

Transferring Atlantic maritime pine improved material to a region with marked Mediterranean influence in inland NW Spain: a likelihood-based approach on spatially adjusted field data

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Abstract The inland region of Galicia (NW Spain) marks the boundary between the Atlantic climate of the coastal area and the typical Mediterranean climate of central Spain. Compared to the Atlantic coast, climate in this area has a pronounced summer drought, lower annual precipitation, and higher annual thermal oscillation. Despite the high productivity and ecological importance of maritime pine in inland Galicia, local forest reproductive material (FRM) of high genetic quality is not available for this area. Seed sources originating elsewhere and of unknown adaptation to this area are commonly used for reforestation. With the aim of finding new sources of FRM for this region and exploiting the genetic gains of existing breeding programmes, we analysed the performance in field conditions of improved families of the Coastal Galicia (CG) and Western Australia (WA) breeding programmes. Growth, stem characteristics and branch habit were evaluated in five progeny trials established following a coastal-to-inland gradient. Likelihood-based analyses were used to estimate genetic correlations between environments and to test

statistically for causes and patterns of genotype \times environment interaction. Because of the strong non-random spatial structures and heterogeneity of residual variances, the analyses were carried out using heterogeneous residual variance mixed models on spatially adjusted data. The results indicated that there is not sufficient evidence to subdivide Galicia into the two current deployment areas. Interaction patterns do not reveal significant differences between zones, and crossover interactions for height growth are present both between and within areas. On the inland sites, the Atlantic improved materials clearly outperformed unimproved seedlots tested in adjacent provenance trials, suggesting the feasibility of using both the CG and WA breeding materials as sources of FRM for reforestation in inland Galicia. Of the two, the WA material showed excellent results for all traits. The inclusion of this material into the Galician maritime pine breeding population should be strongly considered.

Keywords *Pinus pinaster* · Progeny trial · Iterative spatial analysis · Genotype \times environment interaction · Spatial autocorrelation · REML estimation · Genetic correlation · Variance-covariance matrix · Heterogeneous variance models

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Introduction

Maritime pine (*Pinus pinaster* Ait.) is one of the most important forestry species in Galicia (NW Spain) where nearly 400,000 ha of pure and mixed stands are present (27% of the wooded area in Galicia), producing up to $2 \cdot 10^6$ m³ of round-wood per year (Sanz et al. 2006). Galicia is located in the extreme North West of Spain and is typically characterized by an Atlantic climate, although

two main climatic regions can be distinguished. The coastal region (up to 600 m above sea level) has high annual rainfall (1,500–2,500 mm) and short annual thermal oscillation (10–11°C), corresponding with regions of identification and utilization (RIUs) of forest reproductive material number one and three (Fig. 1; García et al. 2001). The inland region of Galicia (corresponding with RIU number two, Fig. 1) is a boundary area where the Atlantic climate of the coastal area meets with the typical Mediterranean climate of Central Spain. Climate in this area has a pronounced summer drought (summer precipitation of 60–100 mm), lower annual precipitation (600–1,200 mm), and higher annual thermal oscillation (13–14°C) owing to the greater continental influence. Maritime pine is abundant and has large productive relevance in both areas. Previous studies have identified important differences in terms of growth patterns and genetic structure between both regions (Alía et al. 1996; Álvarez-Gonzalez et al. 2005).

A genetic improvement programme of *P. pinaster* in the coastal area of Galicia was initiated in 1985. It has included phenotypic mass selection in wild stands and use of this material for seed production in clonal seed orchards. The breeding objectives were focused mainly on improving growth traits, stem form, and branch quality (Zas and Merlo 2008). By contrast, in the inland area, although the annual rate of plantation is also high, local reproductive

material of good quality is not available. Foresters need to introduce material from other Spanish provenances, often with questionable adaptation to this area, and typically showing low growth rates and being of poor quality stem form. Looking for alternative material that performs well in the inland region has become a primary objective in order to provide immediate seed sources suitable for planting in this area.

The coastal region breeding programme has been progressing in recent years, based on the results of a series of progeny trials established in the coastal area. Using the information obtained in these trials, a new depurate clonal seed orchard has been recently installed, and the selection of a second breeding generation has been started (Zas and Merlo 2008). The important gains and good results achieved in the Coastal area encourage us to explore the possibility of using the coastal breeding material in the inland region, combining the breeding efforts and minimizing the overall costs. Galicia would constitute a single breeding area, and new material for the inland region would be available in a short space of time. However, the coastal seed orchard's families have only been tested in the coastal zone, and no information is available regarding their performance within the inland region. Previous results revealed a large genotype \times environment ($G \times E$) interaction in the breeding population both in field conditions within the coastal area (Zas et al. 2004; Martins et al. 2009), and in a drought experiment under controlled conditions (Zas and Fernández-López 2005). However, $G \times E$ interaction was found to be originated by a small number of families especially sensitive to environmental variation, whereas most of the families tested showed a stable behaviour. Selecting for stability has been thus suggested as an option for obtaining material suitable for both climatic regions (Zas and Fernández-López 2005).

A breeding programme in Western Australia (WA) started in the 1950s using a plus tree selection from within the Leiria (Portugal) provenance and has now completed several breeding selection cycles, achieving important genetic gains in growth, stem form and branching habit (Butcher 2007; Butcher and Hopkins 1993; Perry and Hopkins 1967). The Leiria provenance is a clear Atlantic provenance, whereas the current areas reforested with *P. pinaster* in WA are medium-to-low rainfall (400–600 mm) ex-agricultural sites with a marked Mediterranean influence (Butcher 2007). Drought tolerance is therefore an important trait for selection in this breeding programme (Butcher 2007; Butcher and Chandler 2007). The WA example therefore supports the feasibility of achieving successful results through recurrent selection upon Atlantic material for use in more Mediterranean conditions with strong summer droughts (Butcher 2007; Butcher and Chandler 2007).

