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1 **Composition of hybrid larch (*Larix x eurolepis* Henry) Forest**  
2 **Reproductive Materials: how much does hybrid percentage affect**  
3 **stand performance?**

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20

21

1 **ABSTRACT**

2

3 The performance of hybrid larch (*Larix x eurolepis*) stands may be altered by the fact that the  
4 seed lots produced in hybridization seed orchards always contain a proportion of non-hybrid  
5 seeds. We studied the effect of such hybrid/non-hybrid mixtures on the productivity and  
6 quality of young plantations, through a three-step process: determination of tree species  
7 identity with cytoplasmic DNA markers, comparison of hybrid and non-hybrid tree  
8 performance, and finally, assessment of thinning impact on hybrid percentage. Overall, we  
9 analyzed progenies from eight commercial seed orchards at three sites.

10 Huge variations in hybrid percentage were found among orchard progenies. Globally, the  
11 results concerning survival, susceptibility to drought and to *Meria laricis*, growth, stem form  
12 and wood stiffness were clearly in favor of hybrids. In particular, they grew faster than the  
13 non-hybrid trees and exhibited a more homogeneous growth than the mixed progenies.  
14 Hybrid superiority was highest for orchards composed of a single maternal clone of a species  
15 which was poorly adapted to the planting site. Hybrid percentage was enhanced by successive  
16 thinnings. However, a serious loss of income is expected when planting progenies  
17 characterized by low initial hybrid percentage and high hybrid superiority. Moreover,  
18 variations in hybrid percentage proved to be problematic in seed orchard testing as seed  
19 orchard ranking may change depending on whether it is based on the performance of all trees  
20 or hybrids only. These variations reduce the reliability of the recommendations typically  
21 made to forest owners. For commercial and silvicultural reasons, we suggest setting a  
22 minimal threshold level of 60-70% for hybrid percentage in marketed Forest Reproductive  
23 Materials.

24

- 1 **Key-words:** hybrid larch, hybrid percentage, Forest Reproductive Material, seed orchard,
- 2 thinning, growth
- 3

## 1 INTRODUCTION

2 Since their discovery in the Dunkeld estate in the early 1900's (Henry and Flood 1919;  
3 Edwards 1956), interspecific hybrids between European larch (EL) (*Larix decidua* Mill.) and  
4 Japanese larch (JL) (*Larix kaempferi* (Lamb) Carr.) have generated considerable enthusiasm  
5 among foresters and, later on, tree breeders. Not only does hybrid larch (*Larix x eurolepis*  
6 Henry) feature good resistance to larch canker (*Lachnellula willkommii*) (Edwards 1956;  
7 Sylvestre et al. 1999), it has shown better growth than either parent species in many instances  
8 (e.g. Ferrand and Bastien 1985; Pâques 1992; Baltunis et al. 1998; Pâques et al. 2013) and  
9 sometimes has a better form (Lacaze and Birot 1974) and a better survival rate (Miller and  
10 Thulin 1967; Zaczek et al. 1994). For wood properties, the hybrid is usually intermediate  
11 between EL and JL or better than the best parent (Nanson and Sacré 1978; Reck 1980;  
12 Bastien and Keller 1980).

13  
14 Interspecific hybridization breeding programs between European and Japanese larches have  
15 been developed since the forties and plantations dedicated to hybrid seed production, named  
16 hybridization seed orchards, have been established. About forty first-generation orchards,  
17 covering more than 100 ha, have been planted in eleven European countries (Pâques et al.  
18 2013). These orchards differ in the number of genotypes used as male and female (from one  
19 to several dozens), the species from which the cones are collected (EL or JL, and sometimes  
20 both) and the planting design. EL and JL clones may be intimately mixed, arranged in  
21 alternating rows or even planted in separate units especially managed for pollen or seed  
22 production (Nanson 2004). The seeds are produced by open-pollination or exceptionally by  
23 supplemental mass pollination (SMP) (Philippe and Baldet 1997).

24

1 Planting EL and JL genotypes in mixtures allows interspecific crossings to occur and does  
2 result in some hybrid seed production as long as their flowering periods are not too much  
3 asynchronous (Pâques et al. 2013). However, the proximity of individuals of the same species  
4 within the orchard also makes intraspecific crossings possible: among ramets of different  
5 clones, among ramets of the same clone or within the same tree. As a consequence, the seed  
6 lots produced in hybridization orchards are always somehow composed of a mixture of hybrid  
7 seeds and of seeds from one or both parent species (Pâques et al. 2006). The risk of obtaining  
8 non-hybrid seeds is a priori limited in orchards including a single maternal clone where  
9 intraspecific crossings are limited to selfing if the orchard is well-isolated from incoming  
10 pollen. As self-pollinated ovules often produce empty seeds due to embryo abortion (Kosinski  
11 1986; Owens 1995; Slobodnik and Guttenberger 2005; Williams 2007) or seedlings with a  
12 low vigor (Dieckert 1964, Nanson 2004), this design is considered optimal for the production  
13 of high percentages of hybrid seeds.

14

15 The proportion of hybrid seeds also varies according to 1) the respective contributions of the  
16 parent species to the pollen cloud (Lewandowski et al. 1994), 2) the extent of the flowering  
17 overlap among genotypes of the two species (Nanson 2004, Pâques et al. 2013), and 3)  
18 pollination success (Hall and Brown 1977). Those processes involve genetic factors such as  
19 clonal flowering ability, phenology and ‘selfing ability’; climatic factors at flower initiation,  
20 in winter and during pollination (Owens and Blake 1985; Philippe et al. 2006); and in some  
21 cases, anthropic factors (effectiveness of flower stimulation treatments and SMP). Therefore,  
22 hybrid percentage in commercial seed lots fluctuates from one orchard to another but also, for  
23 a given orchard, from year to year (Bergmann and Ruetz 1987, Pâques et al. 2006).

24

1 For a long time, hybrid percentage could not be quantified because morphological  
2 identification of hybrids, EL and JL is impossible for seeds, difficult for young seedlings  
3 (Pâques *et al.* 2006) and very uncertain for saplings. Isozyme markers developed in the 1990's  
4 were a considerable improvement (*e.g.* Häcker and Bergmann 1991) but they were not  
5 adapted to all kinds of orchards. Isozymes have now been replaced by molecular markers  
6 based on mitochondrial (maternally inherited) and chloroplastic (paternally inherited) DNA  
7 that allow reliable taxonomic identification of any seed or seedling (Acheré *et al.* 2004). The  
8 numerous analyses performed from then on with markers show tremendous fluctuations in  
9 hybrid percentage among orchard progenies, ranging from less than 10% to more than 90%  
10 (Pâques *et al.* 2006).

11  
12 In the European Union, seed lots may be marketed with any hybrid percentage provided that it  
13 is specified in the official document produced by the seed dealer (Council Directive  
14 1999/105/CE, 2000). However, some Member States have imposed additional, more stringent  
15 requirements. For example, in France, state grants for planting are contingent upon the use of  
16 forest reproductive materials (FRM) with a hybrid percentage higher than 60%.

17  
18 Besides the problem of genetic conformity of the marketed FRM with the certified seed  
19 orchard, producing seed lots with low and variable hybrid percentages may have adverse  
20 consequences for foresters: reduced or unstable stand performance, heterogeneous final  
21 products and increased susceptibility to specific diseases (*e.g.* larch canker (*Lachnellula*  
22 *willkommii*) and *Meria laricis* on EL; *Phytophthora ramorum* on JL). Therefore, it is important  
23 to understand the effect of hybrid percentage on stand productivity and quality. In our study,  
24 we attempted to answer the following questions:

1 1) How severely does the mixture of hybrid and non-hybrid seeds in FRM affect stand  
2 performance?

3 2) How does thinning impact hybrid percentage over time?  
4

5 Furthermore, in the context of seed orchard testing that is required for certification and  
6 deployment recommendations, it is justified to investigate to what extent the ranking obtained  
7 in genetic tests varies according to hybrid percentage. Hence, we compared the seed orchard  
8 performance considering all trees (mixed progenies) or hybrids alone, both before and after  
9 the start of inter-tree competition.  
10

## 11 **MATERIALS AND METHODS**

12

### 13 **Seed orchard composition**

14

15 Our study included commercial seed lots collected from eight seed orchards with diverse  
16 designs. Their description and codes are provided in Table 1. The orchards were composed of  
17 one or several clones of the mother species (either European larch (FH, E, V, FP, S), Japanese  
18 larch (M, NT) or both (H)) and one or several clones of the paternal species.  
19

20

### 20 **Trials**

21

22 Our study was based on three trials originally established to compare seed orchard progenies.  
23 The three trials were planted at mid-elevation sites, between 600 m and 1000 m, with  
24 favorable climatic conditions for larch (Table 2). The Peyrat and La Courtine trial sites are  
25 subjected to an oceanic climate, more favorable to JL, while the Brenod site, which is more



1 continental, is more suited to EL. La Courtine and Brenod are twin trials in the sense that they  
2 were established with the same genetic materials, i.e. with seedlings deriving from the same  
3 seed lots, and raised in the same nursery. Both trials were planted in spring 1995 according to  
4 a randomized complete block design with four blocks and 64 seedlings per plot. The 2-year-  
5 old seedlings were planted at a 2m x 3m spacing (1,667 individuals per hectare). The third  
6 trial at Peyrat was established in spring 2002. Owing to limited planting stock, each genetic  
7 unit was planted in a single plot containing 71 to 153 seedlings (3m x 3m spacing). A first  
8 thinning was carried out at La Courtine and Brenod, in 2005 and 2008, respectively, but not at  
9 Peyrat.

