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Hybrid breakdown and inbreeding depression for seed yield and early growth in 2nd-generation interspecific hybrids of larch (*Larix x eurolepis* x *L. x eurolepis*)

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Abstract

• **Key message** Advanced hybridization between first-generation interspecific hybrids is an attractive alternative to breed and mass-produce hybrid seed lots. Nevertheless it faces two major drawbacks, namely hybrid depression and inbreeding depression. Their impact may be highly detrimental on some traits (seed yield) especially for the highest level of consanguinity, but much less on some others (seed quality and early growth) as shown in this study for hybrid larch (*Larix x eurolepis*).

• **Context** Mass-production of 1st-generation larch hybrids (*Larix x eurolepis*) in interspecific hybridization orchards is problematic. Alternatives are searched such as advanced-generation orchards combining 1st-generation hybrid clones.

• **Aims** Our aim was to investigate how important hybrid and inbreeding depressions can be in 2nd-generation hybrids with a special focus on reproductive success (and early growth) and their consequences on the success of advanced-generation hybridization seed orchards.

• **Methods** Second-generation hybrids were created by control crosses among 18 1st-generation (F_1) hybrid clones with 3 different levels of relatedness (OC (outcrossed), HS (half-sib) and FS (full-sib)). Seed yield and quality and growth in nursery were assessed for the 3 groups and compared with related F_1 crosses. Hybrid breakdown and inbreeding depression were then estimated.

• **Results** F_1 hybrids are fertile and mating among them is feasible. Nevertheless hybrid depression is observed when passing to 2nd-generation hybrids for crossing success rate (-20%) and for seed yield ($>50\%$ less filled seed/cone). Hybrid depression is reinforced by inbreeding and more severely with increasing consanguinity levels: negligible for HS, a supplementary drop of over 50% of seed yield is observed for FS. In contrast, seed quality traits as well as first-year growth are little impacted by both hybrid and inbreeding depressions. Nevertheless a great variation in inbreeding depression level exists among mother clones. Overall, total depression overpasses 70% for the FS parent group but is limited to less than 34% for OC parents for a synthetic trait (number of germinated seed/cone) and remains negligible for growth.

• **Conclusion** Advanced hybridization is possible with larch but with a more or less severe impact on reproductive success depending on level of relatedness among the parents. Growth seems much less impacted than reproduction and this is a promising result. The proper choice of 1st-generation parents is unavoidable for success of 2nd-generation seed orchards.

Keywords Interspecific hybridization · Hybrid breakdown · Inbreeding · Reproduction · Seed · Height

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Contribution of the co-authors The author planned the experimentation, analysed the data sets and wrote down the manuscript.

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1 Introduction

Interspecific hybridization proves to be a powerful breeding strategy for several forest tree species (Dungey 2001; Potts and Dungey 2004): it allows catching heterosis and combining complementary traits between two different gene pools. Interspecific hybrids (HL) between European (*Larix decidua* Mill.; EL) and Japanese (*Larix kaempferi* (Lamb.) Carr.; JL) larches in particular show a great potential in terms of growth,

adaptation and wood properties when compared with their parental species (Pâques et al. 2013; Marchal et al. 2017). It is increasingly used in reforestation, especially in lowland France and Western Europe. However, whereas hybridization orchards have been established in many European countries for several decades, seed production and trade remain limited impeding deployment of this promising species.

First-generation hybridization clonal orchards of larch suffer in fact from two main drawbacks: on one side, compared with other *Pinaceae*, seed yield in larch is known to be naturally low with many empty seeds. It is reinforced in hybridization orchards where some mismatching in flowering phenology between European and Japanese larch parents is frequent. Besides frost damages to flowers and young cones, pre- and/or post-zygotic abortions are also frequent as demonstrated by Hall and Brown (1977) and Kosinski (1986). At maturity, these seeds distinguished from filled seed by a degenerated but more usually absent endosperm and embryo. On the other side, the structure itself of hybridization orchards where one to several parents of each species are intermixed, in addition to mismatching of phenology, leads to hypothetical hybrid seed percentage. As shown previously (Philippe et al. 2016), it highly varies from orchard to orchard and from year to year, ranging from less than 20% to up to 70–80%. As a remedy, the option in France was to separate species parents into 2 orchards: one (the Japanese larch: several clones) to collect pollen and the other (the European larch: one clone) where supplemental pollination is applied. The advantage of close to 100% hybrid percentage seed lots obtained by this way is however nearly wiped off by the extra cost of supplemental pollination.

Alternative approaches for mass propagation of hybrids are thus attempted. Besides the vegetative approach ('bulk' propagation by cutting (Verger and Pâques 1993) and somatic embryogenesis (Lelu-Walter et al. 2013)), generative propagation through 2nd-generation hybridization orchards looks attractive: a way already attempted by Nikles (1984) for sub-tropical pines in Australia. These seed orchards are composed of 1st-generation hybrid clones, which firstly proved to be fertile and secondly, which should hopefully reproduce as in classical monospecific orchards through open pollination and under the hypothesis of panmixia. Due to the large number of clones (several dozens), mismatching of phenology among clones should not be worse than in classical orchards, and all seed produced in this way should be hybrid and cheap. In addition, some old and preliminary experimental clues (Dietze 1974) indicated that 2nd-generation hybrids obtained from non-related or half-sib hybrid larch parents compete well with 1st-generation hybrids for vigour. This context has thereby encouraged some breeders in Western Europe to establish the first 2nd-generation larch hybridization orchards (Nanson 2004).