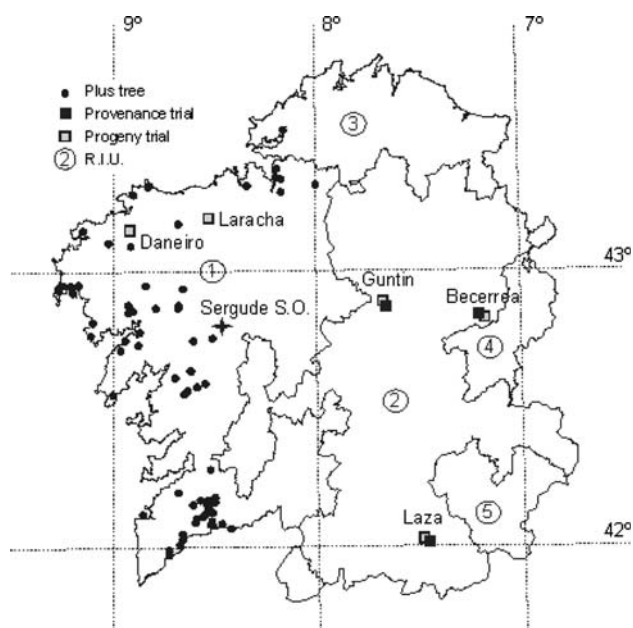


Fig. 1 Location of the *P. pinaster* plus trees (black dots), the five progeny trials (grey squares), the three provenance trials (black squares), and the clonal seed orchard (black star) from which CG families were obtained. Encircled numbers indicate the number of the 'identification and utilization regions' (RIUs) of forest reproductive material (García et al. 2001)

The present study aims to assess the field performance of improved materials with different levels of selection [Coastal Galician (CG) and WA breeding programmes] in the inland region of Galicia. We also analysed in detail the magnitude and relevance of the $G \times E$ interaction within and between the two current deployments areas (Coastal and Inland Galicia) in order to assess the suitability of this zonification. To this end, we evaluated the performance of 111 half-sib CG *P. pinaster* families and six WA families in five sites, two located in the coastal and three in the inland region of Galicia, three and 7 years after planting. The feasibility of the Atlantic breeding materials in the inland sites was assessed through comparisons with unimproved seed sources planted in adjacent provenance trials.

Materials and methods

Genetic material and test sites

The study material consists of half-sib families obtained from 111 selected plus trees represented in a first generation clonal seed orchard (Sergude, 42.82°N, 8.45°W) which provide high genetic quality seed for reforestation in the coastal area of Galicia (Fig. 1). All these plus trees were individually selected from natural stands or from plantations within the Atlantic region of Galicia, for their superior growth, stem form, and branch characteristics.

In addition, six improved families from the WA breeding programme were also included in all the trials (WA1–WA6). The six families were open pollinated in the second generation clonal seed orchard at Manjimup (34.24°S, 116.14°E, WA). Parents were selected for vigour, stem form, small branching and resistance to drought within family trials established in WA upon open or cross pollinations between the original plus trees selected in Leiría and plus trees selected within the WA land race. Seedlot WA6 was a mixture of different families and can be considered to be representative of the average performance for the Manjimup clonal seed orchard (T. Butcher, personal communication).

One-and-a-half-year-old containerized seedlings of the 111 half-sib families and of the WA material were planted in five sites in 2001. Site characteristics are presented in Table 1. Daneiro and Laracha sites were within the RIU number 1 which constitutes, a priori, the deployment area for the selected material of the coastal seed orchards (Fig. 1). These sites have a typical Atlantic climate characterized by high annual precipitation, low summer drought, and low temperature oscillation (Table 1). The other three sites, Becerreá, Guntín, and Laza, were located within the RIU number 2 which includes most of the inland area of Galicia and constitutes a transitional region towards the more continental and Mediterranean climate characteristics of the central areas of the Iberian Peninsula, with prolonged summer drought and wide thermal oscillation (Table 1). Becerreá is the coldest site, while Guntín and

Table 1 Location, main climatic features, and overall means for height at ages 3 and 7 of the *Pinus pinaster* progeny trials

	Coast		Inland		
	Daneiro	Laracha	Becerreá	Guntín	Laza
Longitude (W)	8°55'06"	8°31'47"	7°10'32"	7°40'42"	7°29'32"
Latitude (N)	43°9'35"	43°12'09"	42°50'16"	42°54'02"	42°02'24"
Altitude (m)	210	252	900	550	770
Aspect	SEE	SW	S	W	NE
Slope (%)	0	2	30	3	18
Annual precipitation (mm)	1,528	1,505	1,047	1,000	783
Summer precipitation (mm) ^a	144	140	132	99	76
Gausson index ^b	1	0	0	17	37
Annual mean temperature (°C)	13.2	12.4	10.4	11.6	11.1
Annual temperature oscillation (°C) ^c	10.1	10.8	13.1	13.3	13.7
No. of frost days ($T_{\min} < 0^{\circ}\text{C}$) per year	10	23	58	38	29
Absolute minimum temperature (°C)	−2.8	−4.3	−7.8	−7.1	−4.8
Overall height mean 3 years (cm) ^d	78.6 ± 24.0	133.9 ± 30.9	109.1 ± 23.0	106.1 ± 20.4	88.0 ± 22.2
Overall height mean 7 years (cm) ^d			295.1 ± 60.1	350.3 ± 77.1	300.1 ± 120.8

^a Rain fallen during the months of June, July, and August

^b Gausson = $\Sigma(2T-P)$ for each month where $2T > P$ [P : monthly precipitation (mm), T : monthly mean temperature (°C)]

^c Difference between the mean of daily maximum of the warmest month and the mean of daily minimum of the coldest month

^d Overall mean and standard deviation of height at ages 3 and 7 without spatial correction

Laza show low annual and summer precipitation, with Laza being the drier of the two.

All five plantations follow a randomized complete block design with 25 replications (except Daneiro, with 23 replications) of one tree-plots with 3×2 m spacing (except Laza, with 2.5×2 m spacing), which is the typical spacing for *P. pinaster* plantations in this region.

In the three inland sites, a provenance trial was simultaneously planted adjacent to the progeny trials. Six Spanish provenances (Bajo Tiétar, Sierra de Gredos, Montaña de Soria-Burgos, Serranía de Cuenca, Albarracín, and Sierra Segura-Alcaraz) of the Mediterranean area were tested in each site. These provenances were selected on the basis of their quality for timber production within their natural stands and of their performance in previous provenance trials (Alía et al. 2001; Molina 1965). All three trials follow a randomized complete block design with 10 replications and five tree-row-plots. Seedlings of these provenance trials were cultivated together with the progenies following the same nursery protocols. Both the progeny and the provenance trials were planted at the same time, with the same spacing and soil preparation, and were measured at the same dates. Data from these trials were used to predict the performance of unimproved seed sources and to compare the Atlantic materials (see below). A specific analysis of these provenance trials are presented in a companion paper (de la Mata and Zas 2009).