10

### 11 **Taxonomic identification**

12 In the three trials, the genetic status of individual trees was determined at the seedling/sapling  
13 stage with cytoplasmic DNA markers. The combination of information from chloroplastic  
14 DNA, inherited from the male parent in the *Pinaceae* (Owens 1995), and mitochondrial DNA,  
15 transmitted by the female parent, enabled us to determine the species of the male and female  
16 parents of each tree, and thus its status: EL ( $\text{♀ EL} \times \text{♂ EL}$ ), JL ( $\text{♀ JL} \times \text{♂ JL}$ ) or HL (for  
17 interspecific crossings  $\text{♀ EL} \times \text{♂ JL}$  or  $\text{♀ JL} \times \text{♂ EL}$ ) (Acheré et al. 2004). At all sites, the  
18 analyses were carried out on buds. At Peyrat, the buds were collected from all seedlings  
19 before planting (Table 2). After determination, each seedling was carefully labelled in order to  
20 keep track of its identity in plantation. At the other two sites, the buds were collected a few  
21 months before thinning (Table 2). At that time, overall survival averaged 96% at La Courtine  
22 and 87% at Brenod. Taxonomic identification was carried out for all the orchard progenies  
23 tested at Brenod but only for four progenies at La Courtine (H83, FH, E, M) to limit costs. In  
24 both trials, DNA analyses were restricted to trees from two blocks out of four.

1 Prior to seed sowing, taxonomic identification had been carried out for each seed lot used in  
2 the Peyrat trial. That enabled us to detect a possible difference in hybrid percentage between  
3 the seed and seedling stages. The percentages of hybrid and non-hybrid seeds had been  
4 determined with the only marker available at that time, i.e. isozyme marker (SKDH; 200  
5 seeds per seed lot) as described by Bergmann and Ruetz (1987).

6

## 7 **Measurements**

8

9 The data used in our study were collected at ages 2, 7, 10 and 12 at Peyrat, at ages 6 and 13 at  
10 Brenod, and at ages 6 and 10 at La Courtine. The traits recorded at all three sites were related  
11 to site adaptation (survival), growth in height and circumference, and stem straightness.  
12 Additional traits were assessed at Peyrat: crown dieback following the severe heat wave and  
13 drought in summer 2003, needle damage caused by a severe attack of *Meria laricis* in 2008,  
14 and wood quality (modulus of elasticity at age 12).

15

16 The impact of the 2003 heat wave was estimated through the presence/absence of damage (1-  
17 0) and its severity (height of top dieback). Needle yellowing or loss caused by *M. laricis* was  
18 recorded using a scoring scale from 1 (severe) to 5 (light) in July 2008. In addition, the  
19 subsequent growth reduction was estimated by comparing the length of the terminal shoot in  
20 2008 and from the two previous years. Stem straightness was assessed using a scoring scale  
21 from 1 (crooked) to 5 (straight). Total tree volume (V) was estimated from total height (H)  
22 and circumference at breast-height (C) according to Pauwels and Rondeux (1999):  
23  $V = 0.40678^{-5} * C^2 * H$ , with V in m<sup>3</sup>, C in cm and H in m. To estimate modulus of elasticity  
24 (MOE), a bending test was carried out on samples of standing trees (18 trees per species and  
25 per orchard progeny, on average) with a dedicated device, the Rigidimeter, described by

1 Launay et al. (2000) and Pâques and Rozenberg (2009). Deviation at 1.3 m after bending was  
2 recorded three times for each of two cardinal directions (N and E) together with the pressure  
3 necessary for bending. MOE (MPa) was estimated as follows:

4  $MOE = (32 * R * F * a) / (\pi * D^4)$  with R = radius of curvature of the trunk under flexure  
5 (mm) that is a function of the length of the measurement bar (100 mm) and of stem  
6 deformation (mm), F = force applied to the tree (Newton), a = distance between the fixation  
7 system to the tree and the extremity of the Rigidimeter (300 mm) and D = tree diameter (mm).  
8 As a result, for a given force and a given stem diameter, MOE is inversely proportional to  
9 trunk flexion.

10

## 11 **Thinning impact**

12

13 In order to estimate the effect of thinning on hybrid percentage, we considered both simulated  
14 thinnings (at all sites) and actual thinnings (at La Courtine and Brenod). For the simulations  
15 made for Peyrat, we fixed the thinning rate at 30% of the living trees in each plot. Three  
16 criteria, measured at age 10, were considered successively: tree volume, stem straightness and  
17 an index combining growth and stem straightness. As height had not been measured at age 10,  
18 tree volume (V) was estimated on the basis of circumference at breast height (C) (Thill and  
19 Palm 1984):

20  $V = 19.784 - 3.1514 * C + 0.12589 * C^2 - 506^{-7} * C^3$  with V in dm<sup>3</sup> and C in cm.

21 The growth-form index, drawn from Magnussen (1990) was calculated as follows:

22  $I = 0.955 * C + 16.096 * SS$  where I is the index, C the circumference expressed in cm and SS  
23 the stem straightness score.

24 For La Courtine and Brenod, the proportion of hybrid to non-hybrid trees was determined  
25 after the actual thinnings. They eliminated 42% and 45% of the living trees in 2005 and 2008,

1 respectively; the proportion of planted trees remaining after thinning were 56% and 47%, or  
2 940 and 790 stems per ha. In both trials, thinning was partly systematic (elimination of one  
3 row per plot to facilitate further management) and partly selective (one third of the trees in the  
4 other rows were felled on the basis of vigor, stem form and spatial distribution of the retained  
5 trees). In addition, simulations were made using a thinning rate of 50% (of the living trees)  
6 and considering two criteria successively: circumference and stem straightness. We used data  
7 collected just before actual thinnings, at ages 10 and 13 for La Courtine and Brenod,  
8 respectively. We then simulated second and third thinnings according to the same procedure,  
9 considering that tree density would be reduced to 600 and 400 stems per hectare, respectively.  
10 The traits taken into account were circumference and stem straightness at age 16 for La  
11 Courtine and age 17 for Brenod. For these simulations, we hypothesized that tree ranking  
12 would remain unchanged between data collection and the second and third thinnings which  
13 are approximately done at ages 18 and 25, respectively.

14

## 15 **Data analysis**

16

17 For quantitative traits, we compared the performance of hybrid and non-hybrid trees within  
18 each orchard progeny using analyses of variance performed on individual data as follows:

19  $X_{ijk} = \mu + s_i + b_j + sb_{ij} + \varepsilon_{ijk}$  where  $\mu$  is the overall mean,  $s_i$  is the fixed effect of species  $i$ ,  $b_j$  is  
20 the fixed effect of block  $j$ ,  $sb_{ij}$  is the interaction effect between species and block, and  $\varepsilon_{ijk}$  is  
21 the random error. Species means were compared using Bonferroni test. At Peyrat where the  
22 orchard progenies were not repeated in blocks, the model was restricted to:  $X_{ij} = \mu + s_i + \varepsilon_{ij}$ .

23 For proportions, the data were analyzed with Chi-square tests.

24

1 The effect of hybrid percentage on the ranking of seed orchard progenies was studied at  
2 Brenod and La Courtine. We compared progeny performance by analyses of variance  
3 considering either all trees or hybrid trees only. The following model was used:

4  $X_{ijk} = \mu + p_i + b_j + pb_{ij} + \varepsilon_{ijk}$  where  $\mu$  is the overall mean,  $p_i$  is the fixed effect of orchard  
5 progeny  $i$ ,  $b_j$  is the fixed effect of block  $j$ ,  $pb_{ij}$  is the interaction effect between progeny and  
6 block and  $\varepsilon_{ijk}$  is the random error. We used Tukey or Bonferroni tests to compare progeny  
7 means, depending on whether the number of data differed or not.

8

## 9 **RESULTS**

10

### 11 **Hybrid percentage in the seed orchard progenies**

12

13 Hybrid proportion varied considerably in the studied seed orchard progenies, from 12% to  
14 96% at the seedling/sapling level (Table 1). It was low for FP, S and NT; low to moderate for  
15 H according to annual crop; moderate for M and high for V, FH and E. In addition, hybrid  
16 percentage was higher at the seedling stage than at the seed lot level for most of the progenies  
17 tested in the Peyrat trial. The difference was particularly large for FH and V whose seeds were  
18 collected from a single clone.

19

### 20 **Comparison of hybrid and non-hybrid tree performance**

21

#### 22 *Site adaptation*

23

24 Ten years after planting, overall survival was good at Peyrat (92.9% on average) with few  
25 differences among species (92.5%, 91.5% and 94.1% for EL, JL and HL, respectively).

