Wood density variability in successive breeding populations of maritime pine

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24

1   **Abstract**

2

3   Growth and form are the two main traits used for genetic improvement of maritime pine

4   (*Pinus pinaster* Ait.) in the Southwest of France. In this paper, we studied ring wood density

5   to answer two main questions: Is there a general trend for density variability throughout tree

6   development? Has selection indirectly reduced wood density variability over breeding

7   populations due to genetic unfavourable correlation with growth? Wood density and its

8   components were studied in three polycross tests, each representative of one of the successive

9   breeding populations. Density was measured with an X-ray densitometer on about 50 families

10   per test with more than 1900 trees.

11   A preliminary study showed that bark-to-pith ring indexing allows for a better estimation of

12   genetic effects than pith-to-bark indexing. Genetic variability of wood density appears highly

13   dependent on the year considered and no general pattern can be detected over years. Whereas

14   variability of selected traits is known to have decreased over breeding populations, no

15   significant change was found for variability of wood density.

16

17   *Pinus pinaster* **Ait. / wood density / genetic variability / breeding populations**

18

1   **Résumé**

2

3   La croissance et la rectitude sont les deux principaux critères utilisés pour l'amélioration

4   génétique du Pin maritime (*Pinus pinaster* Ait.) dans le sud-ouest de la France. Dans cet

5   article, la densité du bois a été étudiée pour répondre à deux questions principales : La

6   variabilité de la densité du bois montre-t-elle une tendance générale au cours du

7   développement de l'arbre ? La variabilité de la densité a-t-elle été modifiée indirectement par

8   la sélection en raison d'une corrélation défavorable entre la densité du bois et la croissance ?

9   La densité du bois et ses composantes ont été analysées dans trois tests polycross représentant

10   les populations d'amélioration successives. Au total environ 50 familles par test, soit plus de

11   1 900 arbres, ont été mesurées par microdensitométrie.

12   Une étude préliminaire a permis de montrer que l'annotation des cernes de l'écorce vers le

13   cœur de l'arbre permet une meilleure estimation des effets génétiques par rapport à une

14   annotation du cœur de l'arbre vers l'écorce. La variabilité génétique de la densité du bois

15   apparaît fortement dépendante de l'année considérée mais notre étude ne révèle aucune

16   tendance générale au cours des années. De plus, alors que la variabilité génétique des

17   caractères sélectionnés a diminué au cours des populations d'amélioration, aucune évolution

18   significative n'a été mise en évidence pour la variabilité de la densité du bois.

19

20   *Pinus pinaster* **Ait. / densité du bois / variabilité génétique / populations d'amélioration**

21

1 Introduction

2

3   Maritime pine (*Pinus pinaster* Ait.) is cultivated in artificial plantations throughout the

4 southwestern part of France (Aquitaine region). Overall the cultivated area amounts today to

5 one million hectares. Every year about 20 000 ha are planted mostly with reproductive

6 material coming from second generation seed orchards. For these seed orchards, gain

7 expectations reach 30% on volume and stem straightness (GIS 2002). The maritime pine

8 breeding programme consists in a recurrent selection scheme developed since the 1960's

9 (Durel 1992; GIS 2002) to steadily maintain genetic gain and preserve diversity in the

10 breeding population. This programme has reached today its third generation including the

11 original selection of plus trees. The third generation seed orchards are now being planted.

12

13   Wood density was not taken into account in the first two generations of the breeding

14 programme, where selection was based on volume and stem straightness only. However wood

15 quality has received more and more attention (Pot et al. 2002). Among many traits involved in

16 wood quality, wood density is a trait of interest because it is positively correlated with pulp

17 yield, pulp quality and timber strength (Zobel and Jett 1995; Amstrong et al. 1984; Hatton and

18 Cook 1992).

19 Consequences of directional selection for growth on wood density can be investigated at two

20 levels: either through the successive commercial varieties or through the breeding populations

21 (a variety is constituted with the best genotypes of a breeding population). Changes in wood

22 density mean should rather be studied through the varieties because they may lead to different

23 economic benefits. On the contrary, evolution of wood density variability relates mainly to

24 breeding strategies. Therefore, it must be studied throughout the breeding populations.