Assessments

All trees from each site were assessed for growth, stem form, and branch characteristics at ages 3 and 7, except those dead or badly suppressed. Growth traits included total height (H) measured with a pole in centimetres and diameter at breast height (D) measured with a calliper in millimetres (only assessed at age 7). Stem form and branch traits were assessed following Galera et al. (1997). Stem form of each tree was evaluated by a straightness score (STR: 1 = straight to 6 = very crooked) and a stem leaning score (LEN: 1 = vertical to 4 = severe lean). The number of whorls (WH) was also recorded as a measure of the branch abundance. The spatial position of each tree was determined using a total station (Pentax R-315).

The two coastal sites, Daneiro and Laracha, were significantly affected by pests and diseases, so were only assessed at age 3. Trees in Daneiro were attacked by the large pine weevil, *Hylobius abietis* L., which kills trees by girdling the stem of the young seedlings. In Laracha, the root rot fungus *Armillaria ostoyae* (Romagn.) Herink caused the death of up to 65% of the plants 3 years after planting (see Zas et al. 2007). In addition, the Becerreá provenance trial was affected by the construction of a forest access track in 2005, affecting several of the trial

trees. Data on provenance performance in this site are only available for the 3-year-old assessment.

Statistical analyses

Correcting the spatial dependence

We examined the spatial structure of the dependent variable in each site by constructing the empirical semivariogram for the residuals adjusted for family effects with the SAS VARIOGRAM procedure (SAS-Institute 1999). Those variables that were spatially dependent were corrected using the iterative spatial analysis (ISA) method (Zas 2006). Briefly, this method first fits a theoretical semivariogram model to the observed residual semivariogram using the SAS NLIN procedure (SAS-Institute 1999). Using the theoretical semivariogram model, the spatial variation of the dependent variable is then modelled by the kriging method using the KRIG2D procedure of SAS (SAS-Institute 1999). The original variable is then adjusted for its spatial autocorrelation, subtracting the kriging estimate in each position. Finally, the new corrected variable is reanalysed, and a new estimate of the family effects is obtained and used to generate new residuals. The process is repeated iteratively, until convergence of the BLUPs estimates of family effects. A detailed description of the method can be consulted in Zas (2006). The spatial adjustment was carried out for each site independently, including both the trees of the progeny and the adjacent provenance trials, and considering a single genetic effect (family or provenance) to obtain the original residuals. Once the dependent variable was spatially corrected, the progeny and provenance trials were analysed independently as shown in the following sections.

Likelihood-based analyses of the site by family interaction

Spatially adjusted data (for traits with non-random spatial structures), or original unadjusted data (for spatially independent traits), were analysed by fitting mixed models with site as a fixed factor, and block within site, family and family \times site interaction as random factors (Crossa et al. 2004; Yang 2002). The mixed models were fitted using the MIXED procedure of SAS (Littell et al. 2006), accommodating the SAS Programmes of Yang (2002) to our experimental design. Variance components were estimated using the REML method. The estimation of the family covariance structure (variances and covariance across sites) was achieved by including the SUBJECT and TYPE option in the RANDOM statement. Heterogeneity of residual variances across sites was implemented with the GROUP option of the REPEATED statement. Initial values (derived from single site analyses) were specified in the PARMs statement to facilitate convergence and speed calculations.

In order to explore and interpret the $G \times E$ interaction, different reduced models constraining different elements of the family covariance structure were fitted. Constraints to the family covariance structures were specified by choosing appropriate predefined covariance models for the TYPE option in the RANDOM statement, and/or by fixing specific covariance parameters to certain values using the HOLD option of the PARMs statement (Crossa et al. 2004; Fry 2004; Yang 2002). Out-of-bond family correlations (>1 , or <-1) were avoided by including the UPPERB and LOWERB option in the PARMs statement. A detailed list of the

different models and the corresponding hypothesis analysed is given in Table 2. Hypothesis testing regarding the constraints imposed on the family covariance structure was done by comparing the restricted log-likelihoods (RLL) of the constrained model and the unconstrained model (usually the full model with an unstructured family covariance structure, see later). Under the null hypothesis that the full covariance model is not different from the reduced covariance model, the log-likelihood ratio $LLR = -2(RLL_{reduced\ model} - RLL_{full\ model})$ is distributed approximately as χ^2 with degrees of freedom given by the

Table 2 Description of the full model and reduced models for testing different hypotheses on the relevance and interpretation of the genotype by environment interaction

Model and hypothesis tested	Constraints	CovStruc	Parameters to be estimated	# parms	
				5 sites	3 sites
H0 Full model. All causes of genotype \times environment interaction are allowed	None	UNR	$\sigma_{G1}^2, \sigma_{G2}^2, \sigma_{G3}^2, \sigma_{G4}^2, \sigma_{G5}^2$ $\rho_{12}, \rho_{13}, \rho_{14}, \rho_{15}, \rho_{23}, \rho_{24}, \rho_{25}, \rho_{34}, \rho_{35}, \rho_{45}$ $\sigma_{e1}^2, \sigma_{e2}^2, \sigma_{e3}^2, \sigma_{e4}^2, \sigma_{e5}^2$	20	9
H1 No family by environment interaction	$\sigma_{Gi}^2 = \sigma_{Gj}^2 = \sigma_G^2 \forall i, j; \sigma_{GxE}^2 = 0$	CS*	σ_G^2 $\sigma_{e1}^2, \sigma_{e2}^2, \sigma_{e3}^2, \sigma_{e4}^2, \sigma_{e5}^2$	6	4
H2 Homogeneity of family variance across sites	$\sigma_{Gi}^2 = \sigma_{Gj}^2 = \sigma_G^2 \forall i, j$	CS	$\sigma_G^2, \sigma_{GxE}^2$ $\sigma_{e1}^2, \sigma_{e2}^2, \sigma_{e3}^2, \sigma_{e4}^2, \sigma_{e5}^2$	7	5
H3 Perfect family correlation between all site pairs	$\rho_{Gij} = 1 \forall i \neq j$	CSH*	$\sigma_{G1}^2, \sigma_{G2}^2, \sigma_{G3}^2, \sigma_{G4}^2, \sigma_{G5}^2$ $\sigma_{e1}^2, \sigma_{e2}^2, \sigma_{e3}^2, \sigma_{e4}^2, \sigma_{e5}^2$	10	6
H4 Homogeneity of family covariance across all site pairs	$\rho_{Gij} = \rho_{Gi'j'} = \rho_G \forall i \neq j, i' \neq j'$	CSH	$\sigma_{G1}^2, \sigma_{G2}^2, \sigma_{G3}^2, \sigma_{G4}^2, \sigma_{G5}^2$ ρ $\sigma_{e1}^2, \sigma_{e2}^2, \sigma_{e3}^2, \sigma_{e4}^2, \sigma_{e5}^2$ $\sigma_{G1}^2, \sigma_{G2}^2, \sigma_{G3}^2, \sigma_{G4}^2, \sigma_{G5}^2$	11	7
H5 Perfect family correlation between sites within areas	$\rho_{Gij} = 1 \forall i, j \in same\ area$	UNR*	$\rho_{14}, \rho_{15}, \rho_{24}, \rho_{25}, \rho_{34}, \rho_{35}$ $\sigma_{e1}^2, \sigma_{e2}^2, \sigma_{e3}^2, \sigma_{e4}^2, \sigma_{e5}^2$	16	-

