

# Juvenile Genetic Parameters and Genotypic Stability of *Pinus pinaster* Ait. Open-Pollinated Families under Different Water and Nutrient Regimes

Rafael Zas and Josefa Fernández-López

**Abstract:** The purpose of this study was to estimate the impact of genotype  $\times$  nutrient and genotype  $\times$  water availability interactions in the maritime pine (*Pinus pinaster* Ait.) breeding program in Galicia (northwestern Spain). Twenty-nine open-pollinated families, randomly selected in a seed orchard, were cultivated for 23 weeks in a greenhouse in a split-plot design under controlled conditions. Two water regimes (high and low water supply) combined with two nutrient regimes (high and low nutrient supply) were applied by subirrigation. Both the irrigation and the fertilization treatments had a strong effect in all the assessed traits. Heritabilities for growth and biomass traits were moderate to high (0.2–0.5) in individual treatments, especially in those treatments that led to intermediate growth. However, when analyzing all treatments together, the impact of the family  $\times$  treatment interactions led to an important reduction (0.1–0.2) of the heritability estimates. The results indicated that the genotype  $\times$  water and genotype  $\times$  nutrient interactions may be important and could not be ignored in the Galician maritime pine breeding program. However, the study of the family ecovalence values indicated that interactions are mainly due to some interactive families (7 to 31% of families). Culling several of the female parents with high ecovalence values would result in a stable breeding population and would be enough to overcome the interaction handicap in the breeding program. FOR. SCI. 51(2):165–174.

**Key Words:** Early selection, half-sibs, genotype  $\times$  environment interaction, drought, fertilization.

**M**ARITIME PINE (*Pinus pinaster* Ait.) is the most important forest tree species in Galicia (northwestern Spain). It occupies nearly 400,000 ha (27% of the Galician wooded area) with an annual volume increment estimated around  $3 \cdot 10^6 \text{ m}^3 \cdot \text{year}^{-1}$  (Xunta de Galicia 2001). The climate in Galicia ranges from Oceanic in the coast with high annual precipitation and lack of summer drought, to a clear Mediterranean influence in the southeast interior, where the summer drought becomes important and annual precipitation is much lower (Martínez et al. 1999) (Figure 1). Maritime pine is present and is planted under both climates.

Genetic improvement of *P. pinaster* in the Atlantic area of Galicia was initiated in 1985 and has included phenotypic mass selection in wild stands and use of this material for seed production in clonal seed orchards (Vega et al. 1993). No breeding program has been started to date for the interior area of Galicia. Because of important summer drought differences between the Atlantic and the Mediterranean areas of Galicia (see Figure 1), the study of the stability of the improved genetic material across different water regimes and drought intensities is essential to determine whether it is possible to use this coastal material in the interior area as well. At the provenance level, a significant differentiation was found between *P. pinaster* seed sources for growth and

physiological adaptation to water stress (Nguyen and Lamant 1989, Fernández et al. 1999, 2000). However, there is limited evidence of an important adaptation to water availability within populations in *P. pinaster* (Harfouche 2003) and other conifers (Joly et al. 1989, Sonesson and Eriksson 2000, Sonesson et al. 2002), suggesting that, with suitable selection, it may be possible to use improved material over a wide range of moisture regimes.

Within each climate region, maritime pine is planted over a wide range of soil fertilities, from fertile abandoned agricultural lands to infertile forest soils. It is possible that genetic families may react differently to soil fertility conditions (Mullin 1985, Li et al. 1991b, Jonsson et al. 1992, 1997, Eriksson et al. 1993, Karlsson et al. 2002, Mari et al. 2003b). If the difference between genetic entries in this respect is pronounced, separate breeding programs for different fertility types might be needed (Jonsson et al. 1992). The knowledge of the relevance of the genotype  $\times$  nutrient interaction within the Galician *P. pinaster* breeding population would be necessary before any recommendation of use can be made.

The aim of the experiment presented here was to analyze the significance and practical importance of the genotype  $\times$  water and genotype  $\times$  nutrient availability interactions in a random subset of the *P. pinaster* breeding population in

Rafael Zas, Dpto. Producción Forestal, Centro de Investigaciones Forestales e Ambientais de Lourizán, Apdo. 127, 36080 Pontevedra, Spain—Phone: +34-986-805067; Fax: +34-986-856420; rzas.cifal@siam-cma.org. J. Fernández-López—Phone: +34-986-805002; ffina.cifal@siam-cma.org.

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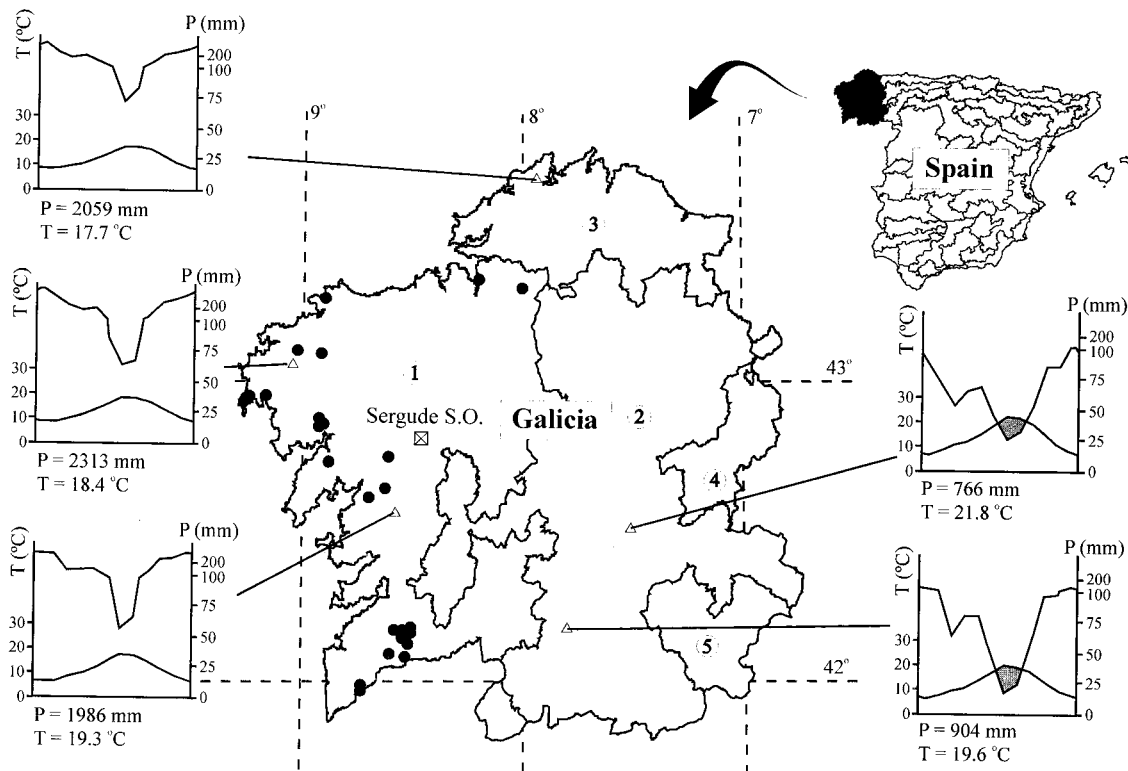