1 Bouffier et al. (2008*b* submitted) foresaw a decrease up to 6 % of the average wood density in

2 the commercial varieties in comparison to unimproved varieties as a result of two main

3 causes. First, the selection on growth rate can indirectly induce a reduction of wood density if

4 those two traits are unfavourably correlated. This was observed on maritime pine even if the

5 magnitude of this correlation was found to be weak (Bouffier et al. 2008*b* submitted). Second,

6 growth rate improvement leads to the shortening of rotation age, thus increasing the juvenile

7 wood proportion in the final product (Bendtsen 1978). As juvenile wood is less dense than

8 mature wood (Zobel and Van Buijtenen 1989), whole-tree density is expected to be lower.

9

10   In this paper, we will focus on the change of genetic variability of wood density over

11 the first three breeding populations (P0, G0 and G1). For the sake of clarity, we will name:

12 (i)   P0 population (the number "0" indicated the generation considered): the original

13   unimproved population (namely the Aquitaine maritime pine forest) where the

14   plus tree were initially selected.

15 (ii)   G0 population: the subset of 635 "plus" trees, phenotypically selected during the

16   sixties in the P0 population. P0 and G0 belong to the same generation but G0 is a

17   subpopulation of P0 whose members follow a normally shaped distribution

18   placed to the right of the P0 distribution curve.

19 (iii) G1 population: the new breeding population of about 2 600 trees individually selected

20   within the progenies of G0 trees based on their genetic value.

21

22   Wood density variability can be described according to three references: "cambial

23 age" (cambial age at time of ring formation), "calendar year" (year of ring formation) or "tree

24 age" (age of the tree at the time of ring formation). These references are not strictly equivalent

25 because samples are always collected at breast height whereas growth rate varies among trees.

1   Most genetic studies on wood density have been done so far using cambial age as a reference

2   (Louzada and Fronseca 2002; Dungey et al. 2006; Li and Wu 2005; Hylen 1999; Kumar and

3   Lee 2002; Rozenberg and Cahalan 1997). In this contribution, we will compare the three

4   references in regards to the estimation of genetic variability.

5

6   The three main objectives of this paper are:

7   1/ to determine the best suited reference to study variability of ring density

8   2/ to describe the development of variability of ring density traits across years

9   3/ to study evolution of variability of wood density across successive breeding

10   populations.

11

Materials and methods

Plant material

Three maritime pine experimental tests were studied: Rousset, Hermitage and Pissos corresponding to the progeny of three successive populations of the breeding programme, respectively P0, G0 and G1. Rousset is a 30-year-old progeny test comprising 74-open-pollinated families collected all over the Aquitaine forest (P0 population). The plantation at Hermitage is a 29-year-old polycross test of plus trees (G0 population). The 261 mother trees were crossed with a pollen mix collected on 28 trees of the G0 population. Pissos is a 12-year-old polycross test of G1 trees established with 129 families; the mix of pollen was collected on 20 G0 trees.

These three tests are arranged in randomized complete blocks design with a mean of ten-tree row plots per family. Rousset and Hermitage plantations were established on a humid sandy moor site and are geographically close (less than 5 km). There is only a 1 year difference between the plantation dates in both sites. The Pissos plantation is more distant (about 50 km far from the two other tests), and is located on dryer sandy soil. The plantation is also younger and there were some differences in spacing and seedling installation (Table 1).

Tree measurements

All the trees of the three studied tests were measured at about 11 years old (10, 12 and 11 years old for, respectively Rousset, Hermitage and Pissos) which is about the selection age. Two growth traits - total height (H) and girth at breast height (D) – and stem deviation to verticality (S) were measured.

1	*Wood density measurements*

2	A random sub-sample of the trees from each test was measured for wood density.

3	From 44 to 51 families, chosen at random, were sampled per test with a mean of 14 trees per

4	family (Table 1). In winter 2004, one increment core was collected in each tree at breast

5	height from pith to bark with a 5 mm diameter increment borer. In fact we often sampled

6	several times the same tree to collect a core including the pith or close to it. Samples were cut

7	to a constant thickness of 2 mm using a double blade saw and then resins were removed by

8	soaking the core into pentane during 24 hours. The wood density was measured using an

9	indirect-reading X-ray densitometer (Polge 1966). The radial density profile was then

10	obtained by analysing the scanned images (one density measurement is collected every

11	25.4 µm) with Windendro (Guay et al. 1992). Ring limits were automatically determined,

12	then manually checked and corrected using this software.