Nevertheless, advanced interspecific hybridization raises at least two main concerns linked on one side to hybrid breakdown and on the other side to inbreeding depression. The first phenomenon measures the decrease of fitness (e.g. fertility, viability) of advanced-generation hybrids relative to F₁'s (Avisé 1994) and results from segregation and recombination in a highly heterozygous hybrid population. Few but contrasting results exist in the forestry literature (see Kain 2003 for a review) with strong hybrid breakdown observed in outcrossed F₂ poplar hybrids (Stettler et al. 1988) but with no or little hybrid breakdown in pines (Hyun 1974; Kain 2003) in relation to the degree of dominance exhibited by alleles: strong for poplars, weak for pines.

The proper choice of the parental F₁ hybrid clones when establishing these orchards is also an issue. Indeed, when the first orchard of this type was established in Belgium, clones were mass-selected at nursery stage in open-pollinated progenies from a single 1st-generation hybridization orchard. Question about the impact of possible relatedness among hybrid clones on reproductive success first and then on growth performances is therefore raised. The impact of inbreeding has been studied by many authors on several coniferous species and for several traits (e.g. survival, growth, stem form, pest and disease resistance, wood density): while inbreeding depression is consistently shown to increase with levels of consanguinity, its intensity varies from species to species, from trait to trait and for a given trait from year to year. However, much less results are available on inbreeding effect on reproductive traits in trees—except in the case of selfing (Orr-Ewing 1954; Sarvas 1962; Franklin 1970; Sorensen 1999)—and particularly in the context of interspecific hybrids (Appendix 1). The most studied trait is the percentage of full seeds for which inbreeding depression is consistently shown to be particularly strong for *F* levels ≥ 0.5 ; for other levels, it can be positive or negative but weaker and less consistently as it varies from species to species (see for example Griffin and Lindgren 1985).

A dedicated mating design among 1st-generation hybrid larches was created by control crossing to study the level of hybrid breakdown and the impact of different levels of inbreeding on reproductive success and later on on growth performances. The objective of this paper is to present results on reproductive success up to germination and early growth in the nursery and to discuss their implication in designing 2nd-generation hybridization orchards in larch.

2 Material and methods

2.1 Mating plan

Controlled crosses among hybrid larch (*Larix x eurolepis* Henry) trees were achieved in clonal archives at INRA

(Orléans) from 1997 to 2001. In total, 18 1st-generation hybrid clones selected from 6 full-sib hybrid progenies variously related were involved in these crosses. They were crossed following either factorial or diallel mating designs (Fig. 1), so that 3 types of 2nd-generation hybrids could be obtained showing different levels of inbreeding: outcrossed (OC; $F = 0$), half-sibs (HS; $F = 0.125$) and full-sibs (FS; $F = 0.25$). Selfing was not attempted in this study because results from previous experimentation showed the near-impossibility to get selfed progenies. For some combinations, crossings had to be repeated up to 4 times to get some filled seeds. In addition, two 1st-generation hybrid combinations (F0008 and F0010) were reproduced together with 7 backcrossed progenies (BC), using each of the 4 Japanese larch grandfathers and one of the three European larch grandmothers.

Well before female receptivity, branches with female buds were bagged together with a home-made heating system to prevent frost damages; care was taken to remove all male buds. Pollination with cold-stored pollen was realized with a brush when female flowers started to be receptive: usually pollination was repeated 2 to 3 times over time to cover the variability of receptivity of female flowers along branches. When the cones started to be formed and in absence of frost risks, bags were removed and replaced by a light permeable tissue bag to prevent animal damages (mostly squirrel) and early release of seed especially during late summer.

2.2 Seed processing and nursery height measurement

Mature cones collected during falls were counted and left in a storage room during several weeks to dry off. Seeds were then manually extracted from cones, de-winged, cleaned and sorted into sound and empty seeds with a seed blower and a gravimetric table. Sound and empty seeds were counted; then seed lots were dried down to 6% RH and finally weighted.

Several parameters were then calculated: number of filled seeds per cone, percentage of filled seed and P1000 and the weight of 1000 seeds.

Finally, all seed lots were pre-treated before sowing by soaking seed into running cold water for 24 h and then by a 3-week storage in wet peat into a cold room at 2 °C. Seed lots were then sown in April 2001 in containers under a greenhouse. One month later, young seedlings were counted to estimate the percentage of germinated seeds. The few which germinated from empty lots were then re-incorporated into the calculation of seed traits.

At the end of the year, total height of seedlings was measured. Seedlings were then transplanted for one more year in the nursery and re-measured for height at the end of the year.