Figure 1. Location of the *Pinus pinaster* plus trees (black dots) and the clonal seed orchard (S.O.) over the bioclimatic regions of Galicia. The encircled numbers denoted the number of the Identification and Utilization Regions (RIUs) of forest reproductive material (García et al. 2001). The ombrothermic Gaussen diagrams of five representative climatic stations are also included. P = annual precipitation, T = mean summer (July–Aug.) temperature. The shadow area of the climatic diagrams denotes the summer drought intensity. Note the precipitation and summer drought differences between the coast and the southeast interior area.

Galicia. Specifically, the objectives were (1) to study how the different growth environments affected genetic parameter estimates, and (2) to study the genotypic stability for growth and biomass traits obtained in seedlings grown in the greenhouse under different water and fertilization treatments.

## Materials and Methods

### Materials

The study material consisted of open-pollinated *P. pinaster* families obtained from 29 randomly selected clones included in the Sergude clonal seed orchard (Figure 1). This seed orchard provides seed of high genetic quality for reforestation in the Atlantic region of Galicia (northwest Spain). One hundred and sixteen plus trees selected within this region for superior growth, stem form, and branch characteristics are represented in the seed orchard following a randomized complete block design with 10 blocks and one ramet of each clone per block. There are no other *P. pinaster* stands close to the seed orchard, so pollen contamination is supposed to be minimal (Merlo and Fernández-López 2004). The seeds were collected from two to three ramets per clone and the mean seed weight for each family was recorded. Genetic parameter estimates of this material when planted in the field have been reported by Zas et al.

(2004a), whereas the impact of the genotype  $\times$  environment interaction has been reported by Zas et al. (2004b).

### Treatments and Experimental Design

The seeds were individually sown in a greenhouse on  $7 \times 7 \times 8$  cm pots filled with perlite and covered with a thin (2–4 mm) layer of sand. A bushy net was placed at the bottom of the pots to impede the roots exit from the pots. During the 7-week germination period, a misting nozzle was used for the daily spraying of the material with water. Temperature was regulated to 22°C during the daytime and 15°C at night. During this pretreatment period, the seedlings were sprayed each 3–5 days with a pretreatment fertilizer solution containing  $100 \text{ mg N kg}^{-1}$  with macronutrient proportions of 100N:20P:70K:7Ca:9Mg:9S and micronutrients. Fungicides were flushed weekly to prevent diseases.

Seven weeks after sowing, the successfully germinated pots were placed following the final experimental design. Seedlings were grown for 23 weeks under two fertilization treatments crossed with two irrigation regimes. The fertilization and irrigation treatments were applied together by subirrigation. The two different irrigation regimes were the well-watered treatment (water high, w:H), with watering to field capacity every day, and the drought treatment (water low, w:L), with a periodical drought (see below). The two

different nutrient solutions were the high-nutrient treatment (nutrient high, n:H) and the limited treatment (nutrient low, n:L). The n:H treatment consisted of a complete nutrient solution (macroelement proportions by weight 100N:20P:70K:7Ca:9Mg:9S and microelements) containing  $100 \text{ mg N kg}^{-1}$ . The low-nutrient availability treatment (n:L) was a  $10\times$  dilution of the n:H solution.

To regulate the drought treatments, a procedure similar to that described by Sonesson and Eriksson (2000) and Sonesson et al. (2002) was used. Ten randomly selected seedlings per treatment-block combination were selected for water weight control. These control seedlings were weighed every day or every second day to estimate the water content in relation to the previously estimated field capacity. The drought treatment seedlings were watered to field capacity when the water weight reached between 20 and 40% of the field capacity weight (adapted from Sonesson and Eriksson 2000), increasing the drought intensity with time (Figure 2a). The control seedlings were weighed again after irrigation to determine the actual field capacity weight. The water weight at field capacity decreased with time (Figure 2b) because of a degeneration of the water-retention capacity of the substrate. After 18 weeks, a technical breakdown in the fog-press system led to an unplanned watering of some blocks of the w:L-n:L treatments. To homogenize the watering regime among the four blocks, it was decided to irrigate all the w:L-n:L seedlings, although the mean water content was around 50% (Figure 2a).