Under the full model, the matrices for the family (Σ_G) and error (Σ_e) covariance structures are as follow:

$$\Sigma_G = \begin{matrix} & \begin{matrix} \text{Inner sites} & \text{Coastal sites} \end{matrix} \\ \begin{matrix} \sigma_{G1}^2 & \rho_{12} & \rho_{13} & \rho_{14} & \rho_{15} \\ \rho_{12} & \sigma_{G2}^2 & \rho_{23} & \rho_{24} & \rho_{25} \\ \rho_{13} & \rho_{23} & \sigma_{G3}^2 & \rho_{34} & \rho_{35} \\ \rho_{14} & \rho_{24} & \rho_{34} & \sigma_{G4}^2 & \rho_{45} \\ \rho_{15} & \rho_{25} & \rho_{35} & \rho_{45} & \sigma_{G5}^2 \end{matrix} \end{matrix} \quad \Sigma_e = \begin{matrix} & \begin{matrix} \text{Inner sites} & \text{Coastal sites} \end{matrix} \\ \begin{matrix} \sigma_{e1}^2 & 0 & 0 & 0 & 0 \\ 0 & \sigma_{e2}^2 & 0 & 0 & 0 \\ 0 & 0 & \sigma_{e3}^2 & 0 & 0 \\ 0 & 0 & 0 & \sigma_{e4}^2 & 0 \\ 0 & 0 & 0 & 0 & \sigma_{e5}^2 \end{matrix} \end{matrix} \left. \vphantom{\Sigma_e} \right\} \begin{matrix} \text{Inner sites} \\ \text{Coastal sites} \end{matrix}$$

where σ_{Gi}^2 and σ_{ei}^2 are the family and residual variances in site i , and ρ_{ij} is the family correlation between sites i and j . The reduced models constrain different elements of the family variance–covariance structure by specifying in the RANDOM statement of PROC MIXED different types of covariance structures (CovStruc), and/or constraining different covariance parameters with the HOLD option of the PARMs statement. Further constrains in specific elements of the variance–covariance matrix are denoted by an * in the CovStruc type. The parameters to be estimated in each model are shown for analyses involving 5 sites (traits assessed at age 3). The total number of parameters to be estimated (# parms) for analyses involving 5 and 3 sites is also given. All models assume heterogeneity of residual variances across sites. All hypotheses are tested by comparing the reduced models with the full model, except the H2 hypothesis which is tested by comparing the H2 model versus the H4 model

difference between the number of covariance parameter specifying the full model and the reduced model (Fry 2004).

Because we are dealing with multiple environments (three or five sites depending on the trait) the following strategy was employed (Yang 2002). Firstly, we analysed our data with the conventional mixed model typically used in tree breeding, which assumes a constant family variance and covariance across sites [i.e. a compound symmetry (CS) covariance structure], and homogeneity of residual variance. This model was compared with a less restrictive model in which residual variances were allowed to vary between sites. Homogeneity of residual variance is a main assumption of conventional statistical analyses, and the non-fulfilment of this requisite is known to be a possible cause of spurious interactions (Yang 2002). Significance of family and family \times site interaction was analysed comparing the RLL of each factor included versus excluded from this model. This test of $G \times E$ interaction, although commonly used, may be not appropriate in cases of heterogeneous family variance and/or covariances across sites. A more precise analysis of the significance of the $G \times E$ interaction in a broad sense was implemented comparing the CS model without the term $G \times E$ (Model H1 in Table 2) with the unstructured covariance model (the full model H0), in which all possible causes of $G \times E$ (i.e. family heterogeneity across sites and deviations from perfect family correlations between environments) are allowed (Yang 2002). Secondly, we specifically tested for the contribution of each of the different possible causes of $G \times E$. Heterogeneity of family variance was analysed by comparing the CS family covariance structure model (model H2 in Table 2) with the heterogeneous compound symmetry covariance structure model (CSH, model H4 in Table 2). Deviations from perfect correlations, which can be interpreted as a test of crossover interactions (Yang 2007), were analysed by comparing a model in which all family correlations are fixed to 1 (model H3 in Table 2) with the full model H0. Finally, if significant deviations from perfect correlations were detected, we examined whether family correlations between sites were constant across all pairs of environments (model H4 in Table 2) or whether they differed depending on the site pairs (full model H0). Additionally, for traits assessed in both Coastal and Inland sites (i.e., traits assessed at age 3), we also investigated whether the crossover interactions were due to deviations from perfect correlations between sites of different areas. This hypothesis was analysed comparing the full model with a model in which all family correlations between sites of the same area were fixed to 1, whereas family correlations between sites of different areas were unconstrained (model H5 in Table 2).

SAS codes for performing all these analyses are available from the second author upon request.

Comparisons between WA and CG materials

In order to analyse the statistical differences between the families of the WA and CG breeding programmes, we re-analysed the data including the breeding programme and the programme \times site interaction as fixed effects in the mixed models, and nesting families within breeding programmes. Additionally, the best linear unbiased predictor (BLUP) of each WA family was statistically compared with the BLUP of the average of all the 111 CG families using the ESTIMATE statement of the MIXED procedure (Littell et al. 2006, Chap. 6).

Superiority of the improved materials

Because no control seedlots were included in the trial series, we used the adjacent provenances tests to obtain predictors of unimproved seed sources with which to compare the selected materials. Despite the provenance and the progeny trials being two independent trials, with independent experimental designs, statistical comparisons between them were reliable due to the fact that common spatial adjustments accounted for the eventual microenvironmental differences between both trials. We estimated the confidence intervals at 95% for BLUPs of each family and provenance across the three inland sites using the ESTIMATE statement of the MIXED procedure (Littell et al. 2006). Non-overlapping confidence intervals were interpreted as significance differences between the improved families and the unimproved provenances. Becerreá was not considered for traits assessed at age 7 because data for the provenance trial from this site at age 7 were lacking.

