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11 **Genetic parameters and QTL analysis of  $\delta^{13}\text{C}$  and ring width**  
12 **in maritime pine**

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**1Abstract**

2Classical quantitative genetics and quantitative trait dissection analysis (QTL)  
3approaches were used in order to investigate the genetic determinism of wood  
4cellulose carbon isotope composition ( $\delta^{13}\text{C}$ , a time integrated estimate of water use  
5efficiency) and of diameter growth and their relationship on adult trees (15 years) of  
6a forest tree species (maritime pine). We used a half diallel experimental set-up to (1)  
7estimate heritabilities for  $\delta^{13}\text{C}$  and ring width and (2) to decompose the phenotypic  
8 $\delta^{13}\text{C}$  / growth correlation into its genetic and environmental components. We found  
9considerable variation for  $\delta^{13}\text{C}$  (range of over 3‰) and for ring width (range of over  
105 mm) and significant heritabilities (narrow sense 0.17 / 0.19 for  $\delta^{13}\text{C}$  and ring width,  
11respectively, 100% additivity). The significant phenotypic correlation between  $\delta^{13}\text{C}$   
12and ring width was not determined by the genetic component, but was attributable to  
13environmental components. Using a genetic linkage map of a full-sib family, four  
14significant and four suggestive QTLs were detected for  $\delta^{13}\text{C}$ , the first for  $\delta^{13}\text{C}$  in a  
15forest tree species, as far as known to the authors. Two significant and four  
16suggestive QTLs were found for ring width. No co-location of QTLs was found  
17between  $\delta^{13}\text{C}$  and growth.

**18Keyword Index :**

19stable isotope,  $^{13}\text{C}$ , growth, water use efficiency, heritability, quantitative trait, Pinus  
20pinaster, tree rings

## 1 Introduction

2 In given environmental conditions, trees with high water-use efficiency at the leaf  
3 level (intrinsic WUE, defined as the ratio of net CO<sub>2</sub> assimilation rate  $A$  to stomatal  
4 conductance for water vapour  $g$ ) can maintain higher growth rates under water  
5 limited conditions than trees with lower WUE (Sun *et al.* 1996; Nguyen-Queyrens *et*  
6 *al.* 1998). Measurements of plant carbon isotope composition ( $\delta^{13}\text{C}$ ) provide time-  
7 integrated estimates of WUE (Farquhar, O'Leary & Berry 1982; Farquhar &  
8 Richards 1984) that can be applied to adult trees (Zhang & Marshall 1994; Zhang &  
9 Marshall 1995; Guehl *et al.* 1995; Sun *et al.* 1996). Assessments of differences in  
10 WUE among- and within-tree species are facilitated by the crown- and time-  
11 integrative nature of tree ring  $\delta^{13}\text{C}$ .

12 Forest tree species are known to be among the most polymorphic species of the flora  
13 (Hamrick, Godt & Sherman-Broyles 1992). Genotypic differences in leaf  $\delta^{13}\text{C}$  of  
14 conifer species were found among provenances in common garden studies (Zhang,  
15 Marshall & Jaquish 1993; Zhang *et al.* 1994; Zhang *et al.* 1995; Guehl *et al.* 1995;  
16 Nguyen-Queyrens *et al.* 1998). It has been suggested that provenance differences of  
17  $\delta^{13}\text{C}$  might be determined by differences in stomatal sensitivity to changes in vapour  
18 pressure deficit (Zhang *et al.* 1995) and/or differences in plant hydraulic  
19 characteristics (Guehl *et al.* 1995). However, differences of  $\delta^{13}\text{C}$  among genetic  
20 families within provenances of *Picea mariana* (Mill.) were found to be mainly  
21 determined by differences in photosynthetic capacity (Johnsen & Major 1995; Major  
22 & Johnsen 1996). Similar indications were obtained for maritime pine (*Pinus*  
23 *pinaster* Ait.) by Guehl *et al.* (1995).

24 Tree growth is an important goal for forest tree breeding programs. To avoid  
25 inadvertent negative selection for growth when selecting for high WUE, it is  
26 important to know if  $\delta^{13}\text{C}$  and growth are genetically linked. Positive but weak  
27 phenotypic relationships between  $\delta^{13}\text{C}$  and height or diameter growth (Flanagan &  
28 Johnsen 1995; Johnsen *et al.* 1999; Nguyen-Queyrens *et al.* 1998) have been found  
29 among trees within different forest tree species. Genetic parameters calculated for  
30 physiological or morphological traits can disentangle phenotypic relationships into  
31 genotypic and environmental components. Johnsen *et al.* (1999) found strong genetic  
32 correlations between  $\delta^{13}\text{C}$  and tree height or tree diameter. They concluded that  $A$   
33 was determining  $\delta^{13}\text{C}$  and growth performance and thus constituted probably the link  
34 between the two traits. However, since  $\delta^{13}\text{C}$  as an indicator of WUE could be either  
35 controlled by  $A$  and / or by  $g$ , there is not necessarily a strong relationship between  $A$   
36 and  $\delta^{13}\text{C}$ . This suggests that the existence of a genetic correlation between  $\delta^{13}\text{C}$  and  
37 growth is depending on the factor by which WUE is controlled.

38 Adaptive traits, like  $\delta^{13}\text{C}$ , are characterised by high phenotypic variation among and  
39 within populations of forest tree species (Meinzer *et al.* 1992; Zhang *et al.* 1993;  
40 Zhang *et al.* 1995; Flanagan *et al.* 1995; Nguyen-Queyrens *et al.* 1998). Moreover,  
41 high heritabilities for  $\delta^{13}\text{C}$  have been found for non-woody (Matus, Slinkard & Van  
42 Kessel 1995; Asay, Johnson & Palazzo 1998) and woody species (Johnsen *et al.*  
43 1999). The development of genetic mapping (Tanksley 1993) has made it possible to  
44 localize genetic factors controlling quantitative traits (QTLs, Quantitative Trait  
45 Loci). High heritability of a trait is a favourable factor for quantitative trait dissection  
46 analysis.

1In crop plant breeding, improvement of WUE has been an important aim and  
2therefore the first QTLs for  $\delta^{13}\text{C}$  were detected in tomato (Martin *et al.* 1989).  
3Mansur *et al.* (1993) found in a preliminary investigation of  $\delta^{13}\text{C}$  on soybean one  
4large genomic region that could be responsible for as much as 53% of the observed  
5variation. In a study of three weeks old barley plants (Pakniyat *et al.* 1997), twelve  
6AFLP markers were detected for  $\delta^{13}\text{C}$ , two of these markers alone accounted for  
753.2% of the variation. QTLs for water use efficiency, as measured by the ratio of  
8dry weight to water used, were found in soybean (Mian *et al.* 1996; Mian, Ashley &  
9Boerma 1998).

