1 and CZ2 clones show a varying degree of mortality averaging at 22%. The Japanese species *Abies firma* exhibited 0% mortality within the *Phytophthora* screenings. The species seems to be almost 100% resistant to *P. cinnamomi.*

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Received for publication August 10, 2012
Accepted after corrections January 14, 2013

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