JOURNAL OF FOREST SCIENCE, *55*, 2009 (4): 162–170

Recent fir hybridization research in the light of Czech-American cooperation

J. Kobliha, J. Stejskal

Faculty of Forestry and Wood Sciences, Czech University of Life Sciences in Prague, Prague, Czech Republic

ABSTRACT: The project is based on control pollination of different fir species in the sense of interspecific hybridization. The aim of this procedure is a new bred material for specific needs of forestry and Christmas tree production. Concrete breeding aims are represented in this sense by resistance to limiting environmental conditions (e.g. drought) and by resistance to diseases and pests. The experiment follows a traditional hybridization program of the department focused on the genus *Abies* and recently it has been extended by the Czech-U.S. cooperation (North Carolina State University Raleigh). For hybridization mainly Mediterranean fir species are used together with Asian species (e.g. *Abies koreana*) and of course American species (above all *Abies fraseri*). Hybridizations will be followed by *Phytophthora cinnamomi* screenings that have high priority. Hybrid progenies will undergo early testing and their vegetative propagation for cloning purposes is being considered.

Keywords: *Abies*; hybridization; breeding; *Phytophthora cinnamomi*; *Abies fraseri*

European and American firs belong to precious coniferous species by their production, ecological and aesthetic significance. Their cultivation in forests supports wood production as well as the other important functions of forest land. By their high aesthetic qualities firs raise a recreational potential of municipal forests and parks. Firs also have a major role in Christmas tree production in plantations. Several species, above all the European silver fir (*Abies alba* Mill.), are endangered by a long-term decline of forest stands in connection with their historical decline from the forest stands (especially in Central Europe). Breeding is an important tool not only for increasing the production of forests but also for improving the resistance of trees and forest stands. Breeding can also improve the aesthetic quality of forest tree species dramatically, which influences Christmas trees on a large scale. The conservation and sustaining of Central European silver fir populations together with increasing its production belong to the main (global) tasks. The second target of high importance is a requirement for the extraordinary aesthetic value of Christmas trees. Christmas tree plantations are paid a special attention in the U.S., where they annually bring a giant economic income to farmers and land owners.

European silver fir (*Abies alba* Mill.) has been one of our most important forest tree species until quite recently. As a result of its long-term decline influenced by changing (environmental) ecological conditions silver fir lost a great part of its original distribution often showing the poor vitality of surviving local populations. Sadly enough, fir as the only native coniferous species can outperform Norway spruce (*Picea abies* [L.] Karst.) in terms of produc-

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project Program KONTAKT No. ME 914 *Fir Breeding for Forestry and Christmas Tree Production*.

tion and in addition has an outstanding ecological and aesthetic significance.

European silver fir belongs to those native coniferous species whose production utilization is conditioned by increasing their general resistance regardless of the recent slight regeneration of silver fir forest stands in Europe. Conservational and breeding efforts are made more difficult by the fact that no generally acceptable explanation of the historical European silver fir decline exists yet, even though much has already been clarified. At the present time silver fir specific resistance cannot become a breeding goal (like in the case of elm species). Therefore the main target of breeding efforts must be to increase its general vitality.

One of the prominent breeding methods possibly leading to higher resistance of fir is intraspecific/interspecific hybridization. It is well known that hybrids originating from crossing within the genus *Abies* perform extremely well in growth and vitality in comparison with the parental trees, which is explained by the effect of heterosis. It supports the significance of interspecific hybridization. The increased vitality of interspecific hybrids is also related to their higher tolerance to changing environmental conditions. In addition, hybrids are expected to tolerate different stress factors such as air pollution or climate change consequences.

With respect to swift changes in environmental conditions, from the aspect of population genetics it is impossible to rely on a gradual adaptation of native silver fir. With the sudden changes, genetic adaptation of those native populations based on the process of selection and mutation would last for 10 generations at least, but probably more. In this case the introduction of exotic fir species adapted to comparable conditions followed by intensive breeding based on interspecific hybridization, selection and reproduction could play an important role. In the concrete, an example of Mediterranean firs and their hybrids with silver fir could be the alternative option to the impending threat of global warming.