13

14	From the density profile, we calculated the following traits of interest per ring:

15	-	ring width (RW)

16	-	ring density (RD)

17	-	earlywood density (EWD)

18	-	latewood density (LWD)

19	-	latewood proportion (LWP)

20	The criterion used to separate earlywood from latewood was calculated for each ring as the

21	average between maximum and minimum ring density (Nicholls et al. 1980; Vargas-

22	Hernandez and Adams 1992; Kumar and Lee 2002).

23	The analyses were first performed based on annual ring data and then on cumulative data. As

24	rings near the bark represent a higher volume than those closer to the pith, RD was obtained

1 with a weighted mean: each density data was weighted by its respective cross-sectional area

2 (Louzada 2003).

3

4 *Statistic model*

5 Genetic analyses of Rousset, Hermitage and Pissos tests allowed us to estimate the

6 genetic parameters of populations P0, G0 and G1 respectively. The following linear mixed

7 model was used for estimation of genetic parameters:

8 $$Y_{ijk} = \mu + B_i + F_j + B \times F_{ij} + e_{ijk}$$

9 where Y_{ijk} is the phenotypic individual observation, μ the general mean, B_i the block effect

10 and F_j the family effect. B was considered as fixed effect, while F and $B \times F$ were treated as

11 random effects.

12 A variance is fitted for each random effect: σ_F^2 the additive variance, $\sigma_{B \times F}^2$ the variance for

13 bloc × family interactions and σ_e^2 the residual variance.

14 Estimates of phenotypic variance ($\hat{\sigma}_P^2$) and additive genetic variance ($\hat{\sigma}_A^2$) were obtained

15 from:

16 $$\hat{\sigma}_P^2 = \hat{\sigma}^2{}_F + \hat{\sigma}^2{}_{B \times F} + \hat{\sigma}^2{}_e$$

17 $$\hat{\sigma}_A^2 = 4 \times \hat{\sigma}^2{}_F \quad \text{because families are maternal half-sibs}$$

18

19 Annual ring data can be considered as "longitudinal data" as each year's density

20 record is a new measurement of the same tree (Apiolaza et al. 2000; Apiolaza and Garrick

21 2001). A longitudinal analysis was thus first performed with the annual ring density values.

22 Several structures of covariance were considered for random effects. Indeed the most

23 parameterised model is not necessarily the most appropriate: the accuracy of the parameter

24 estimates decreases with the number of covariance components considered. We modelled

1   different covariance structures (Apiolaza and Garrick 2001) and the best model was

2   determined with the Akaike's Information Criterion (Wada and Kashiwagi 1990). A banded

3   correlation structure for additive genetic effects was found to be the more appropriate (data

4   not shown). Nevertheless, in our case, these longitudinal analyses can hardly be applied to

5   long time series, as convergence of the likelihood is seldom attained when the number of

6   variables exceeds 5. This prevented us from studying the yearly variation of genetic variation

7   over long periods of time. Furthermore, we compared univariate analyses with longitudinal

8   analyses and found that estimates of genetic variances were of similar magnitude. Finally,

9   although longitudinal analysis were done for each test, but using only the data of 5 years, we

10   will present exclusively the results of univariate analyses, as they allowed to cover the whole

11   time range (from 4 to 30 years).

12

13   All analyses were conducted with the restricted maximum-likelihood method and

14   standard errors estimating by a standard Taylor series approximation using the software

15   ASReml (Gilmour et al. 2002).

16

17   *Variability parameters*

18   Variability of growth and wood density traits were first estimated ring by ring and then

19   on cumulative data. We considered the additive genetic and phenotypic coefficients of

20   variation ($\hat{CV}_A$ and $\hat{CV}_P$) and individual heritability ($\hat{h}^2$), defined as follow:

21
$$\hat{CV}_A = \frac{\hat{\sigma}_A(X)}{\overline{X}} \qquad\qquad \hat{CV}_P = \frac{\hat{\sigma}_P(X)}{\overline{X}} \qquad\qquad \hat{h}^2 = \frac{\hat{\sigma}_A^2}{\hat{\sigma}_P^2}$$

22   where $\overline{X}$ is the mean of the studied trait for the test considered.

23   The comparison of the variability between years or between populations makes sense because

24   the parameters considered are standardized estimates of variability.