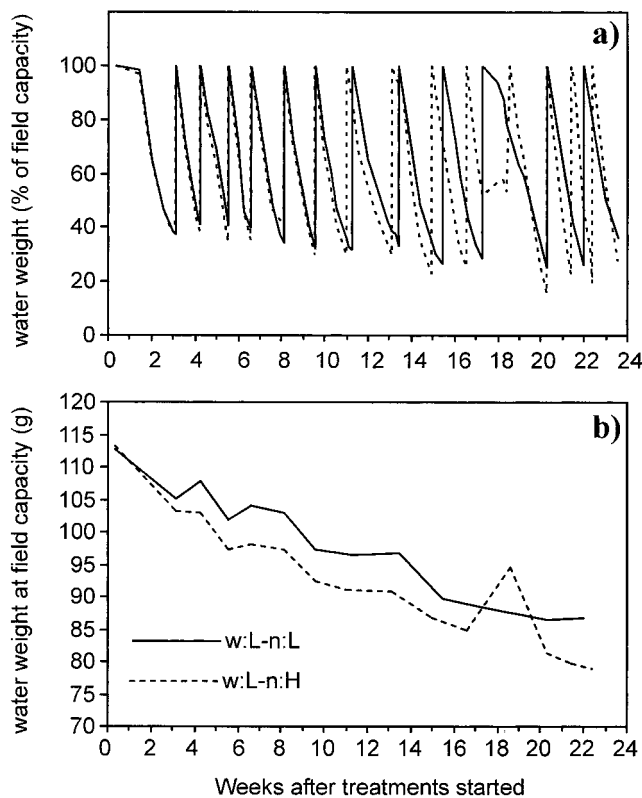


Figure 2. Evolution of the mean water weight (a) and diminution of the water weight at field capacity (b) for the two drought treatments (w:L-n:L and w:L-n:H) during the experiment.

A split-plot design with treatments acting as the whole factor and the maritime pine families as the split factor was replicated in four blocks (Figure 3). The four treatments (w:H-n:H, w:H-n:L, w:L-n:H, w:L-n:L) were randomly assigned to each of the four whole plots in which each block was divided. Within each whole plot, each family was represented by five seedlings arranged in five sub-blocks. Due to poor germination of a few families, the total number of seedlings per family and treatment varied between 15 and 20, with an average of 19.

Day:night temperature was raised to 22:15°C. A minimal 12-hour photoperiod was attained by adding artificial light (Gavita GAN 400 AL lamps) to the period of daylight.

## Assessments

Seedlings were harvested 23 weeks after treatments commenced and total height ( $H$ ) and the root collar diameter ( $D$ ) were measured. The seedlings were clipped at the root collar to separate shoots and roots. The roots were carefully washed out of the substrate with water. Dry weights ( $80^\circ\text{C}$ , 24 hours) of shoots (SDW) and roots (RDW) were measured separately. Several derived traits were calculated: the total dry weight ( $\text{TDW} = \text{SDW} + \text{RDW}$ ), the volume index ( $V = H \cdot D^2$ ), the root/shoot ratio ( $\text{RSR} = \text{RDW}/\text{SDW}$ ), and the height/diameter ratio (HDR). Finally, the total number of branches (BRN) was counted.

## Statistical Analyses

Two types of analyses were carried out, a separate analysis for each treatment and a joint analysis of the four treatments together.

The linear model for the single treatment analyses was

$$Y_{ilm} = \mu + F_i + B_l + \text{sb}_m(B_l) + \text{sw}_i + \epsilon_{ilm},$$

where  $Y_{ilm}$  is the value of single observation,  $\mu$  is the overall mean,  $F_i$  is the random effect of family  $i$ ,  $B_l$  is the fixed effect of block  $l$ ,  $\text{sb}_m(B_l)$  is the fixed effect of the sub-block  $m$  within the block  $l$ ,  $\text{sw}_i$  is the fixed effect of the mean seed weight covariate of family  $i$ , and  $\epsilon_{ilm}$  is the random error term.

The linear model for the joint treatment analyses was:

$$\begin{aligned} Y_{ijklm} = & \mu + F_i + N_j + W_k \\ & + \text{NW}_{jk} + B_l + \text{sb}_m(\text{NWB}_{jkl}) + \text{FN}_{ij} \\ & + \text{FW}_{ik} + \text{FNW}_{ijk} + \text{sw}_i + \epsilon_{ijklm}, \end{aligned}$$

where  $Y_{ijklm}$  is the single observation;  $N_j$ ,  $W_k$ , and  $\text{NW}_{jk}$  are the fixed effects of the  $j$ th fertilization, the  $k$ th irrigation treatments, and their interaction, respectively;  $\text{sb}_m(\text{NWB}_{jkl})$  is the fixed effect of the sub-block  $m$  within the fertilization-irrigation-block combination; and  $\text{FN}_{ij}$ ,  $\text{FW}_{ik}$ , and  $\text{FNW}_{ijk}$  are the random interactions between the family  $i$  and the  $j$ th fertilization treatment, the  $k$ th irrigation regime, and the interaction of both treatments, respectively.

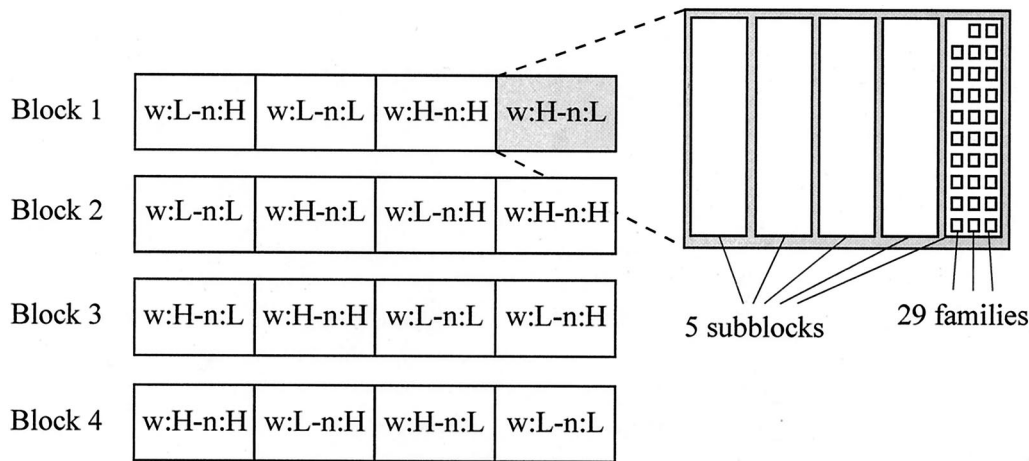


Figure 3. Greenhouse experimental layout. Treatments: w:L-n:L = low water and nutrient supply, w:H-n:L = high water and low nutrient supply, w:L-n:H = low water and high nutrient supply, and w:H-n:H = high water and nutrient supply.