10Detection of QTL on woody species, however, is still in development, due to long  
11generation time and therefore the lack of controlled crosses. Genetic maps have often  
12to be constructed from F1 full-sib progenies. Carlson *et al.* (1991) were the first to  
13show that RAPD primers could be screened for informative markers segregating in a  
141:1 ratio in diploid tissue of full-sib progenies. Grattapaglia and Sederoff (1994)  
15extended this idea in constructing parental maps of an interspecific eucalyptus hybrid  
16family in a mapping strategy named “two-way pseudo-testcross”. It was further used  
17in conifers (Kubisiak *et al.* 1996, Arcade *et al.* 2000) with RAPDs and AFLPs. For  
18maritime pine the genome coverage required for linkage map construction and QTL  
19analysis was achieved by using RAPD markers (Plomion *et al.* 1995a; Plomion,  
20O'Malley & Durel 1995b) and AFLP analysis (Costa *et al.* 2000).

21Our objectives were: 1) To estimate the variability and heritability of  $\delta^{13}\text{C}$  and ring  
22width in a forest tree species (*Pinus pinaster* Ait.) using a half-diallel experimental  
23design. 2) To investigate the phenotypic correlation between  $\delta^{13}\text{C}$  and growth. 3) To  
24separate the phenotypic correlation between  $\delta^{13}\text{C}$  and growth into a genetic and an  
25environmental component. 4) To dissect  $\delta^{13}\text{C}$  and ring width into mendelian inherited  
26components (quantitative trait dissection analysis) using a *Pinus pinaster* Ait. full-sib  
27family. 5) To compare QTLs for  $\delta^{13}\text{C}$  with QTLs for growth.

## 1 **Material and Methods**

### 2 **Half Diallel**

3 A twelve by twelve half-diallel of maritime pine (*Pinus pinaster* Ait.) was used to  
 4 estimate the variability, heritability and genetic correlations among the studied traits.  
 5 Parental trees were crossed in 1980 and seeds from the controlled crosses sown in a  
 6 nursery in spring 1982 and planted in autumn 1982. The 12 parents were trees  
 7 phenotypically selected for stem growth and straightness in the local provenance of  
 8 the Landes de Gascogne. The half-diallel was located in Cestas (Gironde, France,  
 9 44°N44' 0°W44') on a semi-humid podzolic soil. Spacing was 4 m between rows and  
 10 1.1 m between individual trees, i.e. 2272 trees/ha. No selfed crosses were analysed,  
 11 therefore the half-diallel consisted of 66 families (12 female and 11 male parents) of  
 12 125 to 15 individuals each. Three families were not available, therefore only 63  
 13 families were analysed. A parentage test was performed using 3 microsatellites  
 14 (Gerber *et al.* 2000) confirming the authenticity of the progenies used in the half  
 15 diallel. The experimental design consisted of 74 incomplete randomised blocks (the  
 16 large number of blocs is due to the fact that the presented half-diallel is part of a  
 17 much larger complete diallel). For the present study, 564 trees were cut in march  
 18 1997 (trees were fifteen years old). Disks were sampled, dried in a greenhouse and  
 19 analysed for carbon isotopic composition ( $\delta^{13}\text{C}$ ) and ring width as described below.

### 20 **Full-sib family**

21 A three-generation outbred pedigree comprising 202 fifteen-year-old trees was used  
 22 to study the genetic architecture of the studied traits, i.e. the number, genome  
 23 location and effect of Quantitative Trait Loci. The four grand parents were trees  
 24 phenotypically selected for stem growth and straightness in the local provenance of  
 25 the Landes de Gascogne and grafted in clonal archives. These grand parents were  
 26 tested in a polycross progeny test and classified according to their breeding value as  
 27 "Vigor +" (for vigorous trees) and "Vigor -" (for less vigorous trees). Each of the  
 28 parental trees is the result of the cross of one "Vigor +" and one "Vigor -"  
 29 grandparent. The two parental trees were crossed in 1980 and seeds from the  
 30 controlled cross sown in spring 1982. They produced progeny seedlings that were  
 31 planted in autumn 1982. The family was located in Malente (Gironde, France, 44° N  
 32 30' 0° W 47') on a semi-humid podzolic soil. Spacing was 4 m between rows and 1.1  
 33 m between individual trees, i.e. 2272 trees / ha. The trees were felled in March 1997  
 34 and stem discs were cut, dried in a greenhouse and analysed for  $\delta^{13}\text{C}$  and ring width.  
 35 From all the analysed families, 16 trees with no visible growth in the last four years  
 36 before harvest were removed from the analysis.

### 37 **Ring width measurements**

38 Wood subsamples were taken from four positions on the circumference of the stem  
 39 discs for the last four years of growth (1993 to 1996). As the trees were cut in march  
 40 1997, this includes any growth during winter 96/97 utilising reserve material from  
 41 the summer 1996 growth period. Two different methods were used for ring width  
 42 measurements. For the half diallel experiment, ring width was measured using the  
 43 indirect X ray-method first described by Polge (1966). For the full-sib family, the  
 44 ring widths were measured at the four sampling points on the circumference using a

1semi-automatic system consisting of a digitising tablet linked to a computer  
 2(precision 0.1 mm standard deviation). The width was averaged for each ring. To  
 3make the ring width data comparable to the  $\delta^{13}\text{C}$  measurements, for each tree the  
 4mean growth was calculated for the years 1993 to 1996 (mean ring width: MRW),  
 5using an arithmetic mean.