1 Mortality occurred mainly in the first two years following planting. Significant differences in  
2 mortality rates were found for FH and H98 where HL performed better than EL and JL,  
3 respectively (Table 3). At Brenod and La Courtine, hybrid and non-hybrid trees did not differ  
4 in terms of survival or damage since the trials encountered no health problems after DNA  
5 analyses.

6

7 The heat wave and drought of summer 2003 resulted in shoot diebacks and twig drying in  
8 14.4% of the seedlings planted at Peyrat. Within the six seed orchard progenies, HL  
9 performed at least as well as the parent species (Table 3). Considering both the percentage of  
10 damaged trees and the height of top-dieback, the hybrids were or tended to be superior to JL  
11 in H87 and H98. A similar trend was found in most of the progenies when HL and EL were  
12 compared but the differences were not significant.

13

14 In July 2008, 74% of the trees at Peyrat were damaged by *Meria laricis*. Severe foliage  
15 discoloration or loss (scores 1 to 3) was observed for 90.3% and 77.3% of the EL and HL  
16 trees, respectively, while only 42.6% the JL trees were severely affected (Table 3). The lesser  
17 susceptibility of JL was obvious in the two orchards where JL was the maternal species. The  
18 hybrids ranked between the parent species but were closer to EL for needle damage intensity.  
19 However, the impact of *M. laricis* on growth in 2008 proved to be significantly smaller in HL  
20 than in EL for V, H87 and H98 (9.4% loss *versus* 22.3% on average, see Table 3); impact on  
21 growth was not significantly different from JL for H87 and NT (Table 3).

22

23 *Growth, stem form and wood quality*

24

1 In all three sites and for almost all the orchard progenies, the hybrids grew faster than EL  
2 and/or JL (Table 4). On average, hybrid tree volume was higher by 140%, though large  
3 differences occurred depending on site and orchard progeny. In the two sites favorable to JL,  
4 hybrid superiority was very high compared to EL, but only moderate and even sometimes  
5 non-significant relative to JL. This was particularly obvious for H87 and H98 at Peyrat. At  
6 Brenod, where the continental climate is more suitable to EL, the superiority of HL over JL  
7 was more pronounced than at the two oceanic sites, as shown by the results obtained for M  
8 and H83.

9  
10 Whatever the site and the orchard progeny, hybrid superiority over EL was greater for radial  
11 growth than for height growth. As a result, the height:diameter ratio was lower for the hybrids  
12 (Table 4). No clear trend was found when the hybrids were compared to JL. The hybrids also  
13 tended to be less straight than EL but the differences were rarely significant. In addition, the  
14 correlations between stem straightness and volume at age 6 were negative but generally weak  
15 and non-significant, both in hybrid and non-hybrid trees (data not shown).

16  
17 Hybrid modulus of elasticity tended to be or was significantly lower than that of EL. The  
18 reverse trend was observed when HL was compared to JL (Table 4).

19

## 20 **Homogeneity of hybrids relative to mixed seed orchard progenies**

21

22 Compared to overall orchard progenies, composed of a mixture of hybrid and non-hybrid  
23 trees, the hybrids alone were generally more homogeneous (lower coefficients of variation)  
24 for traits related to vigor (Table 5). That was always the case, though to varying degrees, for  
25 the progenies produced in seed orchards composed of a single EL maternal clone. For those

1 progenies, the differences were higher under oceanic climatic conditions, less adapted to EL  
2 (Peyrat and La Courtine). Symmetrically, for progeny M, derived from a single JL maternal  
3 clone, the hybrid population was less variable than the mixed progeny in the continental site  
4 at Brenod, but not at La Courtine. Regarding the hybrids produced in seed orchards composed  
5 of several mother clones, a wide variety of situations was found, even for the different seed  
6 lots produced in seed orchard H.

7

8 The coefficients of variation of the hybrids were most often similar or lower for H:D and  
9 similar for stem straightness. For those two traits, the data showed no clear relationship  
10 between homogeneity and seed orchard design.

11

## 12 **Effect of thinning on hybrid percentage**

13

### 14 *First thinning*

15

16 At the three sites and for almost all the orchard progenies, simulated thinnings based on  
17 growth criteria eliminated a greater proportion of EL and JL trees compared to the hybrids.  
18 Consequently, they led to increased hybrid percentages (Fig. 1, 2). Hybrid proportion reached  
19 nearly 100% in the progenies characterized by a high initial hybrid percentage and a great  
20 superiority of hybrids over parent species (V, E and FH). In most of the other cases, hybrid  
21 percentage increased but remained low or moderate. NT, whose hybrids performed badly  
22 relative to JL, was the only case where thinning resulted in a decrease in hybrid percentage.  
23 When stem straightness was used as the thinning criterion, hybrid percentages remained more  
24 or less stable.

25



1 Simulated thinnings based on the index combining growth and stem form resulted in  
2 increased hybrid percentages at Peyrat (Fig. 1). The increases were generally intermediate  
3 between those obtained with growth and stem straightness separately. At Brenod and La  
4 Courtine, actual thinnings also produced intermediate increases in hybrid percentage,  
5 although it should be kept in mind that the thinnings were partly systematic and took into  
6 account the distribution of the remaining trees in the field (Fig. 2).

7

### 8 *Simulation of second and third thinnings at Brenod and La Courtine*

9

10 The simulations showed the same patterns as for the first thinning. Selecting the trees for  
11 growth resulted in increased hybrid percentages except, of course, for the progenies that  
12 already contained nearly 100% hybrids (Fig. 3). The increase was small for H progenies in  
13 both sites and moderate for M progeny at La Courtine, which is favorable to its maternal JL  
14 species. Even after three thinnings, hybrid percentages remained low or moderate (< 60%) for  
15 these orchard progenies. On the contrary, large increases were observed for M and FP at  
16 Brenod. After the third thinning, hybrid percentage reached 90% for M, though it remained  
17 far below 100% for FP (63%). A thinning selection based on stem form had only limited  
18 effects on hybrid percentages, except for FP for which it increased slightly.

19

### 20 **Impact of hybrid percentage in seed orchard testing**

21

22 Seed orchard progenies were compared in two genetic trials using the data collected at age 6  
23 either from all the trees or from the hybrids alone. At La Courtine, few changes were  
24 observed in the ranking for tree volume (Fig. 4), circumference and stem straightness (data  
25 not shown). The main difference concerned H83 whose performance was poorly estimated

1 because of the small number of hybrid trees. On the contrary, the hierarchy was far less stable  
2 at Brenod (Fig. 4). Analyzing hybrids alone, instead of mixed progenies, led to a dramatic  
3 increase in circumference for FP. Though the mixed FP progeny had a very poor growth, its  
4 hybrids did not differ significantly from any other hybrids.

5  
6 Between ages 6 and 13, when the trees in the plots began competing with each other, a change  
7 in the ranking of orchard progenies appeared for circumference at Brenod, The gap between  
8 the pair FP-M and the three leading progenies (FH, V, E) was appreciably reduced during that  
9 period (Fig. 5). The dynamics was particularly clear for the hybrids: girth increment was  
10 significantly larger for FP and M than for FH, V and E.

11

## 12 **DISCUSSION**

13

### 14 **Hybrid percentage in the seed orchard progenies**

15

16 We found a wide spectrum of hybrid percentages, from 12% to 96%, in the seed orchard  
17 progenies studied. Considering the genetic and physiological mechanisms against self-  
18 pollination that result in embryo abortion and finally in empty or unviable seeds (Dieckert  
19 1964; Kosinski 1986; Slobodnik and Guttenger 2005; Philippe et al. 2006), we expected  
20 hybrid percentage to be high in the progenies from orchards composed of a single mother  
21 clone, and lower in the progenies from orchards with several maternal clones. The limited  
22 data available in the literature generally concurs (Bergmann and Ruetz 1987; Häcker and  
23 Bergmann 1991; Ennos and Tang Qian 1994).

24

1 Our results were partly consistent with the above assumption since we found the highest  
2 hybrid percentages in the progenies collected from a single clone (FH, V, E), and low or  
3 moderate hybrid percentages in the two orchards with several maternal clones (H and NT)  
4 (Table 1). However, low hybrid percentages were also obtained for three seed orchards  
5 composed of a single mother clone (S, FP and, to a lesser degree, M). For those, the non-  
6 hybrid trees could have resulted from self-pollination or pollen contamination, not to mention  
7 other possible problems due to rejected grafts or mislabeling at various stages of the tree  
8 breeding process. None of these explanations could be tested because the markers we used do  
9 not make it possible to distinguish between the two kinds of materials, and the isolation of the  
10 studied orchards from background pollen was largely unknown.

11  
12 Considering the poor full seed yields resulting from controlled self-pollinations (Kosinski  
13 1986; Slobodnik and Guttenberger 2005), it seemed unlikely at first sight that the high  
14 percentages of non-hybrid trees found in several orchard progenies (S, FP, M and FH) derived  
15 from selfing. However, Bergmann and Ruetz (1987) mentioned a very low percentage of  
16 hybrid seeds (19%) in the first crop of a young bi-clonal orchard. The fact that this orchard  
17 was well-isolated from background pollen leads us to think that selfing was responsible for a  
18 large part of the 81% non-hybrid seeds. Myking and Skroppa (2006) also reported that a  
19 seedling lot derived from a seed orchard composed of one single mother clone contained 90%  
20 selfed seeds. Therefore, selfed seeds can represent a larger proportion of the seed lot than  
21 initially thought.