Variance components, standard errors, and significance levels were estimated using the restricted maximum likelihood (REML) method of the MIXED procedure in the SAS software (SAS Institute 1999). The data were previously transformed to equal additive genetic variance (Sonesson and Eriksson 2000, Sonesson et al. 2002) to reduce the scale effects resulting from the strong differences among treatments. For each trait and treatment, the original assessed values were multiplied by the scaling factor  $k = \sigma_{An}/\sigma_A$ , where  $\sigma_{An}$  and  $\sigma_A$  are the additive genetic standard deviation for the w:H-n:H treatment and the given treatment, respectively.

Treatment effects were tested using untransformed data and adding the block  $\times$  nutrient, block  $\times$  water, and block  $\times$  nutrient  $\times$  water interactions as random effects in the model. The mean square errors of these interactions were used as error terms for the nutrient, water, and nutrient  $\times$  water effects, respectively. Because of lack of convergence of the MIXED procedure, the TEST statement (type III sum of squares) of the GLM procedure (SAS Institute 1999) was used to analyze the nutrient and water treatment effects with the appropriate error terms. Significant differences among combined water and nutrient levels were tested using the Tukey's test (SAS Institute 1999).

### Genetic Parameters

Individual heritabilities were calculated as

$$h^2 = \frac{\sigma_A^2}{\sigma_f^2 + \sigma_{fn}^2 + \sigma_{fw}^2 + \sigma_{fnw}^2 + \sigma_e^2},$$

where  $\sigma_A^2$  is the additive genetic variance estimated as  $4 \cdot \sigma_f^2$ ,  $\sigma_f^2$  is the family variance component,  $\sigma_{fn}^2$ ,  $\sigma_{fw}^2$ , and  $\sigma_{fnw}^2$  are the variance components for the three interaction terms, and  $\sigma_e^2$  is the error variance component. The three interaction components were removed in the individual analyses. Approximate standard errors of individual heritabilities were calculated as described in Wright (1976).

Additive genetic coefficient of variations were calculated as

$$CV_A = 100 \frac{\sigma_A}{\bar{x}},$$

where  $\bar{x}$  is the trait mean value. Type B genetic correlation between the same trait in different treatments was estimated from the joint analyses of each pair of treatments (Burdon 1977),

$$r_B = \frac{\sigma_f^2}{\sigma_f^2 + \sigma_{ft}^2},$$

where  $\sigma_f^2$  and  $\sigma_{ft}^2$  are the family and the family  $\times$  treatment variances from the paired-treatment analyses.

To test whether any significant interaction may be a result of only a few families, the ecovalence values (Wricke 1962) for each family and trait were calculated,

$$W_i = \sum_j (Y_{ij} - Y_i - Y_j + Y_{..})^2,$$

where  $W_i$  is the ecovalence value of the  $i$ th family,  $Y_{..}$  is the overall mean of trait  $Y$ ,  $Y_i$  and  $Y_j$  are the mean values for family  $i$  ( $i = 1-29$ ) and for treatment  $j$  ( $j = 1-4$ ), respectively, and  $Y_{ij}$  is the mean value of family  $i$  in the treatment  $j$ . This stability parameter measures the contribution of each family to the interaction and is equivalent for ranking purposes to the Shukla (1972) stability variance (Hill et al. 1998). We have used the Shukla (1972) method to test whether this contribution was statistically significant.

Genetic correlations between different traits across all treatments were calculated from estimates of additive genetic variances and covariances (Falconer 1989) using the option multivariate analysis of variance (MANOVA) in the GLM procedure of SAS (SAS Institute 1999),

$$r_G = \frac{COV_A(x, y)}{\sigma_x \cdot \sigma_y},$$

where  $COV_A(x, y)$  is the family covariance component between traits  $x$  and  $y$ , and  $\sigma_x$  and  $\sigma_y$  are the square root of family variance components for the two traits. Spearman rank correlation was used for analyzing the relation between the ecovalence values of different traits (SAS Institute 1999).

## Results

### General Effects of Treatments

Both the irrigation and the fertilization treatments had a significant and strong effect on the mean values of all traits, except for the number of branches (BRN) and the root:shoot ratio (RSR), for which the water effect was nonsignificant, and for the root dry weight (RDW), for which there were not significant differences between the nutrient treatments (Table 1, Figure 4). The highest values for growth and biomass traits were recorded in the treatment that combined high fertilization and irrigation (w:H-n:H), whereas the lowest values were achieved in the drought and poor fertilized treatment (w:L-n:L). Differences between these two extreme treatments were as high as 2, 5, 7, and 12 times higher for diameter ( $D$ ), height ( $H$ ), total dry weight (TDW), and shoot dry weight (SDW), respectively. The intermediate treatments (w:L-n:H and w:H-n:L) gave similar intermediate results for all the growth and biomass traits except for RDW, which was much higher in the w:H-n:L treatment. The root-shoot ratio (RSR) was higher in the two low fertilized treatments. The fertilization caused a significant increase in the total number of branches (BRN).

### Single Treatment Analyses

All traits showed significant ( $P < 0.05$ ) family variance in almost all treatments with the exception of RSR, for which the family effect was significant only in the w:L-n:L treatment. Heritability estimates were moderate to high for almost all traits and showed low standard errors (see Table 2). For growth traits ( $H$ ,  $D$ ,  $V$ ) the heritabilities were generally higher under low levels of nutrients, whereas, for dry masses, the highest heritabilities were observed in the

w:L-n:H treatment. Heritabilities for RSR were very low in all treatments except in the w:L-n:L treatment. The highest heritabilities for BRN were found in the well-fertilized treatments.

For growth and dry mass traits, the additive genetic coefficients of variation ( $CV_A$ ) followed a similar trend to the heritabilities, but were relatively more stable. The  $CV_A$  values were higher for  $V$  and RDW than for the other growth and biomass traits. Extremely high values of the  $CV_A$  were observed for BRN, ranging from 42 to 71%.