6

## 7Isotope measurements

8The  $\delta^{13}\text{C}$  was measured of a bloc of four rings, which represents a ring-width  
 9weighted mean of the  $\delta^{13}\text{C}$  of each ring. The sampled blocks of wood were cut by  
 10hand into small pieces, pre-ground in a centrifugal mill (Tecator, Cyclotech 1093  
 11Sample Mill, Höganäs, Sweden) and milled to a fine powder in a ball mill (Retsch,  
 12MM2000, Haan, Germany). Cellulose was extracted after an acidic acid /nitric acid  
 13procedure described in Brendel, Iannetta & Stewart (2000). In brief, the method uses  
 14a concentrated nitric acid / 80% acetic acid on-to-ten dilution (0.2 cm<sup>3</sup> in 2 cm<sup>3</sup>) to  
 15digest lignin, proteins and hemicelluloses in 50 mg of powdered wood sample. The  
 16digested molecule fragments are then washed out using ethanol, remainders of acid  
 17are removed during a water wash. The samples are dried chemically with a pure  
 18ethanol / acetone progression and physically in a vacuum centrifugal evaporator  
 19(speed vac) at 100 hPa for 2 h. The original protocol (Brendel *et al.* 2000) was  
 20modified to include two extraction cycles, a 0.5 molar NaOH wash replacing the  
 21water wash to remove acids more thoroughly and prolonging the ethanol washes to 5  
 22min at 60 °C. For  $\delta^{13}\text{C}$  analysis, 1 mg cellulose subsamples were combusted and  
 23analysed for <sup>13</sup>C composition using a continuous flow isotope ratio mass  
 24spectrometer (Delta S, Finnigan MAT, Bremen, Germany). Carbon isotope  
 25composition was calculated relative to the Pee Dee Belemnite standard as (Craig  
 261957):

$$27 \delta^{13}\text{C} = \frac{R_{sa} - R_{sd}}{R_{sd}} \times 1000 [\text{‰}] \quad (1),$$

28where  $R_{sa}$  and  $R_{sd}$  are the <sup>13</sup>C/<sup>12</sup>C ratios of the sample and the standard, respectively.  
 29The discrimination between the  $\delta^{13}\text{C}$  of atmospheric CO<sub>2</sub> ( $\delta_{air} \approx -8\text{‰}$ ) and the  $\delta^{13}\text{C}$  of  
 30plant material ( $\delta_{plant}$ ) was calculated as (Farquhar *et al.* 1984) :

$$31 \Delta = \frac{\delta_{air} - \delta_{plant}}{1 + \frac{\delta_{plant}}{1000}} \quad (2).$$

32Intrinsic WUE was estimated from discrimination using a modified equation from  
 33(Farquhar *et al.* 1982) :

$$34 WUE = \frac{A}{g} = \frac{c_a}{1.6} \left( \frac{b - \Delta}{b - a} \right) \left[ \frac{\text{mol CO}_2}{\text{mol H}_2\text{O}} \right] \quad (3),$$

1 where  $c_a$  is the atmospheric CO<sub>2</sub> concentration (estimated as  $360 \cdot 10^{-6}$  mol mol<sup>-1</sup>),  $b$  is  
 2 the net fractionation caused by carboxylation (27‰) and  $\Delta$  is the discrimination  
 3 between the  $\delta^{13}\text{C}$  of atmospheric CO<sub>2</sub> and the  $\delta^{13}\text{C}$  of cellulose (Equation 2).

4

## 5 Estimation of genetic parameters

6 The normality of the distribution of the traits for both experimental set-ups was  
 7 tested using Smirnov-Kolmogorov test. While  $\delta^{13}\text{C}$  was normally distributed, a small  
 8 distortion from the normality was observed for ring width (p-value = 0.01).  
 9 However, this distortion was considered to be too small to necessitate an adjustment.

10 Analyses of variance for block and family effects in the half diallel were carried out  
 11 with the OPEP software (Baradat 1989; Baradat & Labbé 1995) according to the  
 12 following model derived from the ‘‘Henderson III’’ model (Searle 1971):

$$13 Y_{ijk} = \mu + B_i + F_j + \varepsilon_{ijk} \quad (4)$$

14 where  $Y_{ijk}$  is the value of the trait for the individual  $k$  belonging to the family  $j$ ,  
 15 located in the block  $i$ ,  $B_i$  is the fixed effect of the  $i$ th block,  $F_j$  is the random effect of  
 16 the  $j$ th family and  $\varepsilon_{ijk}$  is the random residual comprising : individual deviation from  
 17 family mean and family x block interactions. When block and family effects were  
 18 significant, data were adjusted to the block effect, prior to the decomposition of  
 19 family effect. The half diallel analysis was carried out with OPEP using the model  
 20 presented below, it is derived from the simplification of the random diallel model  
 21 described by Garretsen & Keuls (1977) (Baradat & Desprez-Lousteau 1997) which is  
 22 adapted to non orthogonal trials with reciprocal crosses :

$$23 Y_{ijk} = \mu + a_i + a_j + s_{ij} + \varepsilon_{ijk} \quad (5),$$

24 where  $Y_{ijk}$  is the value of the trait for the individual  $k$  corresponding to the cross  
 25 between the male  $i$  and the female  $j$ ,  $a_i$  ( $a_j$ ) is the general combining ability (GCA) of  
 26 the  $i^{\text{th}}$  ( $j^{\text{th}}$ ) parent,  $s_{ij}$  is the specific combining ability (SCA) of the cross between the  
 27  $i$ th and the  $j$ th parent and  $\varepsilon_{ijk}$  is the residual term. The additive and dominance  
 28 variances are:  $\sigma^2_A = 4\sigma^2_a$  and  $\sigma^2_D = 4\sigma^2_s$ , whereas the phenotypic variance is :  
 29  $\sigma^2_P = \sigma^2(Y_{ijk}) = 2\sigma^2_a + \sigma^2_s + \sigma^2_\varepsilon$ . The narrow and broad sense heritabilities were  
 30 calculated as  $h^2_{ns} = \sigma^2_A / \sigma^2_P$  and  $h^2_{bs} = (\sigma^2_A + \sigma^2_D) / \sigma^2_P$ , respectively. The percentage  
 31 of additivity is calculated as the additive variance divided by the sum of additive plus  
 32 dominance variances:  $\sigma^2_A / (\sigma^2_A + \sigma^2_D)$ . Genetic and environmental correlations were  
 33 computed with OPEP using a multi-trait analysis of variance and covariance: (1)  
 34 ‘‘estimated’’ genetic and environmental correlations were calculated according to the  
 35 additive and dominance effects assessed in the random model (Equation 5; parental  
 36 level), and (2) ‘‘predicted’’ correlations were assessed from the individual breeding  
 37 values of each tree (individual level; figures 2 a-c). However, as the estimated and  
 38 the predicted correlations gave similar results, only the correlation coefficients based  
 39 on the prevalent ‘‘estimated’’ results were used in the discussion. Standard errors of  
 40 estimates of heritabilities were computed using the robust Jackknife method (Lebart  
 41 *et al.* 1979).