22

### 23 **Performance of hybrids *versus* non-hybrid trees**

24

1 In many studies, interspecific hybrids have been compared to the parent species for various  
2 traits, particularly growth, in order to estimate hybrid superiority and, thus, to judge the  
3 interest of hybrid larch as an afforestation species (e.g. Pâques et al. 2013). Our purpose was  
4 quite different. Indeed, comparing hybrids with EL and JL populations composed of mixtures,  
5 in unknown proportions, of selfed trees and trees derived from non-related parents would be  
6 meaningless in that case. Actually, our study aimed to judge the impact of hybrid percentage  
7 on FRM worth and to detect possible differences according to seed orchard design. This  
8 implies that we know the respective performance of the populations identified as hybrids or  
9 not in the studied orchard progenies.

10

11 At Peyrat, few differences were found between hybrid and non-hybrid trees for survival after  
12 planting, except for FH and H98. For those progenies, the hybrids survived better than either  
13 EL or JL. Mortality mainly occurred in the first two years and can be attributed to the  
14 transplanting shock as well as to the heat wave and drought that happened one year after  
15 planting. Similar results have been obtained for FH in a sister experiment where the hybrids  
16 survived much better than EL seedlings at age 8 (86% versus 38%, data unpublished). Thus,  
17 hybrid percentage increased continuously from the seed lot to the nursery and then in the  
18 field. This is consistent with the fact that selfed seeds do not germinate well and that the  
19 resulting seedlings are generally weak (Dieckert 1964). At Brenod and La Courtine, mortality  
20 was extremely limited in all orchard progenies after age 9 and 13, respectively, when tree  
21 identity was determined. Therefore, there was no direct evidence that the hybrids survived  
22 better than the EL or JL trees. However, although both trials were planted with the same  
23 materials, the proportion of hybrids among the orchard progenies in common appeared to be  
24 slightly higher at Brenod than at La Courtine (61.2% and 56.0%, respectively). At Brenod,  
25 12.7% of the trees had died before sampling for DNA analyses whereas mortality was only

1 4.1% at La Courtine. This suggests that early mortality was less pronounced in the hybrids  
2 than in the non-hybrid trees at Brenod.

3  
4 In addition to mortality, heat and drought caused crown dieback during the second growth  
5 season at Peyrat. On the whole, JL was the most damaged, this species being less drought-  
6 resistant than EL (Pâques *et al.* 2013). The hybrids had less frequent and/or less severe  
7 dieback than JL. Compared to EL, the hybrids resisted that extreme climatic event as well or  
8 even better though the differences were small.

9  
10 A mild, wet spring and a rainy summer favored the development of *Meria laricis* at Peyrat in  
11 2008. In terms of foliage damage and decrease in growth rate, EL was more severely affected  
12 than JL, while the hybrids occupied an intermediate position whatever the orchard progeny.  
13 This ranking is consistent with the known susceptibility to needle cast of EL, JL and HL  
14 (Batko 1955; Lanier 1976; Ridley and Dick 2001; Pâques *et al.* 2013).

15  
16 The hybrids grew faster in height and particularly in diameter in all the orchard progenies  
17 studied except NT. For tree volume, the hybrids were superior by 140% on average at age 6  
18 or 7, i.e. when inter-tree competition was nil or weak. Still, large variations were found  
19 among orchard progenies at each site, and between sites for the progenies tested both at  
20 Brenod and La Courtine. These variations seem to be related to orchard design. Firstly,  
21 considering M and H83, hybrid superiority over JL was much higher at the continental site  
22 (Brenod) than at the oceanic site (La Courtine). In addition, the hybrids produced in orchard  
23 H were more superior to EL than to JL at the second oceanic site (Peyrat). Therefore, we are  
24 inclined to believe that the difference in growth between hybrid and non-hybrid trees will be  
25 more pronounced in sites that are less favorable to the maternal species. Secondly, we

1 assumed that the greatest differences in vigor would be found in progenies originating from  
2 orchards composed of a single mother clone due to inbreeding depression (Dieckert 1964).  
3 This hypothesis was supported by the results we obtained at Brenod, but only partly so by the  
4 results from Peyrat and La Courtine. Knowing the pedigree of the non-hybrid seedlings would  
5 have helped us interpret the data, but the proportion of trees deriving from self-pollination and  
6 pollen contamination could not be determined with the markers we used. Though this theory  
7 must be confirmed with a larger and better balanced set of orchard progenies, we consider it  
8 more than likely. Finally, we expect hybrid superiority over non-hybrid trees to be maximal in  
9 orchards composed of one maternal clone belonging to a species which is poorly adapted to  
10 the planting site.

11

12 Remarks can be made for two orchards for which information is available. Regarding NT  
13 progeny, the small difference in growth between the hybrid and non-hybrid trees might be  
14 explained by the inaccurate identity of some mother clones. Indeed, previous DNA analyses  
15 showed that some were hybrids and not JL as expected (Pâques, pers. comm.). Therefore, NT  
16 progeny was potentially complex, including F1 hybrids and JL trees but also back-crossed  
17 trees and F2 hybrids. Regarding V progeny, the large superiority of hybrids over non-hybrids  
18 at Peyrat and Brenod, as well as the large increase in hybrid percentage between the seed and  
19 seedling stages, lead us to think that most of the non-hybrid individuals derived from selfing.  
20 Therefore, the other 12 EL clones planted on the periphery of the seed orchard (van't Leven  
21 1979) probably contributed little to the seed lot. That is consistent with the fact that seed  
22 orchard grafts are mainly pollinated by close neighbors (e.g. Philippe et al. 2006).

23

24 The hybrids were less slender than EL, a favorable point for wind stability, and similar to JL  
25 for that trait. Regarding stem straightness, the differences were small and rarely significant.

1 The hybrids were no straighter than the parent species, contrary to what Lacaze and Birot  
2 (1974) observed.

3

4 Modulus of elasticity (MOE) is one of the most important mechanical properties of wood.  
5 Though faster growing, the hybrids did not differ significantly from the non-hybrids in MOE  
6 except for three orchard progenies where it was either inferior to EL (FH and V) or superior to  
7 JL (NT).

8

9 If forest owners want straight and fast-growing materials which are well-adapted to the  
10 planting site, they are also interested in homogeneous stands which produce more  
11 homogeneous final wood products and make thinning easier and more efficient. Our results  
12 show that in most of the orchard progenies the hybrid populations were more homogeneous  
13 than the mixed populations in terms of growth traits. This was particularly true when the  
14 hybrids derived from a single clone belonging to a species poorly adapted to the planting site.  
15 In terms of stem form, no clear trend toward homogeneity was found, though the hybrids  
16 were rarely more variable than the mixed progenies.

17

### 18 **Effect of thinning on hybrid percentage**

19

20 The thinning simulations carried out for the three sites showed that the plots became richer in  
21 hybrids when trees were selected for vigor. Logically, hybrid percentage enhancement  
22 depended on three factors: 1) the percentage of trees eliminated in each orchard progeny, 2)  
23 the superiority of hybrids over EL and/or JL trees, and 3) the hybrid percentage before  
24 thinning. In the simulated thinnings for Brenod, the changes in hybrid percentage for FP and,  
25 to a lesser extent, for M were explained by the last factor. Despite the clear superiority of the

1 hybrids, the increase in hybrid percentage remained limited because of the small number of  
2 available hybrids. For FP, for example, even though all the hybrids were retained, they still  
3 represented only 42% of the population after the first thinning. When the trees were selected  
4 for stem straightness, hybrid percentage did not vary much. As growth and stem straightness  
5 were weakly correlated, a selective thinning based on both traits was expected to result in an  
6 intermediate enrichment in hybrids. That is precisely what was obtained in the simulations  
7 carried out on the basis of the vigor-form index at Peyrat, and in the field at Brenod and La  
8 Courtine. However, the effect of actual thinning could not be tested at Peyrat because the trial  
9 has not been thinned yet.

10

11 For the Brenod and La Courtine trials, we simulated second and third thinnings reducing  
12 densities to 600 and 400 stems per hectare, respectively. We based these simulations on the  
13 latest data available and considered that tree ranking would remain unchanged. When trees  
14 were selected for vigor, dramatic increases in hybrid percentage occurred for the orchard  
15 progenies characterized by both a large superiority of hybrids and a low or moderate hybrid  
16 percentage after the first thinning (M and FP at Brenod). Nevertheless, FP progenies were  
17 only 63% hybrids even after the third thinning. In the other orchard progenies, hybrid  
18 percentages remained either very high (FH, V, E), or low (H). As for the first thinning,  
19 selecting for stem form did not produce substantial variations in hybrid percentage. A  
20 thinning based on growth and form would most likely result in intermediate changes.