### Joint Analyses Over All Treatments

Significant interactions were observed in many traits, especially family  $\times$  nutrient interaction, which was significant for  $D$ , BRN, RDW, and SDW (Table 1). The interaction variance estimates were relatively high. The ratio  $(\sigma_{fw}^2 + \sigma_{fn}^2 + \sigma_{fwn}^2)/\sigma_f^2$ , which can be interpreted as the relative importance of the three interactions together in relation to the family variance, was high in all cases, especially for the BRN (Table 1). Height showed no significant interactions and the lowest  $\sigma_{INT}^2/\sigma_f^2$  ratio. When analyzing all treatments together, the significant interaction variances led to a reduction of the overall family effects and the heritability estimates. Nonsignificant family variance ( $P > 0.05$ ) occurred for  $D$ , BRN, RDW, and RSR. Heritabilities were relatively lower than in the single-treatment analyses, ranging from 0.10 to 0.18 for growth and biomass traits. For BRN, heritabilities decreased from 0.34 to 0.76 in the individual analyses to 0.14 in the joint analysis. Volume, height, and total dry weight showed the highest heritabilities, whereas heritability for the RSR was very low.

### Genetic Correlation Among Treatments

Type B genetic correlations among the different treatments (Table 3) were generally high for growth and dry mass traits. Relatively low correlations were observed between the two nutrient regimes, indicating a higher incidence of the genotype  $\times$  nutrient interaction. The w:L-n:H treatment had, on average, the lowest genetic correlation,

**Table 1. Results from the joint treatments analysis. Significance levels for fixed effects, variance components (percentage) for the random effects, individual heritability estimates  $\pm$  standard error, and the ratio of interactions to family variance component  $(\sigma_{INT}^2/\sigma_f^2 = (\sigma_{fw}^2 + \sigma_{fn}^2 + \sigma_{fwn}^2)/\sigma_f^2)$  for the joint linear model of four treatments.**

Trait	Fixed effects			Variance components						
	$N$	$W$	$N \times W$	$\sigma_f^2$	$\sigma_{fw}^2$	$\sigma_{fn}^2$	$\sigma_{fwn}^2$	$\sigma_e^2$	$H_i^2$	$\sigma_{INT}^2/\sigma_f^2$
$H$	***	***	***	4.0**	0.0	0.5	1.8	93.7	0.16 $\pm$ 0.05	57.4
$D$	***	**	*	2.7	0.7	2.3*	0.0	94.3	0.11 $\pm$ 0.04	112.0
$V$	***	**	**	4.4*	0.0	0.9	2.4*	92.2	0.18 $\pm$ 0.06	76.8
HDR	*	***	n.s.	2.1*	0.0	1.2	0.7	96.0	0.09 $\pm$ 0.04	88.8
BRN	***	n.s.	**	3.6	0.9	4.6*	2.1	88.8	0.14 $\pm$ 0.05	214.4
RDW	n.s.	**	n.s.	2.5	2.0*	2.0*	0.0	93.5	0.10 $\pm$ 0.04	163.8
SDW	***	**	**	3.4*	1.7	2.0*	0.0	92.9	0.13 $\pm$ 0.05	109.1
TDW	***	**	*	3.7*	2.2*	1.7	0.0	92.4	0.15 $\pm$ 0.05	108.3
RSR	***	n.s.	n.s.	0.7	0.0	1.3	0.0	98.0	0.03 $\pm$ 0.02	170.8

Significance levels: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; n.s., not significant.

$N$  and  $W$  are the nutrient and water regime effects.  $\sigma_f^2$ ,  $\sigma_{fw}^2$ ,  $\sigma_{fn}^2$ ,  $\sigma_{fwn}^2$ , and  $\sigma_e^2$  are the variance components for family, family by irrigation, family by nutrient regime, family by irrigation by nutrient regime, and error, respectively.

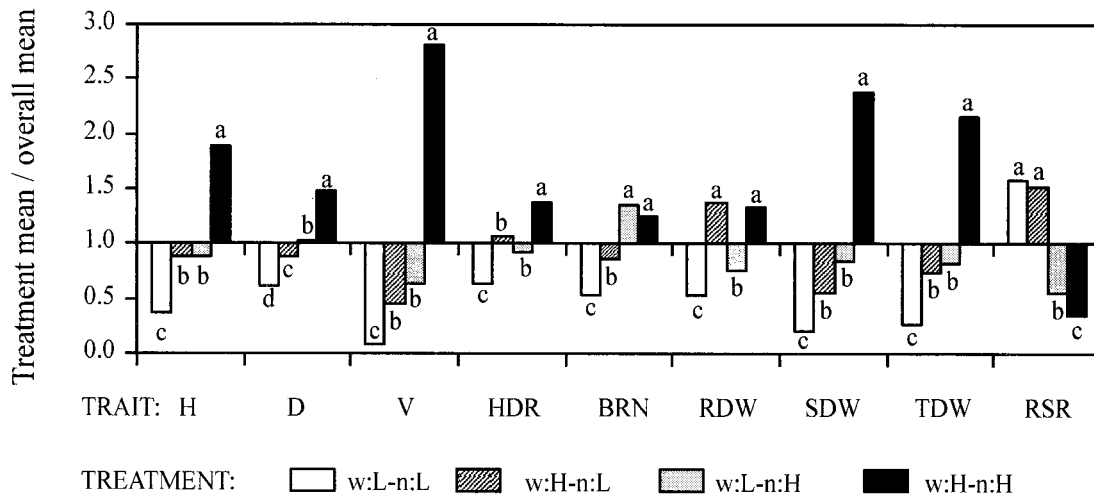
**Table 2. Results from the separate treatment analyses: individual heritability estimates  $\pm$  standard error, additive genetic coefficients of variation, and family significance levels for individual treatments**