## 1QTL detection

2Two genetic maps corresponding to the female and male parents of the full-sib  
 3family were established using AFLP markers, genotyped on a subset of 90 F1. The  
 4whole mapping population was further genotyped with evenly spaced markers to  
 5increase the statistical power of QTL detection (Chagné *et al.* submitted). In order to  
 6reduce the intra-trial environmental background noise, the data were adjusted for the  
 7block effect. We used the two-way pseudo-test cross mapping strategy to construct  
 8the linkage map (Grattapaglia & Sederoff 1994). Twelve linkage groups were found  
 9for the female map, equalling the number of chromosomes for *Pinus pinaster*. For  
 10the male map 15 linkage groups were detected, however the combination of the male  
 11and the female maps into a consensus map using  $3/4/1/4$ -segregating markers yields 12  
 12linkage groups for each parent. For QTL analysis however, only the  $1/2/1/2$ -segregating  
 13markers could be used.

14For QTL analysis, MultiQTL software (A. Korol, <http://www.multiqtl.com>) was used.  
 15In a first step, QTLs were detected by interval mapping using a LOD threshold of 1.5  
 16and a one-QTL-model (one QTL per linkage group). In a second step, these QTLs  
 17were taken as co-factors (composite interval mapping, CIM; introduced by Jansen &  
 18Stam 1994 and Zeng 1994), allowing individual QTL to be detected independently to  
 19the background noise. In a third step, a two QTL model (Korol *et al.* 1998) using  
 20CIM was applied, first testing if two QTLs are significant and then testing if two  
 21QTLs are more significant than one QTL. Standard deviations for the positions of the  
 22QTLs were calculated using a bootstrap method.

23As there are difficulties involved when using asymptotic approximations of LOD  
 24statistics (fixed LOD level) for QTL detection (Doerge & Churchill 1996), a  
 25permutation approach was used to determine appropriate significance thresholds.  
 26Two theoretical critical thresholds were considered, the first corresponding to a per  
 27linkage group type I error of 5% allowing the detection of “suggestive” QTL and the  
 28second corresponding to a genome wise type I error of 5% allowing the detection of  
 29“significant” QTL. Theoretical critical threshold corresponding to a genome wise  
 30type I error of 5% were calculated for each chromosome taking into account the  
 31number of markers in each chromosome. If  $\alpha_m$  is the critical threshold at the marker  
 32level corresponding to a 5% genome wise type I error, the  $\alpha_c$  (critical threshold at the  
 33chromosome level) for a chromosome comprising  $n$  markers would be:  
 34 $\alpha_c = 1 - (1 - \alpha_m)^n$ . These theoretical thresholds were compared to the thresholds  
 35associated with the LOD obtained by CIM at the chromosome level after 1000  
 36permutations of the data. The proportion of phenotypic variance explained by each  
 37QTL was estimated using the coefficient of determination ( $R^2$ , estimated by CIM,  
 381000 permutations), which is based on the partial correlation of a putative QTL with  
 39the trait adjusted for cofactors in the multi-locus model.

## 1 Results

### 2 Trait distributions

3 Means, ranges and variabilities for  $\delta^{13}\text{C}$  were very similar between the half diallel  
4 and the full-sib experimental designs (Table 1, Figure 1). For MRW (mean ring  
5 width) growth was higher in the half diallel by 0.66 mm and also the range of  
6 observed values was larger (Table 1). However, the coefficient of variation was  
7 slightly higher for the full-sib experimental design than for the half diallel. This was  
8 also true when the coefficients of variation for MRW were calculated using the block  
9 effect adjusted data (data not shown).

### 10 Half diallel

11 Analysis of variance including family and block effect (Equation 4) indicated  
12 variation among families for both  $\delta^{13}\text{C}$  and MRW, which justified the decomposition  
13 of the family effect according to Equation 5. Taking into account the significant  
14 block effect for  $\delta^{13}\text{C}$ , data were adjusted prior to the genetic decomposition. The  
15 narrow sense heritabilities were highly significant ( $p < 0.005$ ) for  $\delta^{13}\text{C}$  and for MRW  
16 and close in their values (Table 2). No dominance effects were detected for these two  
17 traits, thus narrow sense and broad sense heritabilities are equal and additivities are  
18 100%.

19 The phenotypic correlation between mean ring width and  $\delta^{13}\text{C}$  associated faster  
20 growth with less negative  $\delta^{13}\text{C}$  values (higher WUE) and was significant with a  
21 coefficient of correlation of  $r = 0.45$  (Table 3 and Figure 2a). The correlation was not  
22 significant (Table 3) for the genetic component (additive effect), whereas the  
23 environmental component was highly significant with a strong correlation coefficient  
24 ( $r = 0.52$ ).

### 25 Full-sib family

26 A significant positive phenotypic correlation between MRW and  $\delta^{13}\text{C}$  of the full-sib  
27 family ( $r = 0.39$ ;  $p < 0.005$ ; Figure 3) was observed. For  $\delta^{13}\text{C}$ , eight QTLs were  
28 found on seven linkage groups (chromosomes) and for MRW six QTLs on four  
29 linkage groups (Table 4). Using the one-QTL model, six QTLs were detected for  
30  $\delta^{13}\text{C}$  and two for MRW. With the two-QTL model, one pair of QTLs was found for  
31  $\delta^{13}\text{C}$  and two pairs for MRW. For  $\delta^{13}\text{C}$ , two of the QTLs detected with the one-QTL  
32 model and the QTL-pair detected with the two-QTL model and for MRW one QTL-  
33 pair are "significant QTLs" at a probability corresponding to a 5% genome wise type  
34 I error. All other detected QTLs are "suggestive QTLs" at a probability  
35 corresponding to a 5% chromosome type I error. For  $\delta^{13}\text{C}$ , QTLs were detected on  
36 the male and the female maps, however not on the same chromosomes. A multi-locus  
37 model, including the male and female maps, explained 51.4% of the phenotypic  
38 variation of  $\delta^{13}\text{C}$ , the major QTL at chromosome 6 alone explaining 12.4%. For  
39 MRW, no QTLs were found on the female map and a multi-locus model for the male  
40 map explained 42.9% of the observed phenotypic variation. No co-localisation for a  
41 QTL of  $\delta^{13}\text{C}$  and of MRW was found.