21

## 22 **Consequences for the forest owner**

23

24 From a silvicultural point of view, hybrid percentage in Forest Reproductive Material (FRM)  
25 will be more or less important depending on the forest owner's strategy and the seed orchard



1 where the seed has been produced. Hybrid percentage does not matter if the production  
2 objective is focused on stem straightness. However, it would be nonsense to use expensive  
3 hybrid larch seedlings for that sole purpose. On the contrary, hybrid percentage is of major  
4 importance when timber production is concerned, though its impact varies according to the  
5 seed orchard progeny and the planting site. When hybrids do not differ much from EL and JL  
6 trees, the potential loss of productivity should be acceptable, even if hybrid percentage in the  
7 planting stock is moderate. In our study, this case corresponds to orchards with several clones  
8 from a mother species which is well-adapted to the planting site. Inversely, the risk of poor  
9 timber production is the highest for orchard progenies in which the hybrids grow much faster  
10 than the non-hybrid trees. According to our results, this concerns the progenies produced from  
11 a single mother clone, especially when it belongs to a species that is poorly adapted to the  
12 planting site. Nevertheless, the consequences will probably remain negligible if the hybrid  
13 percentage is more than 60-70%, which corresponds to the percentage of trees left after the  
14 first thinning in standard silviculture (Pauwels and Rondeux 2000). With such a hybrid  
15 percentage, the plantation will be composed of almost 100% hybrid trees from the second  
16 thinning on, i.e. when the forest owner starts to yield profits. Still, a low hybrid percentage  
17 means that there are fewer possibilities to compensate for mortality or game, insect, frost  
18 damage and also fewer possibilities of choice at the time of thinning. Finally, if the owner's  
19 objective is biomass production, the hybrid percentage should be as high as possible.

20  
21 Furthermore, precautions must be taken for sanitary reasons in afforestation areas favorable to  
22 larch canker, i.e. areas with frequent high air humidity. In such sites, the forest owner must be  
23 cautious in using FRM produced in seed orchards composed of several maternal clones of EL  
24 of alpine origin. Indeed, the non-hybrid seeds or seedlings will be alpine EL and those  
25 provenances are known to be particularly sensitive to *Lachnellula willkommii* (Sylvestre-

1 Guinot and Delatour 1983; Pâques et al. 2013). Therefore, the hybrid percentage threshold  
2 should be set higher, close to 100%, for the above-mentioned orchards in the areas at risk in  
3 order to limit canker development and fungus dispersal.

4  
5 To conclude, we must stress the fact that our study does not allow us to specify the minimal  
6 hybrid percentage required for each orchard progeny to ensure good stand production. To do  
7 so would require establishing several plots per progeny with increasing hybrid percentages in  
8 order to study a gradient of mixture. Such an experiment would be a long and complicated  
9 undertaking and, to our knowledge, has not been attempted to date.

10

#### 11 **Difficulties related to variable hybrid percentages in seed orchard comparison tests**

12

13 In old and middle-aged tests, the hybridization orchards compared are generally represented  
14 by a single seed lot whose hybrid percentage is unknown. As seen above, hybrid percentage  
15 varies considerably among orchards and years, and hybrid superiority differs greatly from one  
16 orchard to another. Therefore, it is quite justified to question the accuracy of results based on  
17 mixed orchard progeny performance and, thus, the reliability of the recommendations made to  
18 forest owners based on such results. To answer this question, we compared the orchard  
19 progenies at Brenod and La Courtine, considering either their overall performance (all trees)  
20 or hybrid performance (hybrid trees only).

21 The comparison was done at age 6, i.e. before among-tree competition had started. At La  
22 Courtine, the orchard progenies ranked similarly for growth and stem form whatever the  
23 populations analyzed. However, only two categories of materials were compared: 1)  
24 progenies characterized by highly superior hybrids but also a low percentage of non-hybrid  
25 trees (FH and E); and 2) progenies whose low hybrid percentage was compensated for by

1 little difference in growth between the hybrids and the non-hybrids (H and M). At Brenod,  
2 where the FP progeny was characterized by both a low hybrid percentage and highly superior  
3 hybrids, things were quite different. Considering hybrids alone, FP did not differ significantly  
4 from the other orchard progenies for radial growth. This indicates that the poor growth FP has  
5 shown in France and other European countries (Philippe et al. 2002) was not due to an  
6 intrinsic characteristic of the seed orchard but rather to the low percentage of hybrids in the  
7 seed lot used in the trials.

8 The above example raises an essential question: should hybrid FRM recommendations take  
9 into account the overall or the hybrid performance? Both approaches have disadvantages. A  
10 given seed lot may be atypical and it would be risky to base a general judgment on its  
11 performance. On the other hand, hybrid performance might never be attained for some  
12 orchards. That is precisely the case for the Halle orchard whose progenies have consistently  
13 low or moderate hybrid percentages. In addition, as it will be discussed below, a judgement  
14 founded on hybrid performance may be biased because of competition effects. To be reliable,  
15 recommendations should be based on the performance of seed lots as similar as possible to  
16 those available on the market. For that, we would need to know the annual variability in  
17 hybrid percentage for the different seed lots produced in each orchard, or to market only  
18 Forest Reproductive Materials with a high hybrid percentage in order to reduce the gap  
19 between overall and hybrid performance. Another alternative would consist in testing seed lot  
20 mixtures instead of single seed lots in order to estimate their "average performance".

21  
22 Furthermore, interpreting data collected in seed orchard comparison tests may be tricky when  
23 the trees get older and are competing with each other. Indeed, competition intensity, which  
24 strongly influences tree growth, may vary with the orchard progeny or, more precisely, with  
25 progeny homogeneity. In our study for example, the V, E and FH hybrids that are surrounded

1 by other hybrids of about the same size are at a disadvantage compared with the few FP  
2 hybrids that dominate neighboring selfed trees and are consequently in a nearly free-growth  
3 situation. Puzzling results or even misinterpretations may result from such differences in  
4 competition intensity. At Brenod for example, seed orchard ranking was altered between the  
5 ages of 6 and 13, i.e. after the onset of competition. The FP hybrids and, to a lesser extent, the  
6 M hybrids, showed the highest growth during that period whereas they were relatively weak  
7 until age 6. It is not impossible that those hybrids grow slowly when young, and then increase  
8 their growth rate as they age. However, FP and M were both characterized by a low hybrid  
9 percentage and a large superiority of hybrids over EL or JL trees. That leads us to believe that  
10 competition was also involved in the sudden, unexpected change in ranking. If this  
11 assumption is correct, a judgement based on hybrid performance would result in an  
12 overestimate of the worth of such seed orchards. Therefore, it seems necessary to take  
13 competition into account. Many competition indices have been developed by tree growth  
14 modelers to estimate the impact of competition on target tree growth (Biging and Dobbertin  
15 1995). Introducing one of these indices as covariable, for instance the basal area of neighbor  
16 trees within a given radius, would help to separate genetic and competition effects.

17

## 18 **CONCLUSION**

19

20 Our results, based on a representative sample of European hybridization orchards of larch,  
21 showed that it is almost always in the interest of forest owners to use hybrid larch Forest  
22 Reproductive Materials (FRM) with hybrid percentages that are as high as possible. Indeed,  
23 the hybrids in our study had several advantages: 1) they were more vigorous and generally  
24 thicker than the non-hybrid trees without degradation in stem straightness; 2) they survived  
25 and resisted the 2003-summer heat and drought spell at least as well as the non-hybrids did; 3)

1 they were less susceptible to *Meria laricis* than the European larch trees; and 4) they were  
2 more homogeneous than the mixed progenies for growth traits. However, the commercial  
3 seed lots studied in this paper had highly variable hybrid percentages. Hybrid percentage did  
4 actually increase over time owing to better seed viability and/or seedling survival and, more  
5 significantly, thinning. Several thinnings are nevertheless necessary to compensate for low  
6 initial hybrid percentage in some orchard progenies and this means a serious loss of income  
7 for forest owners. According to our results, it seems that the risk is maximal for orchards with  
8 a single mother clone from a species which is poorly adapted to the afforestation site. It is  
9 therefore essential that forest owners are informed of orchard composition, and more  
10 particularly of the number, species and origin of the maternal clones.

11

12 Seed orchard managers, foresters and policy-makers should question commercializing and  
13 planting so-called “hybrid larch” FRMs with low hybrid percentage. It would be reasonable to  
14 set a minimal threshold of hybrid percentage for commercialization of at least 60-70%. In this  
15 way, commercial disputes and lawsuits such as those reported by Myking and Skroppa (2006)  
16 could be avoided. Setting that threshold would also reduce the risk of misinterpretation in  
17 seed orchard testing. Different measures can be proposed to help increase FRM hybrid  
18 percentage. These measures can be taken at various steps in the production process, from  
19 genotype selection to seedling selection in the nursery (Table 6).