Results

Mean H were notably different over the different sites, indicating different site indexes. Height means varied from 78.6 cm in Daneiro to 133.9 cm in Laracha at age 3, and from 295.1 cm in Becerreá to 350.3 cm in Guntín at age 7 (Table 1). The dispersion of the individual tree heights within each site was very high, with coefficients of variation up to 40%. For instance, individual tree heights at age 7 varied in Laza from 52 cm to 683 cm, i.e. more than 5 m between the lowest and the highest tree. For each trait, the corrected data adjusted for spatial autocorrelation showed the same site mean as the uncorrected data, but a clearly smaller dispersion, with coefficients of variation of just 15–20% (data not shown).

The survival in the coastal sites was abnormally low (39 and 35% in Daneiro and Laracha at age 3, respectively) because of the sanitary problems explained before. Of the inland sites, survival rates were acceptable in Guntín and Laza with values of around 93% at age 7, whereas it was notably lower in Becerreá (58% at age 7).

Spatial dependence and homocedasticity

Residuals after subtracting family effects revealed pronounced non-random spatial structures for the growth traits and the number of whorls in all sites (Table 3). The spherical theoretical semivariogram fitted well to the observed semivariogram for most of these traits ($r^2 > 0.85$, $P < 0.001$). The variation explained by the spatial pattern varied from 10.6% for WH in Becerreá at age 3 to 73.2% for H in Laza at age 7 (Table 3, see also Figure S1 supplied as supplementary online material). Height at age 7 showed the largest intensities of the spatial dependence, with values of the patch variance to sill variance ratio varying from 41.4 to 73.2%. The range (a_0) or patch size of the theoretical semivariograms was greatly variable, from 29.3 m for H in Daneiro at age 3 to 103.9 m for H in Laza at age 3 (Table 3). Stem form traits revealed random spatial structures with the exception of Becerreá and Daneiro sites, where STR and LEN revealed a slight spatial autocorrelation.

Heterogeneity of residual variances was observed for all traits and ages (see Table S1 as supplementary online

material). Log-likelihood ratios for the heterogeneous variance models were much lower than those for the respective equal variance models, and the differences were highly significant ($P < 0.001$) in all cases.

Because of heterogeneity of residual variances in all cases, all further analyses presented in the following sections were based on heterogeneous error variance models, and in those cases where non-random spatial structures were found (Table 3), spatially adjusted data were employed.

Family variation and family × site interaction

Results of the conventional mixed model including family and family × site interaction as random effects (i.e., assuming a compound symmetry family covariance structure with constant variance and constant covariance) are shown in Table 4. Using this model, family variation was highly significant for all traits and ages, and the family × site interaction was significant ($P < 0.05$) or marginally significant ($P < 0.10$) for 7 out of the 9 traits. The number of whorls at age 3 and the leaning score at age 7 were the traits with no significant family × site interaction. The ratio of interaction to family variance component (σ_{fs}^2/σ_f^2) was relatively low in all cases and varied between 0.10 for leaning at ages 7 to 0.54 for height at age 3.

A comprehensive likelihood-based analysis regarding the relevance and interpretation of the family × site

Table 3 Patch size (range in metres, a_0) and intensity of the spatial pattern structure (patch variance to sill variance ratio, $I(\%) = [C_0 / (C_0 + C_n)] \times 100$) derived from theoretical semivariograms fitted to different traits, adjusted for genetic effects in the five test sites at ages 3 and 7 after planting

	Coast				Inland					
	Daneiro		Laracha		Becerreá		Guntín		Laza	
	a_0 (m)	I (%)	a_0 (m)	I (%)	a_0 (m)	I (%)	a_0 (m)	I (%)	a_0 (m)	I (%)
Age 3										
Height	29.3	19.3	78.9	25.8	53.6	29.5	36.8	24.1	103.9	38.1
No. whorls	Grad ^a	16.5 ^b	SI ^c		61.8	10.6	44.1	11.4	70.0	22.5
Leaning	99.0	26.6	SI		77.6	12.0	SI		SI	
Straightness	Grad	29.8	SI		77.2	15.8	SI		SI	
Age 7										
Height					42.7	41.4	94.6	47.5	47.8	73.2
Diameter					48.0	32.6	88.0	42.1	34.9	57.9
No. whorls					112.5	60.9	Grad	25.6	33.8	35.3
Leaning					113.5	17.7	SI		SI	
Straightness					Grad	18.1	SI		SI	

The shown patch size (a_0) is the a_0 parameter of the spherical model, but is $a_0 \times 3$ for the exponential model (Webster and Oliver 1990)

^a grad = gradient (linear semivariogram)

^b For linear models, the intensity of the spatial pattern structure was calculated for a distance of 100 m as $100 \times C_0 / (100 \times C_0 + C_n)$

^c SI = spatially independent trait

Table 4 Summary of the mixed model analyses for different traits assessed at ages 3 and 7 using a compound symmetry structure for the family variance–covariance matrix across sites

Age	Traits	Site F^a	Family VC \pm SE	Family \times site VC \pm SE
3	Height	1,067.0***	20.316 \pm 3.496***	11.053 \pm 1.937***
	No. whorls	407.0***	0.046 \pm 0.008***	0.005 \pm 0.005 ^{NS}
	Leaning	329.4***	0.001 \pm 0.0003***	0.0005 \pm 0.0004 ^{NS}
	Straightness	75.1***	0.018 \pm 0.003***	0.009 \pm 0.003***
7	Height	635.6***	124.850 \pm 24.946***	52.487 \pm 16.651***
	Diameter	261.0***	4.513 \pm 1.143***	1.617 \pm 1.052*
	No. whorls	109.3***	0.075 \pm 0.015***	0.022 \pm 0.010**
	Leaning	101.5***	0.002 \pm 0.001**	0.0002 \pm 0.001 ^{NS}
	Straightness	86.3***	0.010 \pm 0.003***	0.003 \pm 0.002 ^{NS}

For fixed effects (site), F -ratio and associated significance levels are shown; for random effects (family, and family \times site interaction), variance component estimates \pm standard errors (VC \pm SE) and associated significance levels of the log-likelihood ratio test for significance of each variance component are shown

Asterisks denote the significance level (***) = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; NS = $P > 0.05$) associated to the chi-square value given by the difference in two times the log likelihood of that factor included versus excluded from the model. Because variance components are constrained to be positive, test of variance components are one-tailed (Fry 2004)

^a $F_{4,118}$ for traits at age 3 and $F_{2,67}$ at age 7

Table 5 Likelihood ratios for testing different hypotheses on the relevance and interpretation of the genotype by environment interaction for different traits assessed at ages 3 and 7