## 1 Discussion

### 2 Trait distributions

3 Standard deviations and ranges were similar for half diallel as well as for the full-sib  
4 (Table 1). For  $\delta^{13}\text{C}$  the coefficients of variation were nearly the same, whereas the  
5 coefficient of variation for mean ring width was higher for the full-sib than for the  
6 half diallel (Table 1). Variability of traits might be expected to be higher in a half  
7 diallel with twelve parental trees, than in one full-sib family. However, it has to be  
8 taken into account that parental trees for both experiments were selected from the  
9 same provenance (Landes, Gascogne) and therefore might be genetically close.  
10 Further, with a polygenic complex trait such as  $\delta^{13}\text{C}$ , due to transgression, even  
11 parents with only a small difference in a measured trait can produce offspring with  
12 extreme values (Prioul *et al.* 1997). For  $\delta^{13}\text{C}$ , the average of family variation of the  
13 half diallel (0.55‰ standard deviation within a range of 0.0‰ to 1.0‰ standard  
14 deviations) was similar to the variation found for the 186 trees of the full-sib family  
15 (0.63‰ standard deviation). Similarly, for MRW, the average of standard deviations  
16 within half-diallel families was 0.94 mm, close to the 0.99 mm standard deviation  
17 found for the full-sib family. Using Equations 2 and 3, the measured  $\delta^{13}\text{C}$  values  
18 transformed into a range of WUE of 67  $\mu\text{mol CO}_2 / \text{mol H}_2\text{O}$  to 100  $\mu\text{mol} / \text{mol}$  for  
19 the half-diallel and a range of 65  $\mu\text{mol} / \text{mol}$  to 95  $\mu\text{mol} / \text{mol}$  for the full-sib. This  
20 represents for the half diallel and the full-sib a variation from one to one-and-a half  
21 times the WUE.

22

### 23 Heritabilities and quantitative trait dissection analysis

24 The heritabilities for mean ring width and  $\delta^{13}\text{C}$  were found to be significant, similar  
25 between the two traits and of rather moderate value. Therefore selective crossings  
26 can improve growth and WUE. The heritabilities for ring width are comparable to  
27 values found in the literature for maritime pine or other conifers. Danjon (1994)  
28 found for maritime pine trees from the same provenance as used in the present study  
29 narrow sense heritabilities for diameter growth ranging from 0 to 0.45 for different  
30 experimental set-ups (40 to 100 half or full-sib families). Blada (1999) found for a  
31 *Pinus cembra* L. 10x10 full-diallel narrow sense heritabilities for diameter from 0.23  
32 to 0.32 and broad sense heritabilities from 0.50 to 0.59.

33 For  $\delta^{13}\text{C}$  there are not yet any publications known to the authors that estimated  
34 heritability for maritime pine, and there are only a few publications of estimates of  
35 heritability for  $\delta^{13}\text{C}$  for other species. Narrow sense heritability estimates by Johnsen  
36 *et al.* 1999 for *Picea mariana* are lower for diameter growth (0.14) than for  $\delta^{13}\text{C}$   
37 (0.54). For non-woody species, heritabilities for  $\delta^{13}\text{C}$  can be high (broad sense  
38 heritabilities for *Lens culinaris* Medikus 0.73, Matus *et al.* 1995 and for *Agropyron*  
39 *desertorum* (Fischer ex Link) Schultes 0.90, Asay *et al.* 1998), however it was shown  
40 that water stress could reduce the heritability of  $\delta^{13}\text{C}$  (Ehdaie & Waines 1994,  
41 Johnson *et al.* 1990). An explanation for the moderate heritabilities found in the  
42 present study could therefore be the integrative properties of  $\delta^{13}\text{C}$  measured on  
43 cellulose of several rings, together with the possibility of frequent water stress. The  
44 present study was located in the south-west of France, where summer drought is

1common (Nguyen-Queyrens *et al.* 1998). The half diallel was created from the  
2descendants of trees selected for growth vigour. This might have restricted the  
3genetic base compared to natural populations and hence lowered the detectable  
4heritability of growth.

5Existing QTLs for maritime pine were localized for traits related to growth (Plomion,  
6Durel & O'Malley 1996, Gerber, Lascoux & Kremer 1997). We were able to provide  
7here the first example of QTL observations for  $\delta^{13}\text{C}$  in a forest tree species. The four  
8significant QTLs found for  $\delta^{13}\text{C}$  explained nearly one-third of the phenotypic  
9variation observed for this trait. Several experiments (Prioul *et al.* 1997) have shown  
10that even for complex traits, such as growth or carbon isotope discrimination, the  
11expected number of major loci is quite small, a small number of genetic factors is  
12predominantly determining a quantitative trait. No co-localisations of QTLs for  $\delta^{13}\text{C}$   
13and QTLs for MRW were observed, suggesting no common genetic control for these  
14two traits. However, underestimation of number of QTL is inherent to the  
15methodology of QTL detection.

16

### 17Relationships between $\delta^{13}\text{C}$ and MRW

18The phenotypic correlations between  $\delta^{13}\text{C}$  and growth (ring width) found for the half  
19diallel and full-sib experimental designs are significant with moderate coefficients of  
20correlation (Figure 2a, Figure 3, Table 3) and the estimated regressions are similar in  
21slope and intercept. Among trees in the same environmental conditions, this suggests  
22that an increased growth relates to a higher WUE. Depending on a plants'  
23physiology, a difference in WUE could be predominantly determined by stomatal  
24conductance and/or by assimilation rate. The Farquhar model of carbon isotope  
25discrimination (Farquhar, Ehleringer & Hubick 1989) predicts that an increasing  
26photosynthetic capacity will decrease  $\Delta$ . Positive as well as negative correlations  
27have been found between photosynthesis and growth (Johnsen *et al.* 1995), however  
28when assuming a positive correlation between photosynthetic capacity and growth, a  
29positive correlation between  $\delta^{13}\text{C}$  and growth could suggest a predominantly  
30assimilation rate based control of  $\delta^{13}\text{C}$ . This is in agreement with results for black  
31spruce: differences among families were found to be mainly determined by  
32differences in photosynthesis (Johnsen *et al.* 1995), whereby differences in  
33photosynthesis were rather the result of non-stomatal limitations than of stomatal  
34limitation (Major *et al.* 1996). If  $\delta^{13}\text{C}$  would be controlled by stomatal conductance,  
35the Farquhar model predicts a negative correlation between  $\delta^{13}\text{C}$  and growth.  
36Therefore, the positive correlation between  $\delta^{13}\text{C}$  and growth suggests that the  
37variation of WUE among the measured trees is rather controlled by assimilation than  
38by stomatal conductance. This was the case for the half diallel as well as for the full-  
39sib family.

40The calculated genetic and environmental correlations between  $\delta^{13}\text{C}$  and MRW  
41indicate that the phenotypic correlation is mainly based on environmental influence.  
42This result is in agreement with the lack of colocalisations between QTLs for  $\delta^{13}\text{C}$   
43and for MRW. The bloc effect was included in the estimation of the genetic  
44parameters, hence the environmental correlation is probably due to micro-  
45environmental influences on each individual tree. As the model also accounts for any

1 type of genetic effect, including intra-family genetic variation, the observed large  
2 environmental variation therefore suggests for the two measured traits a high  
3 sensitivity to micro-environmental conditions. This also suggests for growth and  
4 water use efficiency a high non-genetic plasticity to adjust to environmental  
5 conditions. The strong environmental correlation that was found for the two traits is  
6 therefore probably due to a substantial environmental influence of less negative  $\delta^{13}\text{C}$   
7 with increased growth and *vice versa*.