2.3 Data analysis

Univariate two-way analyses of variance were conducted on crossing groups and families nested within groups. The analyses were performed in R Studio version 1.1.456 (R Core Team 2014) using the linear model function *lm*. Means of F_1 and F_2 inbreeding groups were calculated, and when F -test was significant at least at level 0.05, they were compared with a Tukey test.

Hybrid depression or hybrid breakdown was defined as $HD = 1 - F_{2OC} / F_1$ with F_1 being the mean performance of 1st-generation hybrids and F_{2OC} the mean performance of the outcrossed group OC.

Inbreeding depression was calculated as $ID = 1 - F_{2i} / F_{2OC}$ with F_{2i} and F_{2OC} being, respectively, the mean performance of inbreeding group i and of the outcrossed group OC; it has been calculated at the mean inbreeding group level and at the seed parent level.

Finally a total depression index was calculated as $((F_{2OC} - F_1) + (F_{2i} - F_{2OC})) / F_1$ which estimates the relative loss of

F1-hybrid clones		F0008 35(♀) x 3214(♂)					F0001 35(♀) x P(♂)		F0006 35(♀) x 3212(♂)			F0010 35(♀) x 3217(♂)					F0016 103(♀) x 3208(♂)			F0015 108(♀) x 3212(♂)	
		10069	10072	10077	10078	10081	10049	10059	10066	10097	10098	10106	10110	10112	10141	10142	10144	10132	10135		
F0008	10069		FS	FS	x	x	.	HS	HS	HS	HS	HS	x	OC	OC	OC	OC	OC			
	10072			x	FS	FS	.	.	HS	HS	HS	HS	HS	OC	OC	OC	OC	OC			
	10077					FS	FS	.	HS	HS	HS	HS	HS	OC	OC	OC	OC	OC			
	10078						FS	.	.	.	HS	HS	x	HS	HS	OC	OC	OC			
	10081							HS	.	.	HS	HS	HS	HS	OC	OC	OC	OC			
F0010	10097									FS	FS	FS	FS	OC	OC	OC	OC	OC			
	10098										FS	FS	FS	OC	OC	OC	OC	x			
	10106											x	x	OC	OC	OC	OC	.			
	10110													OC	OC	OC	x	.			
	10112												FS	OC	OC	OC	OC	.			
F0016	10141													FS	FS	OC	OC	OC			
	10142														x	x	.	.			
	10144															OC	OC	.			
F0015	10132																	.			
	10135																	.			

Fig. 1 Mating designs used to generate the 3 types of 2nd-generation hybrid sibs between OC (outcrossed), HS (half-sib), and FS (full-sib) F_1 hybrid clones. x means crosses attempted but unsuccessful. These clones

belong to 6 different interspecific F_1 hybrid families (F00..) involving 3 European larch clones as female parents and 5 Japanese larch clones as male parents.

performance due to the use of inbred 2nd-generation hybrids compared with 1st-generation hybrids.

Data availability The datasets generated and/or analysed during the current study are available in the Portail Data INRA repository (Pâques 2019), at <https://doi.org/10.15454/AMNDTO>.

3 Results

3.1 Crossing success

Out of the 114 potential combinations (annotated cells in Fig. 1), 102 were attempted through 180 crosses. Out of these, 143 were considered successful in the sense that they produced at least one filled seed/cone. Nevertheless, a great variation was observed among inbreeding groups with crossing failure rates ranging from 20.8% in OC up to 35.7% in FS. In comparison, F_1 crosses were 100% successful and 25% of BC crosses failed (Fig. 2).

3.2 Average seed yield and quality per inbreeding group

The initial potential for seed production as reflected by the total number of seeds per cone was not significantly different among groups (Table 1). Around 71 seeds/cone can be produced but only 5 full seeds per cone can be expected on average. Nevertheless, the groups significantly differed from each other ($F = 10.079$, $p < 0.0001$), with OC showing the largest number of full seeds (5.5) and FS, the lowest (2.1). In comparison, the number of filled seeds/cone reached 13.1 for F_1 . For percentage of filled seeds, large differences exist also among groups ($F = 10.120$, $p < 0.0001$) with values extending from 3.7% (FS) up to 8.5% (OC). It reached at most 18% for F_1 .

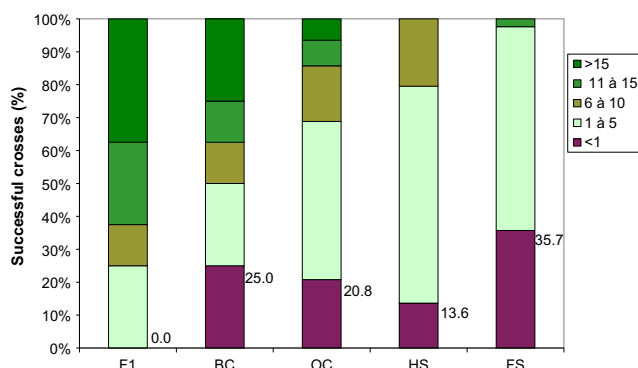


Fig. 2 Percentage of successful crosses per inbreeding groups according to number of filled seeds/cone. Figures correspond to crossing failure percentages. See Table 1 for abbreviations

In terms of seed quality, no significant differences among seed lots could be observed ($F = 1.608$, $p = 0.191$) for 1000 seed weight (P1000) and for percentage of germination ($F = 1.397$, $p = 0.243$).