Trait	w:L-n:L		w:L-n:H		w:H-n:L		w:H-n:H	
	$h_i^2$	CV <sub>A</sub> (%)	$h_i^2$	CV <sub>A</sub> (%)	$h_i^2$	CV <sub>A</sub> (%)	$h_i^2$	CV <sub>A</sub> (%)
H	0.26 $\pm$ 0.12	15.2*	0.16 $\pm$ 0.09	16.0	0.31 $\pm$ 0.12	17.1*	0.24 $\pm$ 0.11	11.4*
D	0.31 $\pm$ 0.12	14.0*	0.15 $\pm$ 0.09	8.5	0.37 $\pm$ 0.14	14.0**	0.21 $\pm$ 0.10	12.1*
V	0.40 $\pm$ 0.14	37.5**	0.22 $\pm$ 0.11	28.2*	0.48 $\pm$ 0.16	38.9**	0.22 $\pm$ 0.11	28.1*
HDR	0.25 $\pm$ 0.11	14.0*	0.06 $\pm$ 0.07	9.9	0.19 $\pm$ 0.10	14.6**	0.33 $\pm$ 0.13	17.1*
BRN	0.37 $\pm$ 0.14	71.3**	0.54 $\pm$ 0.17	45.5**	0.34 $\pm$ 0.13	41.6**	0.76 $\pm$ 0.21	53.8**
RDW	0.23 $\pm$ 0.11	35.3*	0.51 $\pm$ 0.16	41.0**	0.34 $\pm$ 0.13	27.6**	0.15 $\pm$ 0.09	32.1
SDW	0.22 $\pm$ 0.11	26.6*	0.32 $\pm$ 0.13	25.1*	0.20 $\pm$ 0.10	16.6*	0.24 $\pm$ 0.11	30.6*
TDW	0.21 $\pm$ 0.10	28.1*	0.37 $\pm$ 0.14	26.7**	0.38 $\pm$ 0.14	21.3**	0.24 $\pm$ 0.11	30.5*
RSR	0.34 $\pm$ 0.13	25.0*	0.11 $\pm$ 0.08	37.3	0.00 $\pm$ 0.05	0.0	0.04 $\pm$ 0.06	11.8

Significance levels: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

Treatments: w:L-n:L, low water and nutrient supply; w:H-n:L, high water and low nutrient supply; w:L-n:H, low water and high nutrient supply; w:H-n:H, high water and nutrient supply.

Traits: H, height; D, diameter at root collar; V, volume ( $H \cdot D^2$ ); HDR, height/diameter ratio ( $H/D$ ); BRN, number of branches; RDW, root dry weight; SDW, shoot dry weight; TDW, total dry weight (SDW + RDW); RSR, root-shoot ratio (RDW/SDW).



**Figure 4. Effect of treatments on the measured and derived traits. Relative values to overall trait mean are presented. Different letters within the same trait denote significant (Tukey's test,  $P < 0.05$ ) differences among treatments. Treatments: w:L-n:L = low water and nutrient supply, w:H-n:L = high water and low nutrient supply, w:L-n:H = low water and high nutrient supply, and w:H-n:H = high water and nutrient supply.**

except for diameter. For dry masses and volume, there were very low correlations between the two intermediate treatments (w:L-n:H and w:H-n:L). Despite the strong differences in the mean values between the two extreme treatments (w:L-n:L and w:H-n:H) (Figure 4), the genetic correlations between these treatments were quite high, except for diameter. Genetic correlations for RSR were variable and should be used cautiously because of the low family effect in three of the four individual treatments. Low genetic correlations were also observed for the BRN between the two nutrient regimes.

### Genetic Correlation among Different Traits

High genetic correlations were observed among the dry masses and among the growth traits across all four treatments (Table 4). Diameter and volume were strongly correlated with the three biomass traits. The HDR and the RDW were negatively correlated.

### Genotypic Stability

Figure 5 shows the percentage of families that significantly contributed (Shukla 1972) to the family  $\times$  treatment interaction. For all traits, most of the families showed low ecovalence values and hence contributed little to the interactions. Only 2–9 of the 29 studied families (7–31% of families) contributed significantly ( $P < 0.05$ ) to the interaction. Volume and BRN showed the highest proportion of interactive families.

The ecovalence values for the dry masses, V and D, were significantly correlated with each other (Table 4), indicating that the families that significantly contributed to the interactions were, in some extent, the same for all these traits. On the contrary, the ecovalence values for the BRN were not correlated to the ecovalence of the other traits.

If those families that significantly contributed to the interaction were dropped from the analyses, the three interactions (family  $\times$  nutrient, family  $\times$  water, and the triple

**Table 3. Type B genetic correlation among the same traits in different treatments**

Trait	Treatment	w:L-n:H	w:H-n:L	w:H-n:H
<i>H</i>	w:L-n:L	0.32	0.78	0.83
	w:L-n:H		0.50	0.59
	w:H-n:L			0.93
<i>D</i>	w:L-n:L	0.79	0.90	0.36
	w:L-n:H		0.56	0.87
	w:H-n:L			0.39
<i>V</i>	w:L-n:L	0.49	0.80	0.71
	w:L-n:H		0.37	0.56
	w:H-n:L			0.65
HDR	w:L-n:L	0.33	0.88	0.00
	w:L-n:H		1.00	0.74
	w:H-n:L			0.47
BRN	w:L-n:L	0.31	0.57	0.00
	w:L-n:H		0.63	0.90
	w:H-n:L			0.49
RDW	w:L-n:L	0.59	0.78	0.60
	w:L-n:H		0.15	0.61
	w:H-n:L			0.84
SDW	w:L-n:L	0.82	1.00	0.61
	w:L-n:H		0.28	0.72
	w:H-n:L			0.92
TDW	w:L-n:L	0.85	0.93	0.67
	w:L-n:H		0.24	0.66
	w:H-n:L			0.92
RSR	w:L-n:L	0.20	0.99	0.43
	w:L-n:H		0.99	1.00
	w:H-n:L			1.00

See note in Table 2 for trait and treatment codifications.

interaction) became nonsignificant for all traits, and the ratio  $\sigma_{INT}^2/\sigma_f^2$  was zero for the dry masses and diameter, and 15.7% and 24.6% for *V* and BRN, respectively.