8 The non-significant genetic correlation in the presented study is in opposition to the  
9 strong genetic correlation between  $\delta^{13}\text{C}$  and tree growth (height and diameter) found  
10 for black spruce (Johnsen *et al.* 1999). Several factors might have contributed to this  
11 discrepancy. 1) Johnsen *et al.* (1999) suggested assimilation rate as common control  
12 for  $\delta^{13}\text{C}$  and growth. However, correlations between assimilation rate and growth  
13 found in the literature range from negative over non significant to positive  
14 relationships (Johnsen *et al.* 1995). Therefore, even if  $\delta^{13}\text{C}$  is determined by  
15 assimilation rate, if growth is not determined by assimilation rate, then there might  
16 be no correlation between  $\delta^{13}\text{C}$  and growth. 2) Furthermore, genetic control was  
17 rather moderate for both traits, which might have lowered the significance of a  
18 genetic correlation. 3) It has also to be taken into account that in the present study  
19 carbon isotope discrimination measured on cellulose of main stem wood was  
20 compared to the average diameter growth of four years, whereas Johnsen *et al.*  
21 (1999) compared  $\delta^{13}\text{C}$  of needle material with height or trunk diameter. These  
22 complex traits might include the action of a number of slightly different genes and a  
23 common genetic control might exist for the combination of needle  $\delta^{13}\text{C}$  to height or  
24 diameter and not for the combination of trunk cellulose to mean annual growth of the  
25 same growth period.

26 The lack of a genetic correlation between  $\delta^{13}\text{C}$  and MRW found in the half-diallel is  
27 in agreement with the lack of co-localizations between QTLs for  $\delta^{13}\text{C}$  and for MRW  
28 found in the full-sib experimental design. Albeit the lack of a genetic correlation,  
29 both traits were found to be heritable and significant QTLs were detected. This opens  
30 new perspectives for the investigation of the genetic determinism of water use  
31 efficiency and the identification of groups of genes involved in drought responses.

32

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35

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7

## 1 Tables

2 Table 1 Means, standard deviations (SD), coefficients of variation (SD / mean),  
 3 ranges (maximum – minimum) and probability (p-value in %; \* significant at 5%  
 4 level; ns not significant) of block and family effects (Equation 4) of the two  
 5 experimental designs for  $\delta^{13}\text{C}$  (in [‰]) and mean ring width (MRW; in [mm])

|                       |              | N   | Mean   | SD   | Coef. of Var. | Range | Block effect | Family effect |
|-----------------------|--------------|-----|--------|------|---------------|-------|--------------|---------------|
| $\delta^{13}\text{C}$ | Half diallel | 564 | -26.21 | 0.61 | 0.02          | 3.64  | 2.8*         | 2.3*          |
|                       | Full-sib     | 186 | -26.48 | 0.63 | 0.02          | 3.23  | 0.0*         | ---           |
| MRW                   | Half diallel | 564 | 2.88   | 1.10 | 0.38          | 6.77  | ns           | 0.2*          |
|                       | Full-sib     | 186 | 2.11   | 0.99 | 0.47          | 5.15  | 0.9*         | ---           |

6

7 Table 2 Half diallel: Heritabilities for  $\delta^{13}\text{C}$  and mean ring width (MRW) with  
 8 standard deviations and 95% confidence interval in parentheses. As there were no  
 9 dominance effects detected and therefore additivity is 100%, the narrow sense (ns)  
 10 and broad sense (bs) heritabilities are equal.

|                           | $h^2_{ns} = h^2_{bs}$          | %additivity |
|---------------------------|--------------------------------|-------------|
| $\delta^{13}\text{C}$ [‰] | $0.17 \pm 0.06$ (0.06 – 0.29)* | 100%        |
| MRW [mm]                  | $0.19 \pm 0.06$ (0.06 – 0.31)* | 100%        |

11\* significant at the 5% level

12

13 Table 3 Half diallel: Correlations between mean ring width and  $\delta^{13}\text{C}$ ; r is the  
 14 correlation coefficient on the family level, estimated by OPEP software with  
 15 standard deviation and 95% confidence interval in parentheses; r' is the correlation  
 16 coefficient estimated by linear regression analysis using the calculated individual tree  
 17 breeding values which are shown in Figure 2.

|                           | r                                | r'      |
|---------------------------|----------------------------------|---------|
| Phenotypic correlation    | $0.45 \pm 0.057$ (0.33 – 0.56)*  | 0.46*   |
| Genetic correlation       | $0.27 \pm 0.21$ (0.07 – 0.15) NS | 0.02 NS |
| Environmental correlation | $0.52 \pm 0.16$ (0.20 – 0.83)*   | 0.79*   |

18\* significant at the 5% level; NS : not significant

Table 4 Full sib: Results of the composite interval mapping analysis for  $\delta^{13}\text{C}$  and 2mean ring width (MRW) using MultiQTL software. The p-value associated with the 3LODs were calculated using 1000 permutations of the data, standard deviation of 4position (SD) was calculated using the bootstrap method (1000 permutations); in 5case of a significant QTL-pair for a chromosome, Lod and p-value are given for the 6tests of (I) two QTLs *versus* no QTLs and (II) two QTLs *versus* one QTL (difference 7of Lod for two and for one QTL).