Altogether, the yield of germinated seeds/cone ranged from 1.3 for FS to 4.3 for F_1 with OC (3.0) and HS (2.8) being intermediate.

Variability among families within groups can be observed for two traits in Fig. 3. It decreased with increasing levels of consanguinity.

3.3 Nursery height growth

First- and second-year height growth in the nursery is provided in Table 2. Significant differences among groups and families within groups are found for both traits. Growth of the OC group did not statistically differ from F_1 . FS is shown to be the less vigorous group and significantly differed from the others. However its relative difference with OC decreased over time (from 19.3 down to 8.8%).

3.4 Inbreeding depression and total depression

Hybrid depression was estimated as well as inbreeding depression for the 2 levels of inbreeding ($F = 0.125$ and 0.25) in the F_2 hybrid families (Table 3).

Traits in 2nd-generation hybrids (OC) showed a strong hybrid depression (> 0.34) with two major exceptions: one for total height which is close to 0 and one for germination with higher values for OC than for F_1 .

For all traits except germination percentage, inbreeding depression increased with levels of inbreeding. It more than doubled between HS and FS groups. It also varied among traits with the lowest values for the percentage of germination.

A great variation in inbreeding depression is seen among mothers involved in the 3 inbreeding level crosses (Table 4). For example clones 10072 and 10081 showed strong effects in contrast for example to clones 10078 and 10110.

Total depression, cumulating hybrid and inbreeding depressions, is represented in Fig. 4. Except for germination percentage, it was strong for all other seed traits for all groups (> 0.34) but especially for the FS group (> 0.7). It was much more reduced for growth traits (HT1, not shown, and HT2) with the highest value reached by FS (0.13).

4 Discussion

4.1 Seed yield in larch

In this study, seed yield—number of filled seeds/cone and percentage of full seed—is overall low as it did not exceed 13 seeds/cone and no more than 18% of full seed for the best

Table 1 Mean values (and standard errors) of the seed traits for the 4 groups (2nd-generation hybrid progenies from outcrossed (OC), half-sib (HS), and full-sib (FS) parents and 1st-generation hybrid progenies (F₁)). Means with the same letter do not differ significantly (Tukey test, $p = 0.05$)

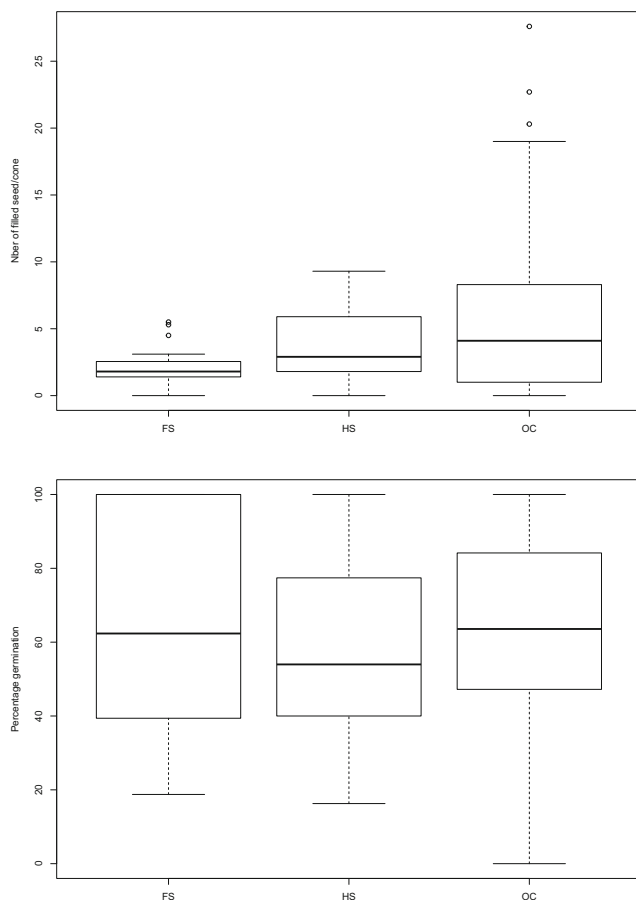
	Total no. of seeds/cone		No. of filled seeds/cone		Percentage of filled seeds		P1000 (mg)		Germination percentage (%)	
	Mean	(SE)	Mean	(SE)	Mean	(SE)	Mean	(SE)	Mean	(SE)
F ₁	87.1 a	13.5	13.1 c	2.0	18.0 c	3.2	722.1 a	503	43.2 a	9.2
OC	72.3 a	4.3	5.5 b	0.6	8.5 b	1.0	664.6 a	176	64.0 a	2.9
HS	69.4 a	5.5	4.6 ab	0.8	9.0 bc	1.3	673.9 a	228	59.4 a	3.7
FS	69.8 a	5.9	2.1 a	0.9	3.7 a	1.4	734.8 a	279	66.5 a	4.0

treatment (F₁). Compared with most other *Pinaceae* species, larch is actually characterized by a low reproductive success (Stoehr 2000; Colas et al. 2008). On one side, whereas male strobili are produced each year, female flowering is highly erratic and greatly varies from year to year; on the other side, as the results of this study showed, seed potential is high and comparable with other *Pinaceae* species but seed yield remains overall weak.