As far back as in 1959 Rohmeder and Schönbach reported interspecific hybrids of *Abies alba* Mill., *Abies veitchii* Carr., *Abies concolor* Lindl. et Cord., *Abies procera* Rehd., *Abies nordmanniana* Spach. to grow much faster than the intraspecific hybrids. So did Mergen and Gregoire (1988), who stated that the interspecific hybrid progeny outperformed intraspecific hybrids. Their results were based on measurements of b.h.d., height and crown shape of 17 years old interspecific fir hybrids taken in 1960 to 1962 in Connecticut, U.S. Crosses of species with neighbouring distribution areas were showing better growth. In the former Czechoslovakia Kantor and Chira (1971) were engaged in fir hybridization research using trees of *Abies alba* Mill., *Abies cephalonica* Loud., *Abies nordmanniana* Spach., *Abies pinsapo* Boiss., *Abies cilicica* Carr., *Abies concolor* Lindl. et Cord., *Abies grandis* Lindl. for interspecific and intraspecific hybridizations.

Kobliha (1988, 1989a) realized the testing of some of these hybrids. Intraspecific hybrid progeny of *Abies cephalonica* showed exceptional results also when γ-rayed pollen was applied. All of the hybrid combinations that genetically included *Abies alba* outperformed the comparative *Abies alba* individuals originating from open pollination. Kobliha (1989b), Kobliha and Pokorný (1990), Snášelová and Kobliha (1990), Kobliha and Snášelová (1991), Kobliha et al. (1991), Kobliha and Králík (1992), Kobliha (1992a,b, 1993a,b) experimented in different methods of reproduction of these hybrids.

In Slovakia Greguss (1984, 1986, 1988a,b, 1992) and Kormuťák (1984, 1985, 1986, 1992) were engaged in fir hybridization. According to Greguss (1986) silver fir significantly fell behind compared to hybrids and at the same time the difference between hybrid progenies and open pollinated single species progenies further increased. Kormuťák (1986) came to similar conclusions. He recommended for our conditions mainly *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*. Aside from combining American species *Abies grandis* and *Abies concolor* he suggested above all using *Abies alba* together with Mediterranean fir species. The same species already played a major role in experiments of Kantor, Chira and Greguss.

The problem of Mediterranean fir species and their hybridization is closely associated with French research (Arbez et al. 1990). French researchers recommended autogamy as a source of fir reproduction for certain inbreeding tolerance.

Hybrids of the second filial generation (F_2) were obtained by Kobliha (1994). F_1 hybrid of *Abies cilicica* × *Abies cephalonica* (or more precisely its fructifying graft) featured as a mother tree. It was successfully self-pollinated and afterwards pollinated by *Abies alba, Abies pinsapo, Abies homolepis* and *Abies concolor*. The pollen of this particular hybrid was also used for pollination of *Abies pinsapo, Abies koreana, Abies grandis* and *Abies lowiana* with the simultaneous crossing of *Abies lowiana* × *Abies grandis* and *vice versa*.

Fady (1993) published details on geographic, orographic and bioclimatic distribution of *Abies cephalonica* and *Abies borisii-regis* in Greece. He also included information on their soil demands, phytocoenology, ecology and colonization strategies, cultivation, natural regeneration, pests and diseases, wood production and wood quality. He described results of Greek provenances from trials located in southern France. The results indicated that these provenances were generally well adapted to rather low precipitation amounts (less than 650 mm) combined with summer drought and they were mostly undemanding on soil substrate. Their growth was rather slow till the age of 15–18 years, but these species have recently become indispensable in the Mediterranean part of France.