## Discussion

### Treatment Effects

The big differences among treatment means (Figure 4) reflected the very different growth conditions among treatments. Besides the significant effect on growth and dry masses of both the irrigation and fertilization treatments, the most interesting effect is the effect on growth partitioning. The low-nutrient treatment (n:L) resulted in smaller seedling size but relatively more biomass allocated to roots than under the high-nutrient conditions (n:H). Nutrient stress generally favors biomass allocation to roots at the expense of needles or both needles and stem (Li et al. 1991a, b, Jonsson et al. 1997, Mari et al. 2003a). Furthermore, as Li et al. (1991a) pointed out, the growth differences among genotypes in different environments might be explained by differences in their patterns of dry matter distribution under different nutrient conditions.

As occurred with the nutrient stress, the water stress usually favored the biomass allocation to roots at the expense of shoots (Joly et al. 1989, Tan et al. 1995, Harfouche 2003). However, in the present study, the RSR values in the w:L-n:L and w:L-n:H treatments were only slightly higher than in the w:H-n:L and w:H-n:H treatments, respectively (Figure 4). The determination of the root dry weight was

somewhat problematic. Pots were too small and may have constrained the normal root development. Although a bushy net was placed at the bottom of the pots to impede the roots exit from the pots, some roots of the plants under the well-watered treatments extended outside the pots, generating somewhat heterogeneous conditions among plants of the same treatments. Roots outside the pots did not die because of the high frequency of the water applications (each day) and the high relative humidity generated by the fog system to reduce the temperature. Watering every second day (Sonesson and Eriksson 2000, Sonesson et al. 2002) may be enough for the high-watering treatment and may reduce the outside root growth. Washing the perlite out of the roots was another problem because some thin roots might have been lost. Indirect measurement of the RDW by weighing dried pots with the substrate before sowing and after harvesting the shoots (Jonsson et al. 1997) is another alternative. Owing to the problems of disentangling the roots, Joly et al. (1989) satisfactorily extracted roots by detonating patterned charges of high-velocity dynamite placed below the soil surface.

### Within-Treatment Genetic Variation

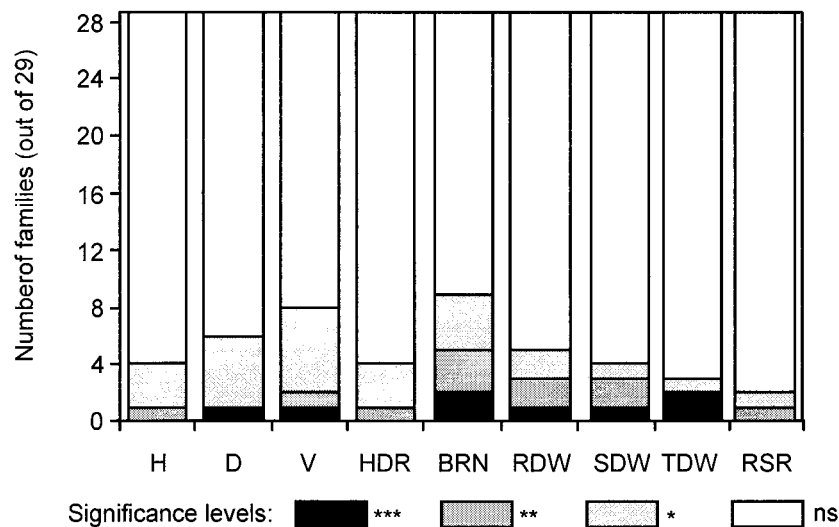
Differences in genetic variation and heritability estimates for growth and biomass traits among different water and nutrient regimes have been reported, but results are somewhat confusing. Some authors found higher genetic variation and heritability estimates when growth conditions were favorable, i.e., under well-watered or full-nutrient regimes (Sonesson et al. 2002, Mari et al. 2003a, Sonesson and Eriksson 2003), whereas others found out the opposite (Li et al. 1991b, Mari et al. 2002). In the present study, genetic variation and heritabilities for growth and dry masses were relatively higher in the two intermediate treatments (Table 2). The genetic variation for growth was higher under the w:H-n:L, whereas for biomass traits, higher variation was found in the w:L-n:H treatment. Similar to our results, Jonsson et al. (1992), studying *Pinus sylvestris* L. full-sib families cultivated at five nutrient levels, found the highest GCA variance components under the nutrient treatments that led to intermediate growth. In a *P. pinaster* families experiment, Harfouche (2003) found larger family variance for biomass traits under water stress. This author also found that the effect of drought stress on root growth and biomass was stronger in less vigorous families than in vigorous ones. It seems to be a general fact, in maritime pine, that vigorous and nonvigorous genotypes develop different strategies for allocation of biomass. The vigorous genotypes invest in shoot growth whereas the less vigorous ones give priority to root development. These differential behaviors are accentuated in a situation of moderate drought.

### Family × Treatment Interaction and Genotypic Stability

As observed in other species (Li et al. 1992, Jansson et al. 1998, Sonesson et al. 2002), *H*, *D*, and *V* exhibited higher

**Table 4.** Genetic correlation between different traits (above diagonal) and Spearman rank correlation between the ecovalence values for different traits (below diagonal) across all four treatments

Trait	<i>H</i>	<i>D</i>	<i>V</i>	HDR	BRN	SDW	RDW	TDW	RSR
<i>H</i>		0.48	0.85	0.57	0.02	0.80	0.18	0.61	—
<i>D</i>	-0.01		1.12	-0.48	-0.19	0.90	0.71	0.82	—
<i>V</i>	0.23	0.82		-0.26	-0.23	1.60	1.02	1.39	—
HDR	0.77	0.14	0.21		0.04	0.11	-0.56	-0.07	—
BRN	0.17	0.04	0.11	0.22		-0.03	0.39	0.08	—
SDW	-0.05	0.78	0.74	0.11	0.18		1.21	1.02	—
RDW	0.19	0.32	0.48	0.36	0.08	0.50		1.11	—
TDW	0.08	0.66	0.66	0.34	0.11	0.87	0.74		—
RSR	0.21	-0.20	0.05	0.05	-0.12	-0.12	0.05	-0.20	



**Figure 5.** Percentage of families that significantly contributed to the family × treatment interaction.

heritabilities (Table 2) relative to older tree growth traits in conventional genetic tests (Zas et al. 2004a). The homogeneity of the growth conditions under controlled environments may explain these differences. However, when all treatments were analyzed together (Table 1), the heritabilities were much lower because of the impact of the family × treatment interaction, especially in the case of dry masses.