Frost damages of reproductive organs which are regularly met in our environmental conditions by this early reproducing species (late winter-very early spring) are certainly one major

cause of failure in nature. Besides that, pre- and post-zygotic abortion due to several causes (e.g. lack of pollination, low pollen viability, self-inviability) (Hall and Brown 1977; Kosinski 1987; Owens 2008) is frequent in larch and leads to significant proportions of empty seeds and seeds with degenerated endosperm and/or embryo. For example Kosinski (1987) reported percentages of empty seeds as high as 62–89% in European larch. For the same species, Sloobodnik and Guttenberger (2005) estimated that 61% of fertilized ovules did not have a viable embryo after open pollination. Owens (2008) observed average proportions of empty seeds around 60 and 56% from lots of western larch, respectively, collected in natural stands and in one seed orchard. However for the same species, Stoehr (2000) reported an average percentage of full seed/cone of 22% (10–34%) for seed lots collected in seed stands and Shearer (1990) found an even lower average rate of 14%.

In this study, female flowers isolated in paper bags without male strobili were protected against frost thanks to individual heating systems directly placed into the bags. Although fresh pollen was not available, the pollen lots used in this study were systematically checked by a conductivity test to assess their viability: in larch, pollen germination in vitro is feasible but hardly obtained (Dumont-BeBoux et al. 2000), and indirect methodologies to assess ‘viability’ such as conductivity test proved to be a good predictor (Bonnet-Masimbert et al. 1998). In addition, pollination was hand-made and repeated 2–3 times to cover the variability of female flower receptivity in a bag. All these cautions should have a priori reduced the

**Fig. 3** Boxplot of mean family performances within groups for number of filled seeds/cone (top) and percentage of germination (bottom). See Table 1 for abbreviations**Table 2** Mean values (and standard errors) of the groups for total height (TH) at ages 1 and 2. Means with the same letter do not differ significantly (Tukey test, $p = 0.05$) (see Table 1 for group abbreviations)

	TH1 (cm)		TH2 (cm)	
	Mean	(SE)	Mean	(SE)
F ₁	21.4 bc	0.7	82.5 c	1.5
OC	21.2 c	0.2	78.7 c	0.5
HS	19.8 b	0.3	76.1 b	0.6
FS	17.1 a	0.4	71.8 a	0.8

Table 3 Hybrid breakdown (HD = $1 - F_{2OC} / F_1$) and inbreeding depression (ID = $11 - F_{2I} / F_{2OC}$) (see Table 1 for group abbreviations)

	No. of filled seeds/cone	Percentage of filled seeds	Germination percentage	Germinated seeds/cone	Total height (2 years old)
Hybrid depression					
OC	0.598	0.550	-0.595	0.342	0.048
Inbreeding depression					
HS	0.176	-0.022	0.059	0.073	0.032
FS	0.607	0.552	-0.036	0.553	0.093

negative impact of low temperature on flower development and fertilization, avoided self-pollination negative effects and enhanced pollination success.

The low seed yield reported in this study may question about a possible negative impact of interspecific crossings. Indeed, Hall and Brown (1977) for example found a much lower full seed set/cone in ELxJL controlled crosses than in ELxEL and JLxJL. Nevertheless, this is contradictory with results we obtained on previous large-scale crossing experiments: firstly in a diallel mating design combining 9 European and 9 Japanese larches in intra- and interspecific crosses, the percentage of filled seeds for interspecific crosses reached on average 11.9% and was even higher than for intraspecific crosses (8.6 and 6.5%, respectively, for EL and JL). The number of full seed/cone was very close (6.8 vs 5.2 and 6.3) (Pâques 1995). Secondly, in the same document, we also synthesized seed yield results from 8 years of bi-parental controlled crosses (800 intra- and interspecific controlled crosses between 1987 and 1994): average percentages of filled seeds reached 8.0, 6.7 and 12.9%, respectively, for EL, JL, and HL (1st generation). So, the results of this study are coherent with these figures for 1st-generation hybrids (F_1) and do not confirm a negative impact of interspecific hybridization on seed yield.

4.2 Hybrid depression for seed traits and nursery vigour in 2nd-generation hybrids

Hybrid depression or breakdown manifests in an advanced-generation hybrid population relative to its ancestral F_1 hybrid population as a reduced viability or fertility (Avisé 1994) and as a deterioration in mean population performance (Levin, 1978). It is supposed to be under multiple gene control, and its effect would be stronger the more distant would be intercrossed taxa (Levin, 1978). Compared with first-generation hybrids (F_1), the rate of crossing success for outcrossed 2nd-generation hybrids (OC) was found to be reduced by about 20%; moreover these hybrids showed a strong hybrid depression for seed yield traits. Nevertheless this was not valid on one side for seed quality traits, namely for seed weight and germination, and on the other side, 2nd-generation hybrids (OC) grew nearly with the same vigour than F_1 (less than 5% less) at least during the first 2 years in the nursery.