Null hypotheses	df	Height		No. whorls		Straightness	
		χ^2	$P > \chi^2$	χ^2	$P > \chi^2$	χ^2	$P > \chi^2$
Age 3							
H1: no family by environment interaction	14	100.7	<0.001	72.1	<0.001	912.8	<0.001
H2: homogeneity of family variance across sites	4	15.8	0.003	60.0	<0.001	19.5	<0.001
H3: perfect family correlation between all site pairs	10	77.3	<0.001	12.6	0.248	67.4	<0.001
H4: homogeneity of family covariance across site pairs	9	14.6	0.102	10.6	0.304	64.2	<0.001
H5: perfect family correlation between sites within deployment areas (coastal and inland Galicia)	4	59.5	<0.001	4.7	0.319	41.0	<0.001
Age 7							
H1: no family by environment interaction	5	21.3	<0.001	26.8	<0.001	256.3	<0.001
H2: homogeneity of family variance across sites	2	4.5	0.100	19.2	<0.001	26.6	<0.001
H3: perfect family correlation between all site pairs	3	12.3	0.006	6.3	0.098	4.2	0.241
H4: homogeneity of family covariance across site pairs	2	1.1	0.576	1.4	0.500	4.2	0.121

The chi-squared values shown are the differences in two times the log-likelihood of the full model (unstructured variance–covariance matrix) and different reduced models constraining different elements of the family variance–covariance structure across sites (see “Methods”). Degrees of freedom (df) associated with the chi-squared values results from the difference between the number of covariance parameters specifying the full and reduced models. P values lower than 0.05 indicate that the null hypotheses should be rejected

interaction for three selected traits is shown in Table 5. When all possible causes of interaction are considered, the family \times site interaction was highly significant in all cases. These results disagree with those presented in Table 4 for the number of whorls at age 3 and straightness at age 7. These discrepancies arise because results presented in Table 4 are based on a model that assumes homogeneity of family variance and covariance across sites, which are clearly inappropriate assumptions in these cases. Indeed, the large heterogeneity of family variance across sites for

these traits (Table 5) is responsible for the significant family \times site interaction. On the other hand, the lack of perfect family correlation between sites contributed significantly to the family \times site interaction in the cases of height growth at both ages, and straightness at age 3, suggesting family rank changes across sites for these traits. Moreover, the results of the H4 hypothesis indicate that the family covariance across sites for height growth was fairly constant for all pairs of sites (Table 5). Thus, family rank changes are likely to be equally distributed among all pairs

Table 6 REML estimates of genetic correlations between sites using an unstructured family variance-covariance matrix across sites for different traits at ages 3 (above the diagonal) and 7 (below the diagonal)

Trait	Site	Inland			Coastal	
		Becerreá	Guntín	Laza	Daneiro	Laracha
Height	Becerreá		0.63***	0.62***	0.77***	0.62***
	Guntín	0.63***		0.60***	0.42**	0.74***
	Laza	0.67***	0.79***		0.84***	0.67***
	Daneiro					0.60***
	Laracha					
No. whorls	Becerreá		0.79***	1.00***	0.24 ^{NS}	0.54**
	Guntín	0.86***		0.95***	1.00*	0.60**
	Laza	0.68***	0.82***		1.00*	0.80***
	Daneiro					1.00 ^{NS}
	Laracha					
Straightness	Becerreá		0.28 ^{NS}	0.27 ^{NS}	-0.41 ^{NS}	0.10 ^{NS}
	Guntín	1.00 ^{NS}		1.00***	1.00***	0.47**
	Laza	0.29 ^{NS}	1.00*		0.89***	0.45**
	Daneiro					0.10 ^{NS}
	Laracha					

Sites are grouped into the two major climatic regions (inland and coastal Galicia). Asterisks denote the significance level (***) = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; NS = $P > 0.05$) associated to the chi-square value given by the difference in two times the log likelihood of that element included versus excluded (fixed to zero) from the model

Table 7 Effects of the breeding programme origin from which families proceed (Coastal Galicia and Western Australia), and its interaction with sites for different traits evaluated at age 7 in the three inland sites

Effects	DF	Height		No. whorls		Straightness		
		F	P > F	F	P > F	F	P > F	
Programme	1	115	11.4	0.001	4.6	0.034	5.1	0.026
Site	2	67	138.7	<0.001	17.1	<0.001	26.0	<0.001
Programme × site	2	230	3.2	0.043	1.5	0.217	2.0	0.132

of sites, and no single site or group of sites is responsible for the crossover interaction (see the low variation in family correlations between sites in Table 6). This is not the case with straightness at age 3, where the common covariance model (H4) fitted significantly less well than the unstructured covariance model (Table 5). The family correlations between sites (Table 6) indicate that Becerreá, which shows no significant family correlations with any other site, has a clearly anomalous behaviour for this trait. Furthermore, in the case of height and straightness age 3, deviations from perfect correlations appeared both within and among the two deployment areas (coastal and inland Galicia). Assuming perfect correlation between sites of the same area and allowing family rank changes between sites of different areas (H5 hypothesis) resulted in clearly and significantly less well-fitting models (Table 5).

Performance of the CG and WA material

Grouping the families into the two breeding programmes which they originated (WA and CG) and including this origin and its interaction with sites as fixed effects in the

mixed models resulted in highly significant differences between programmes for all traits (Table 7). Families from WA performed, on average, significantly better than those from CG (Fig. 2). In the case of growth traits, the programme × site interaction was significant (Table 7), indicating that the superiority of the WA material was not uniform across sites. The superiority in height growth of the WA material was more evident in Laza and Guntín (Table 8). Among the different WA families tested, WA4 always ranked highly for all traits in each of the three inland sites (Table 8), whereas WA1 showed reduced growth and worse stem form. Other WA sources showed intermediate results.

Both the CG and WA materials grew significantly more than the average performance of the unimproved seed sources tested in the adjacent provenance trials, and none of the single provenances performed better than the average of the improved material (Fig. 2). However, although the mean performance in stem form traits of the six provenances was worse than the average of the improved materials, there were some single origins that stand out with very straight stems and low number of whorls (Fig. 2).