1

2 *Ring indexing*

3 Rings were first numbered according to three references (Figure 1):

4 - from pith to bark which corresponds to the "cambial age" reference

5 (cambial age = 1 for the first ring from the pith)

6 - from bark to pith which corresponds either to the "calendar year" reference

7 (calendar year = 2004 for the first ring from the bark) or "tree age"

8 reference (tree age = 12 for the first ring from the bark on the example of

9 Figure 1).

10 In this article, ring indexing refers to the way rings are numbered ("bark-to-pith" or "pith-to-

11 bark") and the reference ("cambial age", "calendar year" or "tree age") is the label chosen for

12 the year of ring formation.

13 The first ring from the pith was systematically removed from the analysis because it was often

14 incomplete.

15 In a preliminary study we attempted to identify the most suitable reference in regards to the

16 objective of the study. We consider that the reference most suitable for our study is the one

17 which minimizes the residual coefficient of variation $\hat{CV}_R$:

18
$$\hat{CV}_R = \frac{\sqrt{\hat{\sigma}_P^2 - \hat{\sigma}_A^2}}{\overline{X}}$$

19 CV_R of RD has been estimated, ring by ring, in each test, considering bark-to-pith and pith-to-

20 bark indexing. Differences between annual estimations obtained with each index were tested

21 with an analysis of variance performed on the three tests. Prior to performing these analyses,

22 normality distribution of CV_R was verified with the Shapiro-Wilk test (W = 0.99, p-

23 value = 0.53).

24

Results

1/ Comparison of genetic variation according to ring indexing

Mean ring density was plotted versus "cambial age" (Figure 2a), "calendar year" (Figure 2b) and "tree age" (Figure 2c) for the three tests analysed. The yearly variation of wood density is lower when indexing from pith to bark ("cambial age" reference) compared to the bark-to-pith indexing (references "calendar year" and "tree age"). Furthermore annual density curves exhibit exactly the same pattern whether indexed by "calendar year" or "tree age"; the only difference is their relative position. Indeed as the ring numbering was done here in the same way (from bark to pith), the annual data gather the same rings for a given test, and for a single-test analysis will result in exactly the same genetic parameters.

We further analysed the data to identify the most reliable indexing method for estimating genetic variation and heritability. The analysis of variance displays a significant "index" effect on CV_R (p-value = 0.01). Annual CV_R of RD is thus significantly lower when considering bark-to-pith indexing rather than pith-to-bark indexing (7.7 % vs. 8.5 %). Table 2 shows that, regardless of the test considered, the lower residual variation for bark-to-pith indexing contributes to higher heritability values. However this difference is not significant considering the standard errors of the estimates.

Thus the comparison of ring indexing based on CV_R analysis suggests to favour bark-to-pith indexing. In this case, two references are still possible: "calendar year" or "tree age". The reference "calendar year" would be most appropriate when the research question has to do with climatic factors. For example, one could investigate climate data for the years 1994 and 1995 where respectively, a sharp increase in density followed by a sharp decrease in density were observed at Rousset and Hermitage (Figure 2b). In this study, the question has to do with genetic variation in different populations at the same stage of development. For

example, we wish to compare patterns observed through age 12 at Pissos (G1) with the

patterns observed at Rousset (P0) and Hermitage (G1). The reference "tree age" (Figure 2c) is

then more appropriate here.

2/ Annual variation of genetic parameters of wood density components

Wood density variability was first studied within each test considering annual ring

data based on the "tree age" reference.

Heritability of RD (Figure 3) and its components (Figure 4) were estimated for each

tree age. Whatever the test considered, annual heritabilities for RD are moderate to high: the

mean values are 0.47, 0.21 and 0.43 respectively for Rousset, Hermitage and Pissos. EWD

shows higher heritability values than LWD and but similar values to RD. LWP shows

moderate heritabilities (between 0.1 and 0.4). Standard errors of heritability estimates range

from 0.11 to 0.18.

To study more precisely the variability of wood density, the phenotypic and additive

genetic variability was also investigated. Variance components were expressed in terms of

coefficients of variation to avoid confounding scale effects (Vargas-Hernandez et Adams

1992; Kumar and Lee 2002). The mean annual CV_P of RD is 10.2 %, 9.6 %, 8.9 % and the

mean annual CV_A is 6.2 %, 4.2 % and 5.7 % for Rousset, Hermitage and Pissos, respectively.

Mean standard error of genetic coefficient of variation is 1.6%.

High inter annual variations can be observed within a test whatever the parameter

considered. For example, heritability of RD estimated at Rousset varies from 0.18 at tree age

11 to 0.82 at tree age 20. No general pattern across tree development arises from the analysis

of annual variability of wood density and its components.