On the other hand, to obtain hybrids is quite a labour-intensive process. Even more problems arise during the reproduction of selected hybrids and especially with their mass propagation. Heterovegetative reproduction with using grafts is well managed by fir, but problems arise when the flowering of grafts is demanded. There is a considerable predominance of male strobiles over female strobiles, which are rather exceptional in occurrence. This problem has been described mainly in silver fir (Kobliha et al. 1991). Rather difficult is also auto-vegetative reproduction by rooted cuttings with respect to the long-term rooting of fir cuttings. Rhizogenesis is very demanding on physical conditions and there is an existing phenomenon of topophysis playing a major role in the fir (Kobliha 1989b; Kobliha, Pokorný 1990; Kobliha, Králík 1992; Kobliha 1992a,b, 1993a,b). Possible *in vitro* reproduction has been unsolved so far (Chalupa, Durzan 1973; Snášelová, Kobliha 1990; Kobliha et al. 1991; Kobliha, Snášelová 1991). Literature dealing with auto-vegetative reproduction of fir on the basis of rooted cuttings or *in vitro* cultures is scarce internationally.

In the elapsed decade a problem of fir hybridization with all the related aspects was studied locally by Kobliha (1998), Kobliha and Janeček (2001, 2002, 2003a,b, 2004, 2005a,b,c,d, 2007), Kobliha et al. (2004) and Janeček and Kobliha (2007).

MATERIAL AND METHODS

Experimental plots

All the Czech seed orchards were founded as biclonal – grafts originated from 2 interspecific hybrids of the first generation F_1 of *Abies cilicica* × *Abies cephalonica*. These grafts fructified many times, which inspired Kobliha (1980) to execute control pollination. F_2 material and new interspecific hybrids were obtained in this way. A part of this material is cultivated at the Truba Breeding Station, Kostelec nad Černými lesy. 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 on further utilization of this material. Secondary grafts were taken to establish the abovementioned hybridization seed orchards. These seed orchards primarily produced F_2 hybrids.

Rootstocks were provided by European silver fir.

Hybridization seed orchards with the presence of female strobiles before 2006 were used mainly for the production of F_2 hybrids. A list of plantations below should outline their historical and present state.

Hybridization seed orchard No. 1 was established in 1994 directly at the Truba Breeding Station near Kostelec nad Černými lesy from the material grafted in 1991 and 1992. The original number of 217 grafts with 4 × 2 spacing was restricted due to secondary waterlogging to the current number of 154. Clone CZ2 is represented to a lower 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 the form of two long rows (one clone in each row) by planting the material grafted in 1993. There has been no mortality so far, though the locality is rather dry. No flowering has been observed so far.

Hybridization seed orchard No. 3 was established in 1997 from the material grafted in 1993 within a nursery by the village 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 flowering was registered in 2003–2008.

Hybridization seed orchard No. 4 was established in May 1999 in the training forest enterprise Kostelec nad Černými lesy in forest stand 20 A 9 by planting 298 grafts (159 clone 2) at a 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 flower in 2008.

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 a significant mortality in the first year a new material – *A. koreana* × *(Abies cilicica* × *Abies cephalonica)* hybrids – was brought in 1997 as 5 years old seedlings. Originally there were 2 plots established

by 25 trees each without significant mortality. These hybrids began flowering in 2004 and female strobiles have been observed annually ever since.

Project background

In this project the guidelines of long-term research of our department are followed. Current activities are connected with a traditional hybridization program within the genus *Abies*, which has recently been extended by our cooperation with a U.S. partner (North Carolina State University Raleigh).

This project solution is based in the long run on bilateral international cooperation between the Czech Republic and U.S. The Czech party is supported by a grant of the Czech Ministry of Education, Youth and Sports of the Czech Republic within the KONTAKT Program. In this particular case there exists a concrete collaboration between the Czech University of Life Sciences in Prague (responsible researcher Prof. Jaroslav Kobliha) and NCSU Raleigh (responsible researcher Prof. John Frampton).

On the Czech side the project is connected to long-time scientific work in the field of fir breeding on hybridization basis of Prof. Kobliha. Concretely there have been several projects recently dealing with this subject. For example the project Fir Breeding (1997–2000) supported by the Ministry of Agriculture of the Czech Republic.