First-generation interspecific hybrids have been reported to be fertile in several forest tree genera; however the reproductive success of outcrossed 1st-generation hybrids is basically not documented in trees although we believe that some unpublished data exist among breeders interested in advanced hybrid breeding. In *Pinus rigida* x *Pinus taeda*, old results from Hyun (1974, 1976) indicated a lower conelet drop and

Table 4 Inbreeding depression for seed traits for separate mother clones

Mother clone	No. of filled seeds/cone		Percentage of filled seeds		Germination percentage		No. of germinated seeds/cone	
	FS	HS	FS	HS	FS	HS	FS	HS
10069	0.553	-0.021	0.506	-0.494	-0.494	-0.569	0.387	-0.762
10072	0.997	-	0.998	-	-1.339	-	0.994	-
10077	0.365	-0.062	0.243	-0.569	0.049	0.399	0.482	0.261
10078	-0.430	-1.576	-0.174	-0.029	0.004	-0.161	-0.941	-2.845
10081	0.873	0.823	0.846	0.764	-0.691	-0.294	0.813	0.768
10097	0.684	-0.160	0.201	-0.542	0.161	0.180	0.696	-0.044
10098	0.730	0.594	0.650	-0.218	0.090	0.155	0.755	0.665
10106	0.359	-1.880	0.466	-1.119	-0.323	0.507	0.319	-0.472
10110	0.286	0.166	0.173	-1.134	0.407	0.331	0.391	0.1454
10112	0.641	0.502	0.761	0.500	-0.208	-0.551	0.541	0.137
10141	0.805	-	0.751	-	-0.162	-	0.797	-

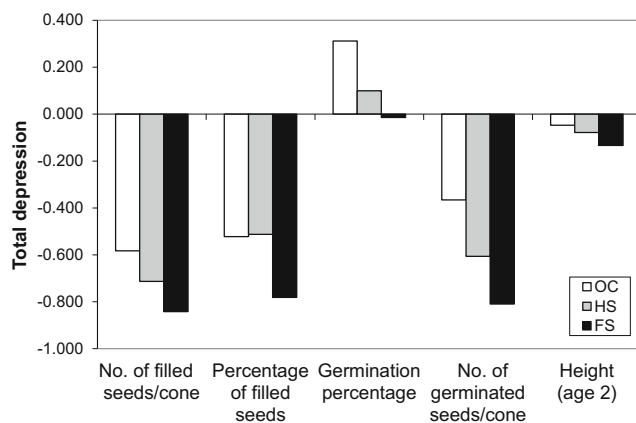


Fig. 4 Total depression $((F_{2OC} - F_1) + (F_{2i} - F_{2OC})) / F_1$ estimated for the 3 inbreeding groups. See Table 1 for abbreviations

a higher fertile seed percentage in F_2 than in F_1 crosses, which is obviously not the case in our study.

Growth performance of 2nd-generation outcrossed hybrids is better documented. Overall it seems that in conifers, hybrid breakdown is weak or moderate when compared with broadleaves. In the most comprehensive study of interspecific breeding in trees with *Pinus elliottii* var. *elliottii* \times *Pinus caribaea* var. *hondurensis* in Queensland, outcrossed F_2 hybrids showed no or little differences with F_1 's for growth traits up to age 12, which was interpreted as an absence of hybrid breakdown (Kain 2003). In larch, several crosses between unrelated first-generation hybrids of European \times Japanese larch had on average a slightly better height growth (9%) than a commercial F_1 hybrid used as reference (Dietze 1974). Nevertheless, F_2 's derived from *Pinus rigida* \times *Pinus taeda* showed some heavier depression varying with sites from -0.01 to -8.4% (Hyun 1976). By contrast, hybrid breakdown is much more severe in broadleaves such as Eucalypts (Potts et al. 2003) and Poplars (Stettler et al. 1988): outcrossed F_2 's (but also F_1 's) may indeed exhibit poorer (or intermediate) performance than parental crosses.

4.3 Inbreeding depression

Seed yield in HS- F_2 hybrids was only slightly reduced (but not significantly) compared with outcrossed F_2 hybrids ($ID < 0.18$) but higher level of inbreeding (FS) induced on average a strong depression ($ID > 0.55$). In contrast, inbreeding has no significant influence on seed quality traits: no significant differences were observed between HS and FS and OC hybrids. Several authors have well documented the linear decrease in filled seed yield with increased levels of inbreeding: nevertheless, ID varies much according to the species: for example for percentage of filled seeds, ID ranged from -0.37 (for *Picea abies*, Anderson et al. 2010) to 0.30 (for *Pinus patula*, Williams et al. 1999) for $F = 0.125$ and from 0.03 (on *Pinus radiata*, Griffin and Lindgren 1985) to 0.48 (on *Pinus elliottii*,

Squillace and Kraus 1963) for $F = 0.25$ and from 0.21 (for *Pinus pinaster*, Durel et al. 1996) to 0.81 (for *Pinus patula*, Williams et al. 1999) for $F = 0.5$. Compared with that of other *Pinaceae*, inbreeding depression of larch seems thus to be beyond the higher extremes reported in the literature for a still weak level of inbreeding ($F = 0.25$). A higher level of inbreeding such as selfing is highly detrimental in larch: successful selfing in European and Japanese larches is known to be possible but is extremely rare with levels of full seed percentages (0.3 – 0.7% , Pâques 1995) and seed conversion into seedlings (EL 0.7 – 1.9% (Dieckert 1964); 4.3% (calculated from Dietze 1974)) never observed in other *Pinaceae*.