3/ Variability of wood density and growth between successive breeding populations

1   Variability between breeding populations was compared based on cumulative traits

2   (Figure 5 and Table 3).

3   The variability parameters for cumulative RD and RW (Figure 5) are much more

4   stable from one year to another compared to those based on annual data and described

5   previously. RD heritability tends to increase during the first years and then stabilizes; at

6   Hermitage a slight decrease is observed from tree age 10 to 15. RW heritability appears stable

7   over tree age. Phenotypic variability of RW and RD is similar among tests, except Pissos

8   which displays a lower CV_P for RW. Genetic variability estimated with h^2 and CV_A shows

9   differences between tests.

10

11   Additive genetic variability can be compared between Rousset and Hermitage until

12   tree age 29. Rousset exhibits higher genetic variability for both RW and RD. The comparison

13   of the three tests can be done only until tree age 12. We thus summarized the variability

14   values estimated on cumulative data at tree age 12 in Table 3. Rousset displays the highest

15   CV_A for RW followed by Pissos then Hermitage. For RD, Rousset and Pissos have the same

16   level of genetic variability ($CV_A = 5.5\,\%$) which is higher than the one at Hermitage

17   ($CV_A = 4.2\,\%$). Nevertheless, based on the standard errors, differences for genetic variability

18   between tests are not significant ($p > 0.05$ with Z-test).

19

Discussion

1/ Comparison of genetic variation according to ring indexing

Ring indexing is an important issue for wood density analyses as the annual ring population considered for analyses depends on the choice of the index.

Bark-to-pith ring indexing appears to be more appropriated to study genetic variability than pith-to-bark indexing as residuals of genetic analyses are significantly reduced. Similar results were found in other softwood species (Rozenberg, unpublished data). The standard errors of the mean heritability per test are high because very different annual values are mixed. As a consequence, even if the mean heritability is higher with bark-to-pith indexing compared to pith-to-bark indexing, this difference is not significant. In this article, we will thus index rings from bark to pith.

Two references can be considered when indexing from bark to pith: "calendar year" or "tree age". When all trees have the same age, these references are equivalent: a specific "calendar year" corresponds to a specific "tree age". When data comprises tests established at different years, the choice of the reference is important. If the study focuses on the year effect, the "calendar year" reference has to be considered in order to compare ring populations gathered depending on the year. In this study, the "tree age" reference is more suitable as we aim to compare variability depending on tree development and on the breeding population. For example, a comparison of wood density, between the three tests studied at tree age 12, is equivalent to a comparison realized with increment cores collected when the tests were 12.

To best understand the difference between pith-to-bark and bark-to-pith indexing, Figure 6a displays the different calendar years obtained for the cambial age 2; and Figure 6b

1 the different cambial ages obtained for the calendar year 2004. The growth recorded for one

2 particular cambial age (Figure 6a) has been achieved by trees in 3 different calendar years at

3 Rousset and Hermitage (corresponding to respectively 91% and 86% of the samples) and in 2

4 calendar years at Pissos (corresponding to 99% of the samples). Figure 6a and Figure 6b are

5 symmetric, except that some samples have no 2004 ring (the last ring before the bark) because

6 it was incomplete and thus removed from the analysis. Nevertheless, calendar age can be

7 attributed to these samples since some years show a very specific density profile.

8 The cambial age has a "smooth effect" on wood density (meaning not very different for very

9 close cambial ages) whereas year effect can be completely different from year to year

10 depending on the annual climate. This can be clearly visualised, for example, on rings 1994

11 and 1995 at Rousset and Pissos (Figure 2b): the high density difference between these two

12 rings cannot be explained by the cambial age effect, since this effect is minimum between two

13 successive rings, but rather by the year effect i.e. climatic conditions. As a consequence, bark-

14 to-pith indexing mixes rings with similar density (because of neighbouring cambial ages)

15 whereas pith-to-bark indexing mixes rings with highly different density (because of different

16 years). Thus, genetic analyses with pith-to-bark indexing display higher residuals as they

17 contain high variability due to year effect.

18 When analysing plantations where all trees were established at the same time, cambial age

19 and calendar year effects can not be considered together in a statistical model because they

20 would be partially confounded and the data highly unbalanced. The only way to obtain

21 balanced data aiming to correctly separate and estimate these two effects would be to sample

22 trees in a genetic design in which trees have been planted at different times. This kind of

23 experimental design is very cumbersome to establish and to manage over several years as

24 competition for light would be generated by the age differences of trees.