Fig. 2 Best linear unbiased predictors (BLUPs) for the 111 half-sib families of the CG seed orchard (*white bars*) and the six families of the WA breeding programme (*black bars*, WA1-WA6), for **a** height, **b** straightness, and **c** number of whorls at age 7 in two sites (Guntín and Laza) located in the inland region of Galicia. The best linear unbiased predictor (BLUP) of the best provenance and the overall mean of all the provenances tested in the adjacent provenance trials (*grey bars*) are also shown. *x*-axis intersects the *y*-axis at the overall mean of the progeny trials

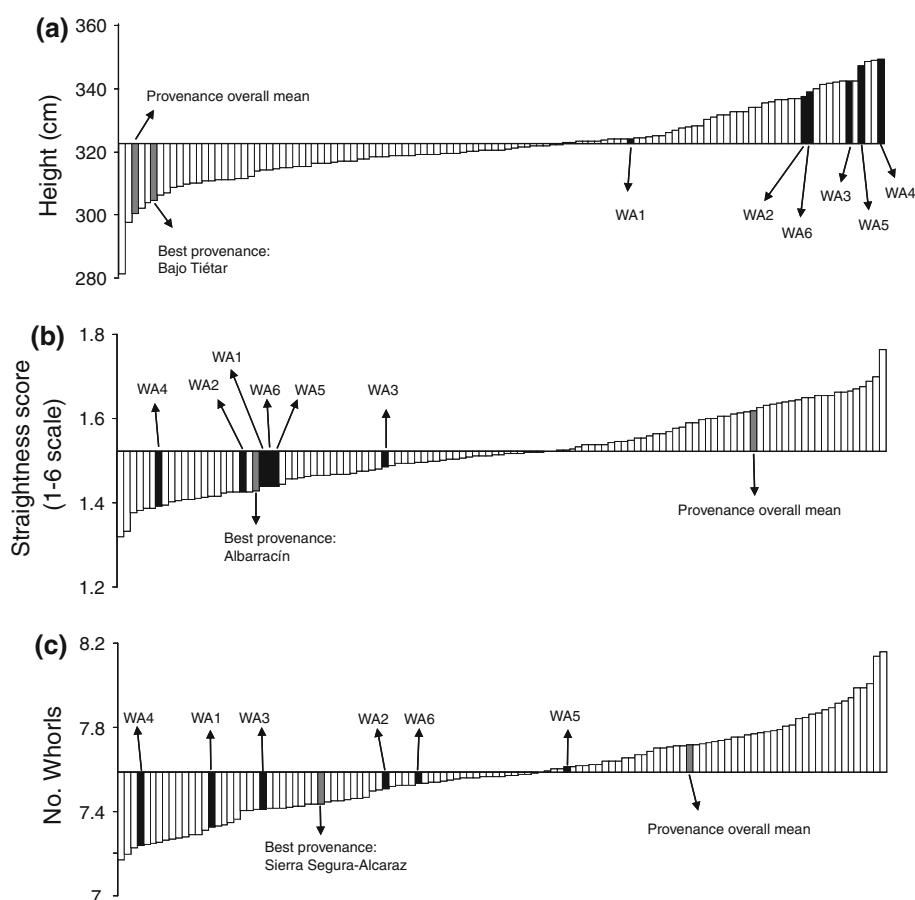


Table 8 Ranking of the BLUPs of the Western Australia (WA) families in the three inland sites for height, straightness and number of whorls at age 7 ($N = 117$ genetic entries)

WA families	Height			No. whorls			Straightness		
	Becerreá	Laza	Guntín	Becerreá	Laza	Guntín	Becerreá	Laza	Guntín
WA1	47	44	43	12	19	9*	39	23	46
WA2	16	14*	15*	83	63	70	7	15	4*
WA3	7*	9**	3***	16	22	21	35	58	31
WA4	4**	3**	1***	1**	1**	6*	3*	6	2*
WA5	61	16*	10*	70	65	74	36	27	35
WA6	15	13*	13*	32	30	59	28	26	21

Asterisks denote the significance level associated to the specific contrast between the BLUP of each WA family and the BLUP of the average performance of the 111 CG families for each trait

Significance levels: *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$

Discussion

This paper provides new information about the performance of Atlantic improved material in the inland region of Galicia, where the Atlantic character is clearly modified by continental and Mediterranean influences. Growth patterns of maritime pine in Galicia are known to differ between the inland and the coastal area, as a consequence

of both environmental and genetic factors (Álvarez-González et al. 2005; Barrio-Anta et al. 2006). Our results are in agreement with this, and the height growth of the studied material in the inland sites was lower than that observed in the coastal region in an earlier trial series at similar ages (Zas et al. 2004). However, mean height growth in the three inland sites was similar to intermediate to high site index *P. pinaster* plantations in this area (Álvarez-

Gonzalez et al. 2005), suggesting that the coastal material also performed relatively well in the drier and colder environmental conditions of the inland region.

Results from the likelihood-based analyses of the $G \times E$ interaction also indicate that there is not sufficient evidence to warrant the subdivision of the region into the two independent deployment areas of coastal and inland Galicia. This subdivision would be only justified if the relevance of the $G \times E$ interaction were much lower within than between areas. The $G \times E$ interaction is of consequence only when it involves rank changes across sites (i.e. crossover interactions), and so, deployment areas should cluster sites into groups with statistically negligible crossover interactions (e.g. Crossa et al. 2004). In this study, family \times site interaction was highly significant for most of the studied traits; however, this interaction was due to departures from perfect family correlations between sites only in the case of height growth and straightness at age 3. Imperfect genetic correlations between environments suggest crossover interactions (Yang 2007; Crossa et al. 2004). Thus, for these traits, there could be major disadvantages to merging both areas into a single deployment zone. However, the analysis of additional hypotheses regarding the genetic correlations between sites revealed that, in the case of height growth, crossover interactions are as important within areas as between areas. Constraining genetic correlations between sites of the same deployment area to 1 (i.e. avoiding family rank changes within areas) significantly reduced the log likelihood of the model, and thus resulted in an inappropriate model compared with the unconstrained full model. Moreover, genetic correlations between sites were similar for pairs of sites of the same or different areas, and the mixed models that assumed a constant family correlation between sites fitted as well as the unstructured full model. Thus, the pattern of the crossover interactions for height growth seemed to be similar between all possible pairs of sites, irrespective of the sites belonging to the same or different deployment areas.

The interpretation of the crossover interactions for straightness at age 3 was different. Assuming a constant family correlation between sites was not appropriate for this trait, and Becerreá seemed to be a site showing clearly anomalous behaviour. Family correlations between this site and the other four sites were not significant and even negative in some cases, whereas the remaining family correlations were significant and positive. The anomalous results for straightness in Becerreá, a site near the upper limit of the distribution of the species, are likely to be caused by the strong and irregular slope and the presence of snow during the winter. Additionally, straightness was assessed as an ordinal trait (1–6 scale), and residuals of the mixed models significantly ($P < 0.05$; Kolmogorov–

Smirnov test) departed from normal distributions, although residual histograms were unimodal and relatively unskewed. Normality of residuals is a main assumption of mixed models (Littell et al. 2006), so the results regarding this trait should be managed with care here. Generalized linear mixed models would have provided a more appropriate way of analysing an ordinal trait such as this (Bolker et al. 2009), but fitting generalized linear mixed models to our large data set and imposing constraints to the family covariance structure resulted in a failure to converge.