|                       | map    | Chr <sup>a</sup> | N <sup>b</sup> | Position $\pm$ SD <sup>c</sup>           | Lod                 | P-value <sup>d</sup>      | Dir. <sup>e</sup> | R <sup>2</sup> | R <sup>2</sup> <sub>total</sub> |
|-----------------------|--------|------------------|----------------|--|---------------------|---------------------------|-------------------|----------------|---------------------------------|
| $\delta^{13}\text{C}$ | male   | 3a               | 149            | 4.3 $\pm$ 13.2                           | 1.78                | 0.021*                    | +                 | 0.047          | 0.268                           |
|                       | male   | 6                | 164            | 102.7 $\pm$ 21.6                         | 4.40                | 0.001***                  | +                 | 0.124          |                                 |
|                       | male   | 8                | 85             | 0.0 $\pm$ 15.7                           | 1.85                | 0.021*                    | -                 | 0.050          |                                 |
|                       | male   | 9                | 183            | 104.6 $\pm$ 28.5                         | 1.90                | 0.033*                    | -                 | 0.047          |                                 |
|                       | female | 2                | 84             | 209.1 $\pm$ 56.4                         | 2.30                | 0.019*                    | +                 | 0.065          | 0.246                           |
|                       | female | 5                | 164            | 99.9 $\pm$ 22.0                          | 1.98                | 0.0033***                 | -                 | 0.062          |                                 |
|                       | female | 12               | 153            | 1: 0.0 $\pm$ 36.7<br>2: 135.4 $\pm$ 27.1 | I: 4.24<br>II: 1.88 | I: 0.002***<br>II: 0.036* | -<br>+            | 0.119          |                                 |
| MRW                   | male   | 2b               | 180<br>153     | 1: 47.2 $\pm$ 15.9<br>2: 51.8 $\pm$ 11.1 | I: 2.49<br>II: 1.57 | I: 0.048*<br>II: 0.027*   | -<br>+            | 0.181          | 0.429                           |
|                       | male   | 5                | 145            | 56.8 $\pm$ 33.4                          | 2.41                | 0.022*                    | -                 | 0.065          |                                 |
|                       | male   | 6                | 174<br>168     | 1: 7.5 $\pm$ 31.0<br>2: 133.7 $\pm$ 22.0 | I: 4.24<br>II: 2.68 | I: 0.001***<br>II: 0.002* | -<br>+            | 0.124          |                                 |
|                       | male   | 11               | 165            | 11.6 $\pm$ 30.9                          | 1.73                | 0.040*                    | +                 | 0.059          |                                 |

8<sup>a</sup> chromosome ID

9<sup>b</sup> number of full-sibs with available data for QTL detection

10<sup>c</sup> Lod score peak position (from the top) of the chromosome (cM)

11<sup>d</sup> Probability for the null hypothesis of no QTL at the chromosome level

12 \*\*\* : probability corresponding to a 5% genome wise type I error (significant QTL)

13 \* : probability corresponding to a 5% chromosome type I error (suggestive QTL)

14<sup>e</sup> direction of influence of presence of the allele for each QTL

**1Figure legends**

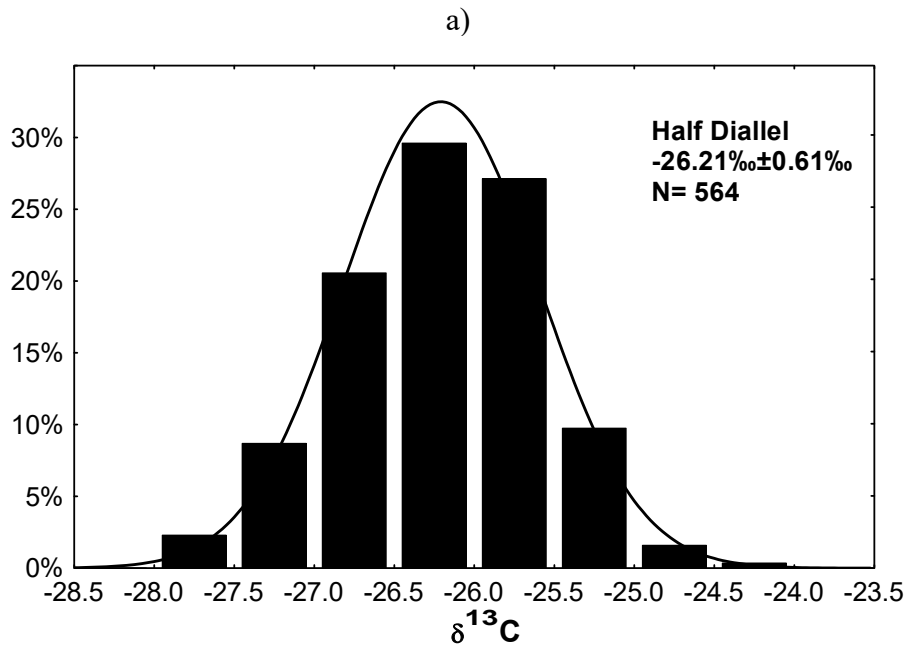
2**Figure 1** Distribution of cellulose  $\delta^{13}\text{C}$  (raw data; not corrected for block effect) for  
3a) the half diallel experiment and b) the full-sib experiment; parameters for the  
4normal distributions as in Table 1

5**Figure 2** Linear correlations between mean ring width (MRW) and  $\delta^{13}\text{C}$  for the half  
6diallel experiment using data corrected with the individual tree breeding values  
7(OPEP software): a) phenotypic, b) genetic (additive effects) and c) environmental  
8correlations; all data are adjusted for block effect, data for genetic and environmental  
9correlations are centred and standardized by the mean.

10**Figure 3** Phenotypic linear correlation between mean ring width (MRW) and  $\delta^{13}\text{C}$   
11for the full-sib experiment; data adjusted for block effect