2006

Fructification in hybridization seed orchards in 2006

Female strobiles occurred in seed orchard No. 1 and No. 3. The highest abundance of female strobiles was observed in seed orchard No. 3. It led to an additional application of *Abies fraseri* pollen. This particular pollen was obtained from a single tree that is situated in the faculty arboretum in Kostelec nad Černými lesy.

2007

In the year 2007 the core of our activities was represented by hybridizations. It was possible to import *Abies fraseri* pollen from the USA (Fraser fir seed orchard in North Carolina, Appalachians). Specifically, we obtained the frozen pollen of clones NC73, NC52, NC84 and polymix (PC – polycross) 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 was frozen and later shipped to the U.S. in 2008. Pollen collected in seed orchard No. 3 was used fresh for pollination at the same place.

Control pollination was performed in spring 2007 in seed orchard No. 1. The pollen of *A. fraseri* (NC73, NC84) was applied. A view of seed orchard No. 1 is given in Fig. 1. Two cones in seed orchard No. 1 originated from open pollination (F_2 Kostelec). In

Table 1. Mating in seed orchard No. 1, Kostelec nad Černými lesy – Truba, 2007

Combination	CZ1 × NC73	CZ2 × NC73	CZ1 × NC84	F_2 (open pollination)
Number of cones	14	5	28	2
Average cone length (cm)	15	17	16	15
Total cone weight (g)	1,120	600	2,410	180
Average weight of 1 cone (g)	80	120	86	90
Total weight of seeds (g)	180	70	390	30
Average weight of seeds in 1 cone (g)	13	14	14	15
Absolute weight of 1,000 seeds (g)	61	60	64	68
Total number of seeds	2,935	1,166	6,082	439
Average number of seeds in 1 cone	210	233	217	220
Full seed share in a sample (%)	7	7	7	24
Expected full seed number	205	82	426	105

F_2 (*A. cilicica* × *A. cephalonica*) × (*A. cilicica* × *A. cephalonica*); CZ1, CZ2 – clones of *A. cilicica* × *A. cephalonica*; NC73, NC84 – clones of *Abies fraseri*

Fig. 1. Control pollination in seed orchard No. 1 (2007) (Photo Kobliha)

Fig. 2. Field germination 2007 – materials from 2005 and 2006 crossings (Photo Kobliha)

seed orchard No. 3 there was a similar situation – the pollen of *Abies fraseri* was used (NC52, PC) plus the pollen of *Abies cilicica* × *Abies cephalonica* hybrid (clones CZ1 and CZ2) was applied, thus creating F_2 Prostějov. Aside from the two main seed orchards, control pollination was performed on an experimental plot in Kostelec nad Černými lesy with *A. koreana* × *(Abies cilicica* × *Abies cephalonica)* hybrids. There was a great majority of *A. fraseri* pollen (NC73, PC) applied with a single exception of open pollination.

2008

During the 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 the North Carolina State University. Specifically, we obtained the frozen pollen of clones NC73, NC52, NC84, NC136 and polymix (PC – polycross) of these clones collected in 2007. 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, in seed orchard No. 3 we used the pollen of other species, concretely of *Abies balsamea* and *Abies fraseri* originating from Kostelec Arboretum and *Abies koreana* from Průhonice Arboretum.

Control pollination was performed in spring 2008 in seed orchard No. 1, No. 3 and for the very first time also in seed orchard No. 4. Applied pollen was *A. fraseri* (NC52, NC73) with a negligible part of open pollinated cones (F_2 Kostelec). In seed orchard No. 3 there was a similar situation – the 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).

Later this autumn cone and seed processing similar to that of 2007 is planned to draw conclusions about this year's pollination results. During December *Phytophthora* screenings are planned by the U.S. partner.