20

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11

12 **Conflict of interest**

13 The authors declare that they have no conflict of interest.

14

15

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23

1 **Table 1** Composition of tested seed orchards and hybrid percentages in the seed lots and  
 2 orchard progenies studied at Peyrat, Brenod and La Courtine

3

Seed orchard (country)	Orchard progeny code	Orchard composition (♀/♂) <sup>a</sup>	Crop year <sup>b</sup>	Test site	Taxonomic identification <sup>c</sup>			
					Sample size	Genetic status (%)		
						ELxEL	JLxJL	ELxJL + JLxEL
FH 201 Barres (France)	FH	<u>1 EL</u> x 1 FS <sup>d</sup> JL	1992	Peyrat	200 seeds 81 seedlings	50.7 28.4	0 0	49.3 71.6
				Brenod	109 trees	14.7	0	85.3
				La Courtine	115 trees	15.6	0	84.4
Maglehem (Sweden)	M	<u>1 JL</u> x 8 EL	1992	Brenod	110 trees	0	57.3	42.7
				La Courtine	121 trees	1.7	60.3	38.0
Esbeek (The Netherlands)	E	<u>1 EL</u> x 4 JL	1992	Brenod	111 trees	2.7	0.9	96.4
				La Courtine	115 trees	8.7	1.7	89.6
Vaals <sup>e</sup> (The Netherlands)	V	<u>1 EL</u> x 26 JL	1996	Peyrat	200 seeds 118 seedlings	32.0 22.3	0 0	68.0 77.7
			1992	Brenod	108 trees	7.4	0	92.6
Halle (Belgium)	H87	<u>15 EL</u> x <u>15 JL</u>	1987	Peyrat	205 seeds 170 seedlings	30.2 26.7	34.2 32.0	35.6 41.3
	H98		1998	Peyrat	205 seeds 160 seedlings	22.4 29.0	30.2 23.0	47.3 48.0
	H80	<u>15 JL</u> x 15 EL	1980	Brenod	117 trees	3.4	70.1	26.5
	H83		1983	Brenod	112 trees	3.6	75.9	20.5
	H83		1983	La Courtine	124 trees	0	87.9	12.1
FP237 (Denmark)	FP	<u>1 EL</u> x 17 JL	1992	Brenod	96 trees	72.9	1.0	26.0
Schnappenhammer (Germany)	S	<u>1 EL</u> x 1 JL	1983	Peyrat	200 seeds 131 seedlings	86.4 82.0	0 1.0	13.6 17.0
Wiston - NT23 (UK)	NT	<u>33 JL</u> x 33 EL	1996	Peyrat	200 seeds 99 seedlings	0 0	77.5 77.6	22.5 22.4

4 <sup>a</sup> This column indicates the number of clones of European larch (EL) and Japanese larch (JL)  
 5 included in the seed orchard; the clone or set of clones used as maternal parent is underlined.

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 7 <sup>b</sup> Crop year denotes the year of seed harvesting

8  
 9 <sup>c</sup> The genetic status of the living trees was determined before the 1<sup>st</sup> thinning at La Courtine  
 10 and Brenod (9 and 13 years after planting, respectively) while all the seedlings were analyzed  
 11 before planting at Peyrat

12  
 13 <sup>d</sup> FS denotes full-sib family

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 15 <sup>e</sup> Vaals seed orchard includes 13 clones of EL and 26 clones of JL but the cones were  
 16 collected in the central part of the orchard composed of a single maternal clone (van't Leven  
 17 1979)

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**Table 2** Main characteristics of the Peyrat, Brenod and La Courtine trials: site conditions, trial composition and design, trial management and historical account of the main events (planting, thinning and taxonomic identification)

Site	Peyrat-le-Château	Brenod	La Courtine
<u>Location</u>			
- longitude	1°44'E	5°38'E	2°19'E
- latitude	45°47'N	46°06'N	45°45'N
- elevation	579 m	995 m	830 m
- type of land	Forest nursery	Agricultural land	Forest
<u>Climate<sup>a</sup></u>			
- mean annual T <sup>a</sup>	9.7°C	6.9°C	7.7°C
- T <sup>b</sup> April-Sept.	13.9°C	11.6°C	12.0°C
- annual rainfall	1196 mm	1682 mm	1058 mm
- rainfall April-Sept.	542 mm	781 mm	501 mm
<u>Soil</u>			
- bedrock	Granite	Limestone (cracked blocks)	Granite
- soil depth	> 40 cm	20-40 cm	> 50cm
- texture	Sandy	Surface loam then clay at depths	Loamy clay
<u>Genetics</u>			
- tested orchards	5	6	6
- orchard progenies (#)	6	7	7
- trial design	1 plot per progeny (71-153 seedlings)	7 progenies x 4 blocks x 64 seedlings per plot	cf Brenod
<u>Trial management</u>			
- planting date	March 2002	April 1995	April 1995
- spacing	3m x 3m	3m x 2m	3m x 2m
- type of seedlings	Bare roots	Bare roots	Bare roots
- seedling age at planting	3 years	2 years	2 years
- 1st thinning date	Planned for 2016	August 2008	May 2005
- % of living trees felled		45%	42%
<u>Taxonomic identification</u>			
- studied genetic units	All	All (2 blocks)	FH, H83, E, M (2 blocks)
- bud collection date	Fall 2000	February 2008	February 2004

<sup>a</sup> All data were obtained from Météo-France (Aurelhy simulations for the period 1971-2000)

<sup>b</sup> T is temperature

**Table 3** Performance of hybrid and non-hybrid trees for adaptive traits for the six orchard progenies studied at Peyrat. The heatwave occurred in summer 2003 and the attack of *Meria laricis* in summer 2008, i.e. two years and seven years after planting, respectively.

Orchard progeny code	Species <sup>a</sup>	% mortality at age 10 <sup>b,d</sup>	Shoot dieback after heatwave		<i>Meria laricis</i> damage	
			% trees affected <sup>b,d</sup>	% of stem length affected <sup>b,d</sup>	% trees severely affected <sup>b,d</sup>	Growth reduction (%) <sup>b,c,d</sup>
FH	HL	15	14	19	86	-7
	EL	55***	22 NS	28 NS	100 NS	-16 NS
V	HL	2	5	5	80	-3
	EL	4 NS	14 NS	10 NS	100*	-26**
H87	HL	4	4	4	85	-11
	EL	9 NS	6 NS	28 NS	100*	-29**
	JL	2 NS	13 NS	10 NS	42***	0 NS
H98	HL	5	9	18	73	-12
	EL	0 NS	5 NS	21 NS	81 NS	-22***
	JL	24*	37***	31 NS	31***	+1***
S	HL	0	5	1	42	-15
	EL	1 NS	6 NS	49 <sup>e</sup>	88***	-18 NS
NT	HL	12	50	30	73	-4
	JL	5 NS	57 NS	31 NS	38*	+11 NS

<sup>a</sup> EL, JL, HL denote European larch, Japanese larch and hybrid larch, respectively

<sup>b</sup> Values rounded to the nearest unit

<sup>c</sup> Growth reduction % = (length of terminal shoot in 2008 - mean length of terminal shoot in 2006 and 2007) \* 100 / mean length of terminal shoot in 2006 and 2007

<sup>d</sup> \*, \*\*, \*\*\* denote that European larch or Japanese larch trees differed significantly from the hybrids at P = 0.05, P = 0.01 and P = 0.001, respectively (NS = non-significant)

<sup>e</sup> The mean comparison test was not applicable due to too few damaged HL trees

**Table 4** Hybrid performance (expressed in % relative to European larch (EL) and Japanese larch (JL)) for the orchard progenies tested at Peyrat, Brenod and La Courtine. The studied characters are represented by their initials followed by the age since planting. H is total height, C is circumference, V is volume, HD is height:diameter ratio, SS is stem straightness and MOE is modulus of elasticity.

Site	Orchard progeny	H7		C10		V7		HD7		SS10		MOE 12	
		EL	JL	EL	JL	EL	JL	EL	JL	EL	JL	EL	JL
Peyrat	FH	43***		73***		185***		-16***		-4 NS		-39***	
	H87	21***	7*	53***	4 NS	145***	41 NS	-20***	7*	-7 NS	3 NS	-16 NS	0 NS
	H98	9**	16***	23***	10 NS	53***	43**	-14***	-2 NS	-8 NS	5 NS	-13 NS	16 NS
	NT		-5 NS		-7 NS		-14 NS		20 NS		10 NS		49**
	S	22***		54***		193***		-22***		-3 NS		3 NS	
	V	32***		81***		275***		-25***		-1 NS		-40***	
		H6		C13		V6		HD6		SS6			
		EL	JL	EL	JL	EL	JL	EL	JL	EL	JL		
Brenod	FH	27***		51***		161***		-12**		-15**			
	H80		5 NS		b		29 (*)		-1 NS		3 NS		
	H83		14***		14***		79***		-9 (*)		9 NS		
	V	17*		50***		122*		-13 (*)		-17 NS			
	E	a		a		a		a		a			
	M		30***		33***		178***		-20***		-13*		
		70***		99***		658***		-33***		-8*			
		H6		C10		V6		HD6		SS6			
		EL	JL	EL	JL	EL	JL	EL	JL	EL	JL		
La Courtine	FH	27***		57***		132***		-6 NS		-4 NS			
	H83		10**		8 (*)		33**		-4 NS		8 NS		
	E	30***		72***		185***		-16***		-2 NS			
	M		7*		5 NS		27*		9*		-3 NS		



(\*), \*, \*\*, \*\*\* denote a significant effect of species at  $P = 0.10$ ,  $P = 0.05$ ,  $P = 0.01$  and  $P = 0.001$ , respectively (NS = non-significant)

<sup>a</sup> The analysis would be meaningless because of two few European larch trees in the orchard progeny

<sup>b</sup> The orchard progeny was not measured

**Table 5** Variability (coefficients of variation) of populations composed of all trees (all) or hybrid trees only (HL) for the seed orchard progenies tested at Peyrat, Brenod and La Courtine. The studied characters are represented by their initials followed by the age since planting. H is total height, C is circumference, V is volume, HD is height:diameter ratio and SS is stem straightness.