Both the results of the joint analyses (Table 1) and the genetic correlations among treatments (Table 3) indicated that the family × nutrient interaction was relatively more important compared to the family × water interaction. It must be noted that, although most of the interaction variances were not significant, the sum of the three interaction variances was very high in relation to the family variance ( $\sigma_{INT}^2/\sigma_f^2$ ) (Table 1), indicating a high relative importance of the interactions. Shelbourne (1972) suggested, as an approximation, that the effects of the family × site interaction are likely to be serious on gains from selection and testing when the interaction component reaches 50% or more of the family variance. With values above 100% there is a need for delineation of different breeding zones with separate breeding in each zone (Eriksson and Ekberg 2001). In fact, if the nutrient and water treatments are considered as a unique factor with four levels, the family × treatment interaction became significant in all cases, except for RSR and HDR

(data not presented). Hence, the genotype × nutrient and genotype × water interactions should not be ignored in the Galician maritime pine breeding program. However, the results of the stability analyses indicated that only a relatively few families (7–31%) significantly contributed to the interactions (Figure 5), and thus, culling the few parents with high ecovalence values would result in a stable breeding population and would be enough to overcome the interaction handicap in the breeding program. Similar results were observed in the field, where the family × site interaction was quantitatively important for growth and quality traits, but was a consequence of a few interactive families that may be particularly sensitive to environmental variation (Zas et al. 2004b). The interaction became negligible when these interactive families were dropped from the analyses.

Significant family × nutrient interaction was observed in *Pinus sylvestris* (Jonsson et al. 1997), *Pinus taeda* L. (Crawford et al. 1991), *Picea abies* (L.) Karst. (Mari et al. 2002, 2003b), and *Picea mariana* (Mill.) B.S.P. (Mullin 1985). In the case of drought, a significant differentiation among maritime pine provenances was found for growth and physiological adaptations to water stress (Fernández et al. 1999). However, at the family level, little evidence of family × water interaction was observed, indicating low adaptation to water availability within populations (Harfouche 2003). The



family  $\times$  water interaction was also of little importance in progeny tests under controlled conditions in *Pinus sylvestris* (Sonesson and Eriksson 2000), *Picea abies* (Sonesson et al. 2002), and *Pseudotsuga menziesii* Mirb. Franco (Joly et al. 1989).

The family  $\times$  treatment interaction was very important for the BRN, especially the family  $\times$  nutrient interaction. Despite the high genetic variation and heritabilities for the BRN in the separate treatment analyses (Table 2), the family variance was not significant in the joint analysis (Table 1). Early selection for the branch number is, thus, very sensitive to the nutrient regime applied. Jonsson et al. (1992) found low family  $\times$  nutrient interaction for quality traits in *Pinus sylvestris* grown under five nutrient levels in a growth chamber.

### **Relationships Among Traits**

Among the traits studied, dry weights showed higher heritabilities and additive genetic coefficients of variation than growth traits (Tables 1 and 2). However, height and volume, which were strongly correlated with dry weights (Table 4), also showed high genetic variation and heritability. Because height and volume were easier to assess, the determination of dry weights for early selection purposes is not recommended. Furthermore, the stability across the water and nutrient treatments was very similar among the different growth and biomass traits (Table 4). However, the analysis of the juvenile–mature (JM) correlations should be considered before discarding any early trait.

### **Validity for Field Performance and Implications for Breeding**

One of the most interesting aspects of this study is the analysis of the genotype  $\times$  environment interaction and the genotypic stability, restricting the environmental variation to a limited and known set of parameters, such as the nutrient and water availability. Early testing may be not only useful for culling half-sib families before establishing more costly genetic tests (Wu 1998), but also for analyzing the genotype  $\times$  environment interaction and discarding the most interactive families. However, this study was performed on very young trees in very artificial environments, and the results may not be comparable to mature field conditions. The efficiency of early selection in forest trees depends on the strength of the statistical and genetic parameters of juvenile traits, and on the significance of the JM correlation (Wu 1998). Thus, the analysis of the greenhouse–field correlations should be carried out before establishing any early selection strategy.

A good deal of effort is being devoted toward determining the optimal early environment and the optimal early traits to be measured that improve the JM correlations. Some authors support the hypothesis that JM correlations would be improved by mimicking the limiting factors in the field (e.g., Tan et al. 1995, Sonesson et al. 2002), whereas others found better JM correlations when the juvenile

growth conditions are favorable (e.g., Li et al. 1992, Eriksson et al. 1993). Recently, Harfouche (2003) concluded that some biomass traits of one-month-old *P. pinaster* seedlings were able to discriminate between vigorous and nonvigorous families at age 17 in the field. The results of Harfouche (2003) did not support the hypothesis that mimicking field-limiting factors improves JM correlations in maritime pine.

As indicated by Sonesson and Eriksson (2000), because we have found significant interaction effects in both the present experiment and the field (Zas et al. 2004b), we would probably not get good JM correlations for all four treatments and the field test sites.

In conclusion, despite the need of the JM correlation analysis, the results of the present article indicated a strong family  $\times$  nutrient and also family  $\times$  water interaction for most of the studied traits that should not be ignored in the maritime pine breeding program in Galicia (northwest Spain). Nevertheless, the interaction is mainly due to a low proportion of interactive families (Figure 5) and became negligible when these interactive families were dropped from the analyses. Hence, this study does not suggest that there is a compelling reason to identify different breeding zones with respect to the nutrient and water availability for maritime pine in the coastal area of Galicia. Furthermore, although it is probably difficult to claim that the dry water treatment mimics the droughty interior climate, the results do not restrain the possibility to use the most stable coastal genetic material in the droughty interior area of Galicia, where there is no breeding program to date.

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