Fig. 3. Inverse X-ray of 2007 hybridization – clones CZ1 × NC73 (Kostelec)

Fig. 4. Field germination 2008 – clones CZ2 × NC52 (Photo Stejskal)

Table 2. Mating in seed orchard No. 3, Prostějov – Seč, 2007

Combination	CZ1 × PC	CZ2 × PC	CZ1 × NC52	CZ2 × NC52	F_2 (control pollination)
Number of cones	14	21	22	10	395
Average cone length (cm)	17	19	20	18	16
Total cone weight (g)	1,950	3,060	3,450	1,450	39,000
Average weight of 1 cone (g)	139	146	157	145	99
Total weight of seeds (g)	140	320	350	110	4,550
Average weight of seeds in 1 cone (g)	10	15	16	11	12
Absolute weight of 1,000 seeds (g)	79	75	72	72	90
Total number of seeds	1,783	4,250	4,838	1,530	50,572
Average number of seeds in 1 cone	127	202	220	153	128
Full seed share in a sample (%)	2	0	0	0	24
Expected full seed number	36	0	0	0	12,137

F_2 (*A. cilicica* × *A. cephalonica*) × (*A. cilicica* × *A. cephalonica*); CZ1, CZ2 – clones of *A. cilicica* × *A. cephalonica*; NC52 – clone of *Abies fraseri*; PC – polycross-*Abies fraser*

RESULTS

2006

In the framework of this project planting stock (1/0) that originated from hybridizations in 2006 is also used (saplings and seedlings). Most of the material is represented by F_2 *Abies cilicica* × *Abies cephalonica*. There is a certain percentage of *(Abies cilicica* × *Abies cephalonica)* × *Abies fraseri* saplings. Field germination assessment of this material was done in spring 2007. Fig. 2 shows this material. In Kostelec (seed orchard No. 1) we harvested 46 cones. From 9,416 F_2 *Abies cilicica* × *Abies cephalonica* seeds collected in seed orchard No. 1 a total of 1,360 saplings originated, which is 14.4%.

Table 3. Mating within a hybrid progeny test, Kostelec nad Černými lesy – Truba, 2007

Combination	1/2 × NC73	3/2 × PC	4/3 × PC	4/4 × PC	4/5 × PC	1/5 (open pollination)
Number of cones	9	1	6	29	21	2
Average cone length (cm)	8	2	2	2	10	2
Total cone weight (g)	320	–	60	310	700	20
Average weight of 1 cone (g)	36	–	10	11	33	10
Total weight of seeds (g)	30	4	59	100	90	2
Average weight of seeds in 1 cone (g)	3	4	10	3	4	1
Absolute weight of 1,000 seeds (g)	30	23	22	22	39	23
Total number of seeds	986	169	2,685	4,582	2,307	85
Average number of seeds in 1 cone	110	169	448	158	110	43
Full seed share in a sample (%)	2	1	0	9	0	3
Expected full seed number	20	2	0	412	0	3

Coordinates within the orchard (ex. 4/5) – (*A. koreana* × [*A. cilicica* × *A. cephalonica*]); NC73 – clone of *Abies fraseri*; PC – polycross-*Abies fraseri*

Table 4. Field germination in 2008 (29. 5.)

Combination	Origin	Number of seeds	Number of saplings	Field germination (%)
CZ1 × PC	Prostějov	1,783	21	1.18
CZ2 × PC	Prostějov	4,250	40	0.94
CZ1 × NC52	Prostějov	4,838	1	0.02
CZ2 × NC52	Prostějov	1,530	123	8.04
F_2 (control pollination)	Prostějov	50,572	2,025	4.00
CZ1 × NC73	Kostelec	2,935	33	1.12
CZ2 × NC73	Kostelec	1,166	0	0.00
CZ1 × NC84	Kostelec	6,082	159	2.61
F_2 (open pollination)	Kostelec	439	35	7.97
1/2 × NC73	Kostelec	986	2	0.20
3/2 × PC	Kostelec	169	0	0.00
4/3 × PC	Kostelec	2,685	0	0.00
4/4 × PC	Kostelec	4,582	114*	2.49
4/5 × PC	Kostelec	2,307	0	0.00
1/5 (open pollination)	Kostelec	85	0	0.00

*Additional number of saplings due to late summer germination

Seed orchard No. 3 yielded 426 cones in total (45 of the *Abies fraseri* combination). From 111,946 F_2 *Abies cilicica* × *Abies cephalonica* seeds sown 13,500 saplings originated (12%). From 5,730 *(Abies cilicica* × *Abies cephalonica)* × *Abies fraseri* seeds sown 270 saplings came up (4.7%).