The pest and disease problems that occurred in the coastal sites may be also distorting the results regarding the comparison of inland and coastal performance. Both the fungus and the pest significantly reduced the survival and growth of the pine seedlings (Zas et al. 2007) and may have also favoured severe deformities in the stems (Sampedro et al. 2009). In addition, pine growth and stem straightness in the five studied sites could also be heavily influenced by rooting problems induced by the relatively long time nursery period (up to 19 months) and the small containers used (Superleach 125 cm³; Climent et al. 2008).

Both across and within site variation in the studied traits was very high. The results indicated a strong spatial autocorrelation for all the growth variables, reflecting the strong within site heterogeneity, whereas no spatial pattern was found for the stem form variables, except in Becerreá and Daneiro. Spatial dependence in stem form traits in these sites may be caused by the strong and irregular slope and the presence of snow during the winter in Becerreá, and by the existence of patchy waterlogged areas in Daneiro, that caused severe deformities in the stem. Non-random spatial patterns in traits assessed in forest genetic trials are very common (Dutkowski et al. 2006; Fu et al. 1999). Augmenting standard analytical models with spatial components has shown to increase the accuracy of genetic parameter estimates and to increase the treatment correlation between tests (Dutkowski et al. 2006; Qiao et al. 2000; Zas 2006). The impact of the spatial autocorrelation on the analyses of forest genetic trials can be so dramatic that several authors stated that leaving data unadjusted in the presence of spatial autocorrelation is clearly unacceptable (Costa-Silva et al. 2001; Dutkowski et al. 2006; Zas 2006). PROC MIXED in SAS allows to fit spatial correlation models in which residuals are allowed to be autocorrelated among themselves, with autocorrelation being a function of the distance that separate them (Littell et al. 2006, Chap. 11; Saenz-Romero et al. 2001). The mixed models used in the present paper could thus have been augmented allowing for an autocorrelation structure of the residual variation within each site. However, both fitting spatial autocorrelation models and fitting heterogeneous residual variation models with unstructured family covariance structure across sites are computationally very demanding. Integrating all these

family and residual covariance structures into a single mixed model would be very difficult with a conventional personal computer. Thus, the procedure used here, in which we first adjusted data for spatial autocorrelation (Zas 2006) and then fitted these complex mixed models, seems to be an operative way to circumvent this problem.

The studied trial series lacks control seedlots of local origin with which to compare the improved materials. Indeed, finding a representative seedlot of the many different origins commonly used in the maritime pine plantations in the inland region of Galicia would be a difficult task. We took advantage of the adjacent provenance tests to get estimates of unimproved seed sources in the area. Comparisons between materials from these adjacent trials with independent experimental designs were possible thanks to the common spatial adjustment, which accounted for the microenvironmental variation between the two trials within each site. The results indicate that both the CG and WA improved materials grew significantly more than all the provenances tested. On average, stem form and branching habit of the improved materials were also better, but some individual provenances from Central Spain performed clearly better regarding these traits. Atlantic origins are known to present poor forms and are clearly surpassed in provenance tests by other mountain origins such as Morocco, Corsica, and Sierra de Gredos (Alía et al. 1995). In fact, the French maritime pine breeding programme, developed upon the basis of the Atlantic Landes provenance, has exploited interprovenance crosses with Corsican origins to improve stem straightness of the local population (Alazard 1988; Harfouche and Kremer 2000). Our results also suggest that the superiority of the Atlantic improved materials in inland Galicia could be complemented by introducing favourable genes for straightness and branching habit through introgression. Interprovenance crosses with Albarraçín (good stem straightness) and Sierra de Segura-Alcaraz (low number of whorls), or with other mountain origins of known stem straightness (e.g. Morocco, Corsica or Sierra de Gredos; Alía et al. 1995; Harfouche and Kremer 2000; Sierra de Grado et al. 2008) should therefore be considered.

The Western Australia breeding programme has achieved, using an Atlantic provenance, very good results and ample gains for use in areas of severe drought within that region (Butcher 2007). The wide genotypic variation within provenances (Mariette et al. 2001; Petit et al. 1995) and the characteristic phenotypic plasticity of the species (Alía et al. 1997; Chambel 2006) offer a plausible explanation for this large flexibility. The performance of the WA material in inland Galicia was also very good, always above the overall average at each site. The superiority of the WA families was evident for the three main studied traits: growth, stem form, and branch habits (Fig. 2,

Tables 7, 8). However, the performance of the WA families was fairly variable across the three inland sites (Table 8). As expected, the best development of this material occurred on the driest sites (Guntín and Laza), while the worst performance was on the coldest one (Becerreá). Among the different WA families, WA4, a full cross between “sexy” clones characterized by a high resistance to drought, good growth and strong apical dominance, was one of the most stable and stands out as one of the best families for the three main traits in the three inland sites. The remaining WA families, except WA1, also show superior growth in Laza and Guntín, but only intermediate performance in terms of stem form. Results from the WA6 seedlot, which is a mixture of different open pollinated families from the 2nd generation clonal seed orchard at Manjimup (WA), indicated that, on average, the material from this seed orchard performed better than that from the Galician coastal seed orchard, although the differences were only significant in the case of growth on the two warmest and driest sites. Because the tree breeding programme for maritime pine in WA has continued to progress in recent years (Butcher 2007), we could expect even greater gains with new WA materials with higher levels of selection. Testing new seedlots from WA is, therefore, highly recommended.

As a practical conclusion, the results of this paper suggest the feasibility of using both the CG and the WA breeding materials as possible sources of forest reproductive material for the inland region of Galicia. Specifically, based on the excellent results of the WA material, importing seed for reforestation in inland Galicia and/or including specific selected WA genotypes into the local breeding population should be strongly considered. Furthermore, judging from the results of the $G \times E$ interaction analyses, there is not sufficient evidence to subdivide Galicia into the two current deployment areas, coastal and inland Galicia. Interaction patterns do not reveal significant differences between zones, and crossover interactions for height growth are present both between and within areas. Although a strategy is undoubtedly needed to deal with the overall crossover interactions, based on the results of the present paper, both zones could be merged into a single breeding and deployment area.

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