Site	Orchard progeny	H7		C10		V7		HD7		SS10	
		all	HL	all	HL	all	HL	all	HL	all	HL
Peyrat	FH	17.5	10.0	23.4	12.2	47.8	31.7	13.9	12.0	17.2	17.7
	H87	16.1	15.1	25.6	21.7	55.2	45.0	18.5	15.5	20.6	19.8
	H98	14.8	11.6	23.2	21.2	52.7	42.9	17.1	15.2	26.5	27.5
	NT	17.9	25.2	20.0	28.3	54.0	75.1	31.8	52.5	24.7	18.8
	S	18.8	13.9	31.4	20.0	84.3	53.2	15.5	13.0	17.2	24.5
	V	15.0	10.2	25.6	15.5	48.2	32.1	18.7	12.9	19.7	20.5
		H6		C13		V6		HD6		SS6	
		all	HL	all	HL	all	HL	all	HL	all	HL
Brenod	FH	18.3	14.2	22.3	17.3	58.3	46.8	23.3	22.5	28.0	28.5
	H80	15.1	18.2	a	a	60.1	71.9	15.8	17.3	33.5	33.7
	H83	16.4	14.5	13.8	12.1	62.5	61.6	19.3	13.2	32.9	29.9
	V	19.3	18.8	21.7	19.4	66.2	61.9	21.6	20.9	34.8	36.5
	E	16.6	16.2	16.3	16.2	49.8	47.5	20.0	18.0	23.7	24.3
	M	24.3	20.2	24.1	17.6	99.6	71.7	27.5	18.5	30.7	36.8
	FP	41.0	25.1	46.5	22.7	201.2	100.7	36.3	22.5	16.2	15.8
		H6		C10		V6		HD6		SS6	
		all	HL	all	HL	all	HL	all	HL	all	HL
Courtine	FH	16.1	12.4	23.4	18.1	48.6	40.6	15.5	16.4	25.5	26.1
	H83	13.9	10.7	15.0	18.3	43.9	39.2	15.2	12.7	33.2	34.3
	E	13.2	9.1	21.1	15.2	41.8	32.7	16.9	15.6	24.1	24.8
	M	18.4	21.5	20.5	23.0	56.7	61.0	20.1	25.9	30.3	32.4

<sup>a</sup> The orchard progeny was not measured

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 2 **Table 6** Measures that can be taken by tree breeders, seed orchard managers, seed plant  
 3 managers and nurserymen to increase hybrid percentage in Forest Reproductive Materials  
 4 (FRM) produced in F1-hybridization seed orchards (options including vegetative propagation  
 5 are voluntarily excluded)

Stage	Orchard composed of EL <sup>a</sup> and JL <sup>a</sup> clones	Separate orchards of EL <sup>a</sup> and JL <sup>a</sup>
Genotype selection	Use a single mother clone “phenologically” compatible with the clone(s) used as pollen producer(s) <sup>b</sup> .	Use a single mother clone selected for its performance, irrespective of phenology.
	Verify the identity (EL or JL) of the parent clones with molecular markers (Acheré et al. 2004).	
Orchard establishment	Verify that the site is well-isolated from incoming pollen.	
	Prefer tree by tree alternation of species.	Consider establishing indoor-orchards in northern countries (Colas <i>et al.</i> 2008) and take measures to reduce epigenetic effects.
Orchard management	Control for and eradicate rootstock suckers (“graft rejection”). Preferably use the alternate species rootstock.	
Flower management	Flower stimulation and SMP <sup>c</sup> are highly recommended (Philippe et al. 2006, Pâques et al. 2013).	Flower stimulation is highly recommended. SMP <sup>c</sup> is mandatory.
	Monitor flower development to estimate the flowering overlap between EL and JL clones (and possible pollen contamination).	Monitor flower development to make decisions for pollen collection (pollen cone maturation) and SMP <sup>c</sup> (seed cone receptivity).
Cone collection	Estimate hybrid seed % in advance with molecular markers (possible as early as August) and collect cones or not according to the result.	
	Avoid collecting cones in case of poor flowering, poor flowering overlap between EL and JL or pollen contamination.	
Cone and seed processing	If the cones are collected from both species, treat EL and JL progenies separately in the seed plant.	
	Determine hybrid seed % after seed processing and do not market seed lots with low hybrid percentage.	
Raising seedlings	Use molecular markers or phenotypic tests to sort out the seedlings in the nursery (Acheré et al. 2004, Pâques et al. 2006).	

6 <sup>a</sup> EL denotes European larch; JL Japanese larch

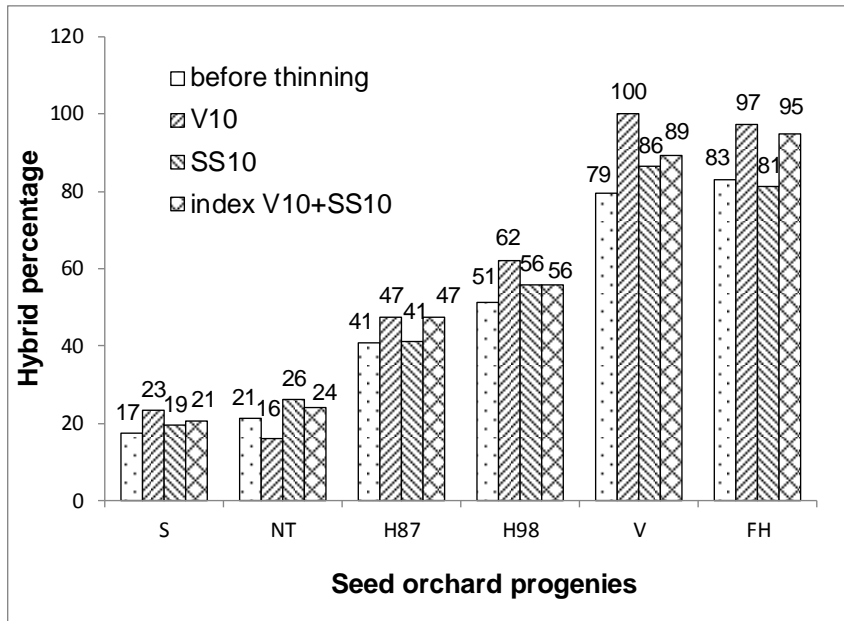
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1 <sup>b</sup> The products of orchards with a single mother clone have a low genetic diversity. Thus, it is  
2 recommended to vary the FRM used at a regional scale to reduce sanitary risks. In addition,  
3 the plantations deriving from such orchards must not be regenerated naturally.  
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5 <sup>c</sup> SMP: supplemental mass pollination  
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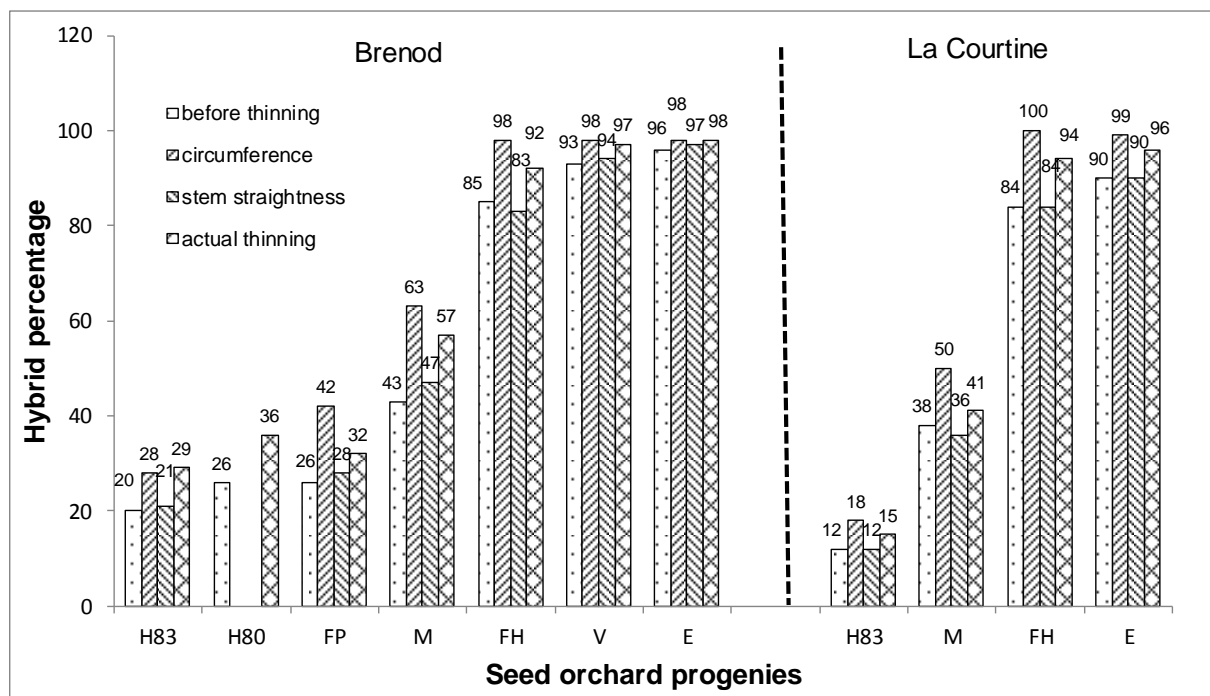
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2 **Fig. 1 (xls)** Effect of simulated first thinnings on hybrid percentage for the seed orchard  
3 progenies tested at Peyrat. The thinnings were based on either tree volume at age 10 (V10),  
4 stem straightness at age 10 (SS10) or an index combining both traits (index V10 + SS10).  
5 Simulations were made considering that thinning would eliminate 30% of the living trees.  
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 2 **Fig. 2 (xls)** Effect of simulated and actual first thinnings on hybrid percentage for the seed  
 3 orchard progenies tested at Brenod and La Courtine. The thinning simulations were based on  
 4 either circumference or stem straightness (measured at age 13 for Brenod and 10 for La  
 5 Courtine). Simulations were made considering that thinning would eliminate 50% of the  
 6 living trees. Thinning could not be simulated for H80 at Brenod because this orchard progeny  
 7 had not been measured. Actual thinnings, carried out at age 14 at Brenod and age 11 at La  
 8 Courtine, were partly systematic and partly selective (criteria: vigor, stem form and spatial  
 9 distribution of the trees); they eliminated 45% and 42% of the living trees at Brenod and La  
 10 Courtine, respectively.