25

1   Bark-to-pith indexing is also more suitable for a technical reason. As it is very difficult

2   to sample the pith while collecting a core with an increment borer, the cambial age of the

3   most internal ring has often to be estimated, while the year of the most external ring (the ring

4   immediately under the bark) is precisely defined by the collection date. This difficulty is

5   generally increased with the asymmetry of the rings due to lack of stem straightness (a

6   common defect of Landes maritime pine), and also with tree age. 10 to 20 % of the increment

7   cores from the two older tests (Rousset and Hermitage) did not sample the pith whereas

8   almost all cores from Pissos did. As a consequence, Figure 6a displays, for cambial age 2,

9   eight calendar years at Rousset and Hermitage against four at Pissos.

10

11   Although "cambial year" reference is more often used in the literature (see

12   introduction), bark-to-pith indexing has also been chosen in several papers (Vargas-

13   Hernandez and Adams 1992; Costa e Silva et al. 1994; Fries and Ericsson 2006; Fujimoto et

14   al. 2006). Authors never clearly justified their choice. Our results demonstrate the relevancy

15   of choosing bark-to-pith indexing for variability studies. When several tests are considered,

16   reference has to be chosen depending on the goal of the study: "calendar year" to focus on

17   climate year effect or "tree age" to focus on tree development effect.

18

19   *2/ Estimation of genetic variability of wood density over tree age*

20

21   *Variability estimated with annual data*

22   Louzada and Fronseca (2002), also working on maritime pine, found higher annual

23   heritabilities for ring density (between 0.53 and 0.74) than in our study. Nevertheless a similar

24   trend was observed between the wood components; indeed, earlywood is much more heritable

25   than latewood and latewood proportion shows intermediate values.

1   Genetic variability of wood density and its components shows no particular trend over

2   age and appears highly variable depending on the year. Phenotypic variability is less variable

3   but still no pattern arises. The important variation displayed by annual estimates can be

4   explained by annual climate differences. As Rousset and Pissos are two tests very close, they

5   have identical climate influence. We thus expected the same trend if ring traits were studied

6   with the calendar year reference. We effectively found a significant positive correlation for

7   phenotypic variability whatever the trait considered: the Pearson coefficient between the CV_P

8   of Rousset and Hermitage is 0.51 (p-value = 0.01), 0.71 (p-value < 0.001) and 0.67 (p-

9   value < 0.001) for RD, EWD and LWD, respectively (Figure 7). On the contrary, annual

10   coefficients of genetic variation were not correlated between these two tests

11   The absence of annual trend found in the literature on various conifer species also highlights

12   the major role of the environmental influence rather than tree age influence. For example,

13   Fries and Ericsson (2006) found no age trend for heritability on the 10 outer annual rings in a

14   30-year-old progeny test of Scots pine analysed with an individual tree model. This study was

15   performed on rings indexed from bark to pith; the references cited hereafter, and conducted

16   using pith-to-bark indexing, present similar results.

17   Zamudio et al. (2002), Li and Wu (2005) and Dungey et al. (2006) estimated heritability of

18   density components across successive cambial ages in radiata pine: no congruent trend was

19   found among these studies, neither for the level of heritability nor for the yearly trend of

20   heritability variation.

21   In *Pinus elliotti* (slash pine), Hodge and Purnell (1993) estimated moderate heritability values

22   ($h^2 \approx 0.2$) for the rings near the pith (rings 3-4) and for the rings composed of mature wood

23   (rings 11-13) while intermediate rings showed slightly lower heritabilities ($h^2 \approx 0.10 - 0.15$).

24   Hylen (1999) found large differences of heritability values of RD (from non significant to

25   0.49) between cambial ages in *Picea abies* (Norway spruce) with no particular trend.

1

2 *Variability estimated with cumulative data*

3   Wood density variability of maritime pine was first studied using basic density:

4 estimations of heritability on several rings of young trees varied from 0.40 to 0.75 (Keller

5 1973; Polge and Illy 1968; Nepveu 1973). More recently, Pot et al. (2002) studied X-ray

6 density and found a heritability of 0.30 on several rings of 14 years old maritime pines. Our

7 values fall within this range.