Pinaceae are known to have high numbers of lethal equivalents (Williams and Savolainen 1996) but larch (in fact *Larix laricina*) has been shown to be one of the species with the largest estimate with 10.8 lethal equivalents (Park and Fowler, 1982, in Williams and Savolainen 1996). In Pâques (1995), we estimated this number as 11.4 for European larch and 13.4 for Japanese larch. This heavy genetic load in larch explains its rather low reproductive success.

Inbreeding appeared to be less detrimental on seed quality traits than on seed yield. This result is consistent with that of Durel et al. (1996): they observed no significant differences for seed weight among inbreeding groups ($F = 0$ to 0.75) in maritime pine. As well, Woods and Heaman (1989) found in Douglas fir a much weaker relationship between seed weight and F ($r^2 = 0.19$) than between full seeds/cone and F ($r^2 = 0.35$). In European larch, Dieckert (1964) observed no differences in seed weight between full seed lots after self-pollination and outcrossing. Weight of full seeds (P1000) is highly dependent on the mother clones. Once the majority of aborted seeds (empty seeds) have been removed during the sorting process, germination rate mostly depends on seed reserve contained in the endosperm, a maternal tissue in conifers, if we assume that dormancy has been properly and similarly released in all inbreeding groups. Park and Fowler (1982) noted as well the lower impact of selfing on germination than on percent full seed.

Nevertheless, contrasted inbreeding depression levels were observed for both seed yield and seed quality traits within inbreeding groups according to the mother clones and specific combinations. Several authors (e.g. Durel et al. 1996; Woods and Heaman 1989; Wu et al. 1998; Ford et al. 2015) also found strong family/founder effects within inbreeding groups.

Levels of inbreeding depression were on average low for height growth including for FS hybrids ($ID = 0.09$). They were in the range of Dietze (1974)'s findings for hybrid larch: from his study, we estimated inbreeding depression at 0.027 , 0.123 and 0.208 , respectively, for $F = 0.125$, 0.25 and 0.5 . Up to F levels lower or equal to 0.25 , Durel et al. (1996) found no significant differences for height growth between 1 and 11 years in maritime pine (inbreeding depression less than 5%); but depression became severe beyond that level. In

Douglas fir, inbreeding depression for height increased with inbreeding levels and with age but seemed to level off after 5 years of age at around 7% for $F = 0.125$ and 10–11% for $F = 0.25$ (Stoehr et al. 2015). In white spruce, Doerksen et al. (2014) estimated a decrease in height growth at age 15 of around 6% for every 0.1 increase in the inbreeding level.

Compared with growth, seed traits are usually more affected by inbreeding as shown in this study. It is a common finding that traits more closely related to fitness are more susceptible to inbreeding (e.g. Durel et al. 1996, Sorensen 1999) even though some evidence of opposite results exists in some species (Sorensen 1999).

4.4 Use of second-generation interspecific hybrids: overall depression

Mass-production of hybrid larch seeds in 2nd-generation hybridization orchards is attractive to circumvent the many problems to successfully mass-produce seed lots with high hybrid percentage in 1st-generation hybridization orchards. Mismatching in flowering phenology between male and female parental species clones (usually a few to increase levels of hybrid vigour) decreases chances to produce hybrid seed lots. Increasing the number of one or both parental clones to enlarge the flowering phenology window may increase interspecific recombination success but usually reduces overall hybrid vigour. Disconnection of parental species in separate orchards and use of supplemental pollination (or controlled crosses) allow high hybrid percentage (over 90–95%) but generate extra costs (Pâques et al. 2013). Second-generation hybridization orchards, composed of several dozens of selected 1st-generation hybrid clones are expected to function as any other coniferous seed orchards, relying on wind pollination. This option is valid as long as firstly, reproductive success is ensured and secondly, 2nd-generation hybrids still show superiority over parental species for growth traits.

The choice of proper parental F_1 hybrid clones with no or low inbreeding levels has been shown to be crucial for establishment of 2nd-generation hybridization orchards. Up to inbreeding levels of 0.125 (half-sibs), one can still expect around 80–85% of successful crosses while it drops down to 65% in case full-sibs are used. The deleterious effect of advanced hybridization and inbreeding (total depression) has been shown to be severe for seed yield without or with inbreeding but use of full-sib parents would reduce seed yield by as much as 70% (number of germinated seeds/cone). Use of non-related (or half-sib) parents would cut nearly by two this reduction. However, individual parents showed different reactions to hybrid breakdown and to inbreeding, and a large variability among them was observed too within inbreeding groups; evaluation and selection of best parents would be thus highly beneficial to increase seed production.