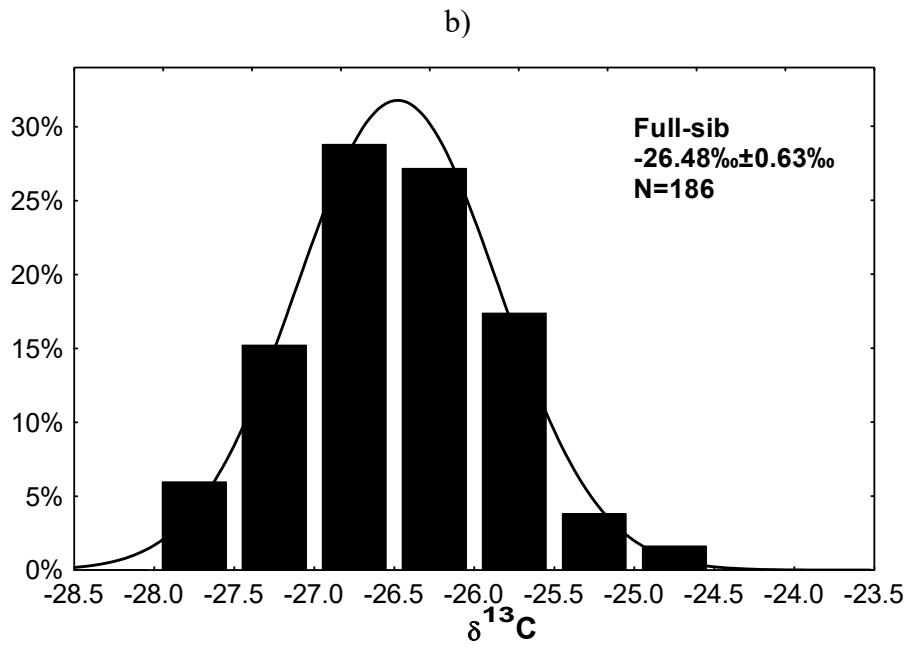
1 **Figure 1 a b**

2



3

4

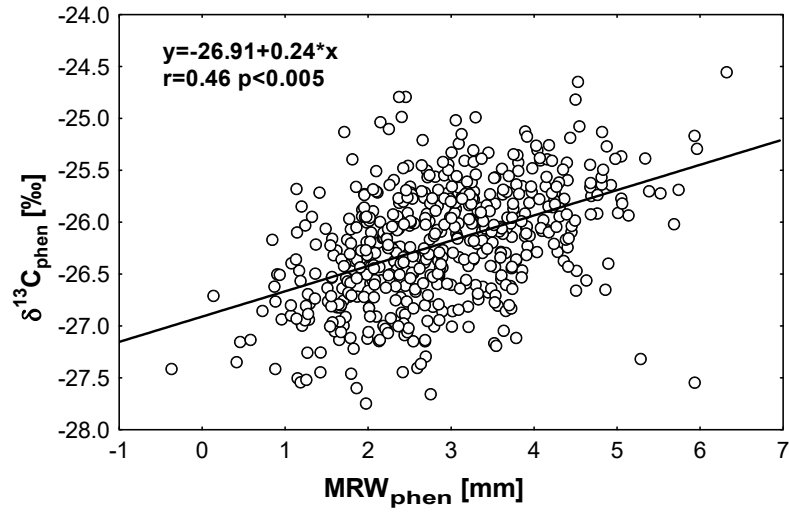


5

1 **Figure 2 a b c**

2

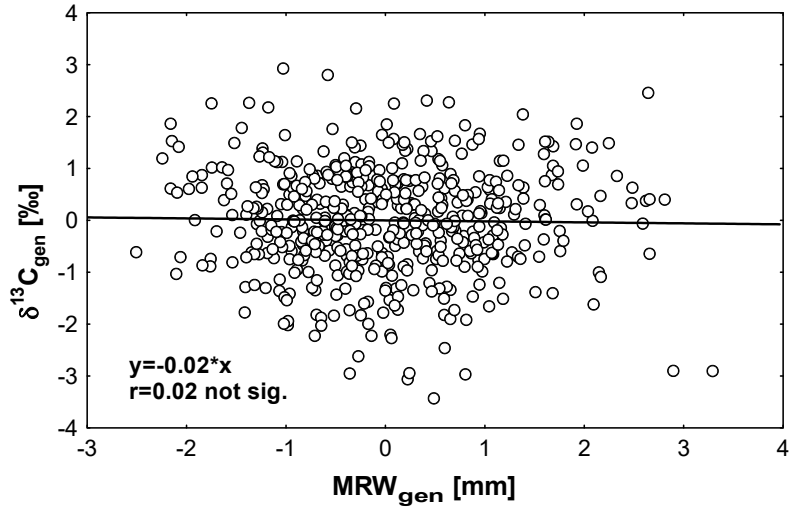
a) phenotypic correlation



3

4

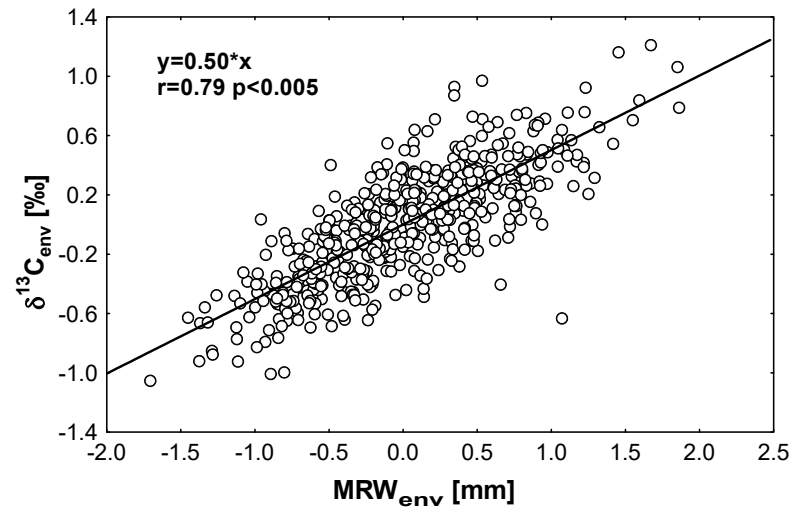
b) genetic correlation



5

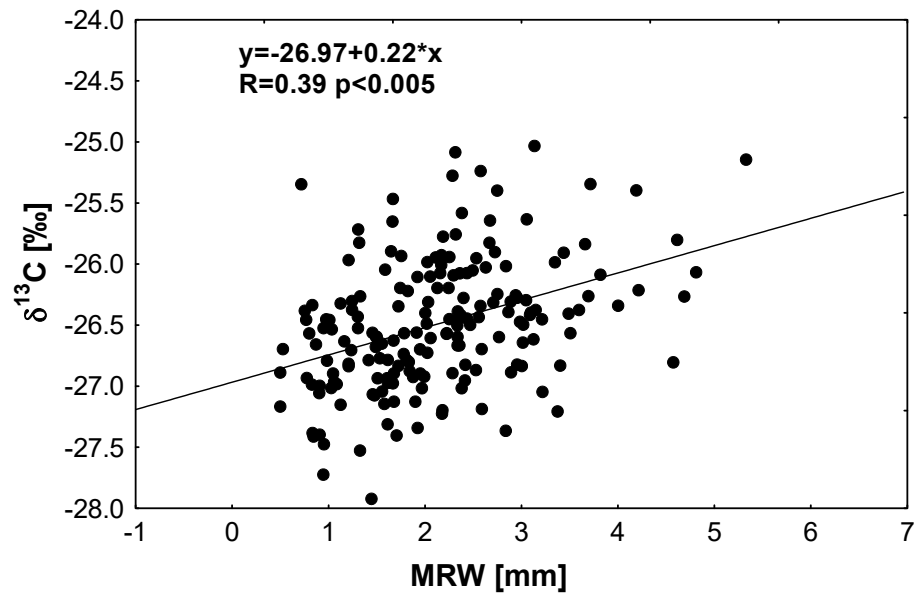
6

c) environmental correlation



7

2

1 **Figure 3**2  
3  
4