8 Variability analyses based on cumulative data show less variation over age. The main trend

9 observed is an increase of wood density heritability in juvenile wood despite a slight decrease

10 between tree age 10 and 15 at Hermitage. No major trend for genetic variability of density

11 over years was found. Two previous studies, carried out on cumulative density data based on

12 bark-to-pith indexing, showed an increase of overall wood density heritability with age

13 (Fujimoto et al. 2006; Vargas-Hernandez and Adams 1992). The trend was explained by a

14 decrease of environmental variability while additive variance remained stable over ages.

15 Studies indexing rings from pith-to-bark also found an increase of wood density heritability

16 (Hylen 1999; Kumar and Lee 2002) whereas for others no obvious trend was highlighted (Li

17 and Wu 2005; Gwaze et al. 2002).

18

19   *3/ Comparison of genetic parameters of wood density and growth between successive*

20 *breeding populations*

21

22   Bouffier et al. (2008*a* submitted) studied variability for growth traits in three

23 populations of the French maritime pine programme: P0 (the Aquitaine forest), G0 (the plus

24 trees) and G1 (the selected trees in the G0 progenies). Based on several tests per population,

25 they found a significant reduction of genetic variability in G0 compared to the unimproved P0

1   population as a consequence of selection on growth traits. In the two successive improved

2   populations, G0 and G1, no significant evolution was observed, most likely because the

3   accuracy of the estimates was not sufficient to detect slight changes. Similarly, King et al.

4   (1998) reported no evolution of variability between two breeding populations of *Pinus radiata*

5   equivalent to the ones we referred to as G0 and G1.

6   In this study, the three tests analysed for wood density allow to compare estimates of

7   genetic variability among these three breeding populations. Indeed, genetic parameters of P0

8   were estimated with Rousset, a progeny test from random trees of the Aquitaine forest.

9   Hermitage is a progeny test of G0 trees and Pissos of G1 trees, thus the analyses of these tests

10   estimate the genetic variability for G0 and G1 populations.

11   Comparisons between wood density means over breeding populations could be

12   hazardous in our present study because only one test per population was studied, hence

13   confounding both test effect and population effect. Genetic variability can be more easily

14   compared because we used standardized parameters (heritability and coefficients of

15   variation). For comparative purposes, genetic variance is more appropriately standardized by

16   trait mean than by phenotypic variance (Houle 1992); we will thus focus our analysis on

17   coefficient of additive variation rather than heritability.

18   As expected, the genetic variability of growth traits is lower for selected populations

19   (G0 and G1 estimated with tests Hermitage and Pissos) than for the unimproved P0

20   population (estimated with Rousset), based on cumulative RW analysis. Nevertheless, the

21   increase of genetic variability for RW between G0 and G1 is unexpected as it has been shown

22   on a large data set (Bouffier et al. 2008*a* submitted) that variability is stable for growth traits

23   between these populations. Regarding wood density, the genetic variability is more similar

24   between tests, suggesting no strong evolution throughout breeding populations. However, due

25   to large standard errors, the evolution of genetic variability must be analysed cautiously.

1   Genetic variability of a trait can be modified in breeding population undergoing

2   selection in two different ways: through indirect selection if this trait is negatively correlated

3   with a selected trait or through population size reduction.

4   It has been shown that correlations between wood density and selected traits (even weak

5   compared to the standard errors, see Table 4) have significantly decreased wood density in the

6   commercial varieties (Bouffier et al. 2008*b* submitted). These correlations did probably not

7   change the variability of wood density throughout the breeding populations because the

8   selection intensity is lower in breeding populations than in varieties. Moreover the status

9   number of the G1 population is about 100, which is assumed to be high enough to maintain

10   variability (White 1992).

11

12   4/ Conclusion

13

14   Our analysis of three maritime pine progeny tests, gathering nearly 2 000 trees,

15   provides answer the questions addressed in the introduction.

16   - The genetic variability of wood density is better estimated when the rings are

17   indexed from bark to pith rather than from pith to bark.

18   - No general pattern arises over years for annual genetic variability of wood density. It

19   appears that the climatic year plays a greater role to explain the magnitude of genetic

20   variability compared to the tree development stage.

21   - Directional selection on growth had no detectable effect on genetic variability of

22   wood density throughout the breeding populations. This is of first concern as wood quality

23   gets more and more importance. Wood density can thus be improved in the future without

24   changing the current composition of the breeding population.

25

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