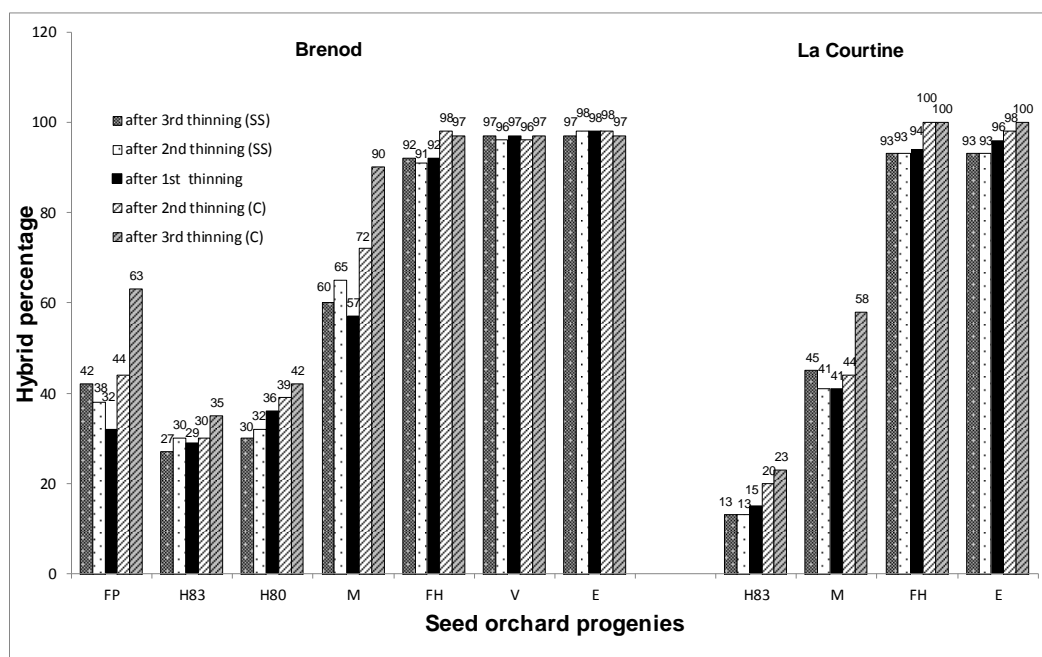
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 2 **Fig. 3 (xls)** Effect of simulated second and third thinnings on hybrid percentage for the seed  
 3 orchard progenies tested at Brenod and La Courtine. Thinning simulations were based on  
 4 either circumference (C) or stem straightness (SS) measured after actual first thinning (at age  
 5 17 for Brenod and 16 for La Courtine). Simulations were made considering that tree density  
 6 would be reduced from 790 trees/ha or 940 trees/ha (density after actual first thinning at  
 7 Brenod and La Courtine, respectively) to 600 trees/ha after the second thinning and then to  
 8 400 trees/ha after the third thinning. For each orchard progeny, the figure can be read from the  
 9 black central bar which represents the hybrid percentage after actual first thinning. The two  
 10 bars on its left indicate the corresponding percentage after 2<sup>nd</sup> and 3<sup>rd</sup> thinnings based on stem  
 11 straightness; the two bars on its right indicate hybrid percentage after 2<sup>nd</sup> and 3<sup>rd</sup> thinnings  
 12 based on tree circumference.

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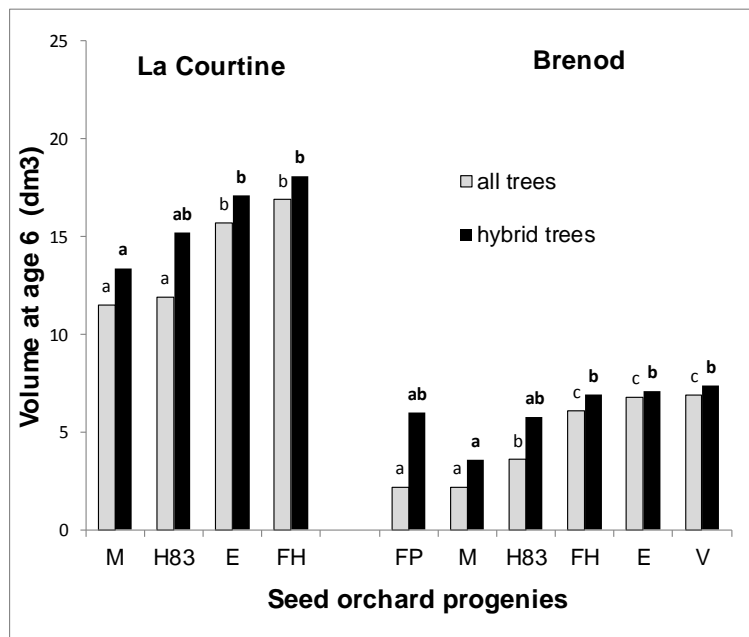


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2 **Fig. 4 (xls)** Ranking of the seed orchard progenies tested at La Courtine and Brenod for tree  
3 volume at age 6, considering populations composed of all trees or hybrid trees only. For each  
4 site, orchard progenies with the same letter were not significantly different ( $P = 0.05$ ). The  
5 homogeneous groups found for all trees and hybrid trees are represented by letters of normal  
6 type and letters in bold, respectively.

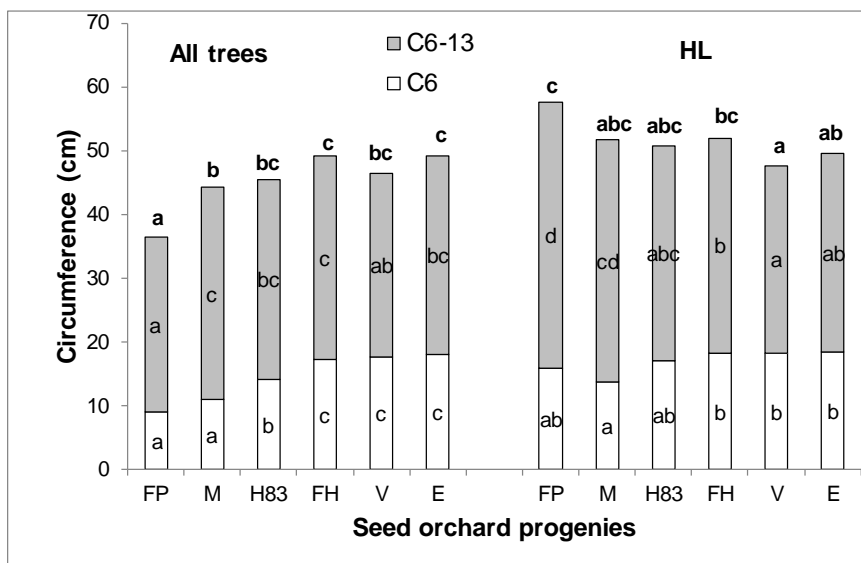
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 2 **Fig. 5 (xls)** Ranking of the seed orchard progenies tested at Brenod for circumference at age 6  
 3 (C6) and girth increment between ages 6 and 13 (C6-13), considering populations composed  
 4 of all trees or hybrid trees only. For each population, orchard progenies with the same letter  
 5 were not significantly different ( $P = 0.05$ ). The homogeneous groups found for circumference  
 6 at age 6 and girth increment are represented by letters in the middle of the corresponding bar;  
 7 those found for circumference at age 13 are represented by letters in bold above the two bars.

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