More positively, our results showed an overall weak depression relative to F_1 for seedling height (4.8–13.4%) but it is still of interest to keep inbreeding at the lowest level, especially if one expects an increasing depression with age (Stoehr et al. 2015).

Limitation of relatedness among reproducing clones in any orchard is a general recommendation (Zobel and Talbert 1984; Nanson 2004). Nevertheless, in advanced hybridization of larch, we know of at least two situations where potentially some inbred F_1 hybrid clones might have been included in 2nd-generation hybridization seed orchards. The first case is a commercial clonal orchard in Belgium issued predominantly from mass selection of top-100 best clones in F_1 hybrid progenies from an open-pollinated hybridization orchard (Nanson 2004). At that time, discriminating molecular markers were not available for larch to investigate levels of relatedness among selected clones. The second is a ‘pilot-scale’ orchard established in France in 1993: it is composed of half-sib F_1 hybrid clones selected in progenies sharing a common mother clone with an exceptional breeding value for stem straightness. When the orchard was planned, this option was privileged to benefit from a good stem form at the expense of some expected loss in growth.

Although a slight reduction (around 11%) in the number of living germs/kilogram was observed in F_2 's commercial crops from the Belgian orchard when compared with crops from a nearby commercial F_1 seed orchard, it was largely compensated by 3–4 times more abundant annual seed yield/ha (over 6 years) on average in the F_2 orchard. Moreover it solved the recurrently low to moderate hybrid percentage (< 42%) registered in the F_1 orchard (Servais, *pers. comm.* 2017). So, at least from a seed production operational point of view, F_2 seed orchards—even with some consanguinity—look as a successful response to the major drawbacks encountered in commercial F_1 seed orchards.

As an extension of these results, we can also conclude that natural regeneration of first-generation hybrid plantations—while biologically and technically feasible—is not recommended for the same reasons mentioned above on top of an actually low genetic diversity of the base material.

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Compliance with ethical standards

Conflict of interest The author declares that there is no conflict of interest.

Appendix. Effect of various levels of inbreeding (*F* level) on reproductive success in the forestry literature. OC crossing between unrelated parents, HS between half-sibs, FS between full-sibs, S1 selfing and S2 2 generations of selfing

		Crossing	OC	HS	FS	S1	S2
		<i>F</i> level	0	0.125	0.25	0.5	0.75
Woods and Heaman 1989	Douglas fir	% cone abortion	No relationship with <i>F</i> ($r^2 = 0.007$)				
Woods and Heaman 1989	Douglas fir	% Succ. crosses	100	94.3	90.0	100	100
Griffin and Lindgren 1985	<i>Pinus radiata</i>	% filled seed	78.9	80.7	76.5	34.2	33.1
Durel et al. 1996	<i>Pinus pinaster</i>	% filled seed	94.1 a	89.7 b	88.4 bc	74.7 bc	56.9 c
Williams et al. 1999	<i>Pinus patula</i>	% filled seed	43.9	30.7	27.6	8.4	
Squillace and Kraus 1963	<i>Pinus elliottii</i>	% filled seed	100	92	52		22
Saito et al. 1973	<i>Pinus thunbergii</i>	% filled seed	100	87	74	66	59
Bingham 1973	<i>P. monticola</i>	% filled seed	100	102	81	60	19
Anderson et al. 2010	<i>Picea abies</i>	% filled seed	100	137	80	25	7
Griffin and Lindgren 1985	Overall mean of studies	% filled seed	100	104	77	50	39
Woods and Heaman 1989	Douglas fir	No. of filled seed/cone	31.6	21.3	16.7	4	0
Griffin and Lindgren 1985	<i>Pinus radiata</i>	No. of seed/cone	77.5	100.1	106.1	84.7	101.4
Durel et al. 1996	<i>Pinus pinaster</i>	No. seed/cone	142.5 a	135.1 a	128.0 a	127.2 a	85.1 b
Williams et al. 1999	<i>Pinus patula</i>	No. of seed/cone	17.7	8.3	10.3	3.4	
Woods and Heaman 1989	Douglas fir	Seed weight	Weak negative relationship with <i>F</i> ($r^2 = 0.19$)				
Durel et al. 1996	<i>Pinus pinaster</i>	P100	5.54 a	5.82 a	5.72 a	5.51 a	5.09 a
Wu et al. 2004	<i>Pinus radiata</i>	% trees producing female flowers age 5	69.6	63.8	54	55.3	49.1
Wu et al. 2004	<i>Pinus radiata</i>	No. of female flower/tree age 5	3.8	4.1	3.3	1.6	2.6
Durel et al. 1996	<i>Pinus pinaster</i>	No. of cones/tree	0.62 a	0.52 a	0.40 ab	0.29 ab	0.07 b

In italics, cited from Griffin and Lindgren (1985) and from Franklin (1970); in %OC. Values with the same letter do not differ significantly (for $p = 0.05$)

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