2007

In September 2007 mature cones of all the hybrid combinations were harvested. After the cones were measured and weighed, the seeds were extracted and examined. Seed samples of the individual combinations were then X-rayed in early October to assess the final share of full seeds. An example of the inverse X-ray photograph of *Abies cilicica* × *Abies cephalonica* crossed with *A. fraseri* is attached as Fig. 3.

The seeds were sown afterwards during late autumn. Most of the seeds logically originated from F_2 *Abies cilicica* × *Abies cephalonica* (F_2 Prostějov). 0.6 kg samples of this origin were either shipped to the U.S. or granted to somatic embryogenesis research of our department. The U.S. party will examine this material for specific resistance to *Phytophthora cinnamomi*, which represents a serious threat to Christmas tree plantations in the U.S.

Details on control pollinations, cone and seed parameters can be found in Tables 1, 2 and 3.

Field germination assessment was carried out in Kostelec in spring 2008. Complete data on various hybrid materials are presented below in Table 4. Germinating seeds of clone CZ2 pollinated by *A. fraseri* (clone NC52) are presented in Fig. 4.

CONCLUSIONS

As the hybridizations of 2007 show some promising results, we assume 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 interspecific hybrids we work with).

In the year 2008 we replaced *A. koreana* × *(Abies cilicica* × *Abies cephalonica)* in the hybridization by more promising F_1 *Abies cilicica* × *Abies cephalonica*. Also one new taxon was included – *Abies balsamea*. This idea was based on its close relationship with *Abies fraseri*, so it can work as a kind of substitute when running out of *A. fraseri* pollen.

As the transport of most seeds from this year's harvest to the U.S. is organized this month, their sowing in our facilities is not a part of the plan. At this point

Phytophthora resistance screenings performed at NCSU are strongly preferred by both parties, for they will provide the most important results and needed feedback to us. After the completion of these tests it will be much easier to pick the most promising hybrid combinations for our future work.

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Received for publication November 10, 2008
Accepted after corrections November 26, 2008

Současný výzkum hybridizace jedle ve světle česko-americké spolupráce

ABSTRAKT: Práce je založena na kontrolovaném opylení různých druhů jedle ve smyslu mezidruhové hybridizace. Cílem tohoto postupu je vyšlechtění jedlového materiálu pro specifické potřeby lesního hospodářství a produkce vánočních stromků. Ke konkrétním šlechtitelským cílům v tomto směru patří odolnost vůči limitním ekologickým podmínkám (např. sucho), odolnost vůči chorobám a škůdcům atd. Práce navazují na dosavadní hybridizační program rodu *Abies*, tradičně rozvíjený na tomto pracovišti, a jsou rozšířeny o aktuální spolupráci s americkým partnerem (North Carolina State University Raleigh). Pro hybridizační práce jsou využívány především mediterránní druhy daného rodu, druhy asijské (např. *Abies koreana*) a pochopitelně druhy americké (především *Abies fraseri*). Na hybridizační práce budou navazovat především testy na rezistenci vůči kořenové hnilobě (*Phytophtora cinnamomi*), časné testy hybridních potomstev a také vegetativní množení hybridního materiálu pro účely jeho klonování.

Klíčová slova: *Abies;* hybridizace; šlechtění; *Phytophtora cinnamomi; Abies fraseri*

Corresponding author:

Prof. Ing. Jaroslav Kobliha, CSc., Česká zemědělská univerzita v Praze, Fakulta lesnická a dřevařská,
165 21 Praha 6-Suchdol, Česká republika
tel.: + 420 224 382 878, fax: + 420 234 381 860, e-mail: kobliha@fld.czu.cz