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Genetic Control of Growth, Wood Density and Stem Characteristics of *Pinus pinaster* in Portugal

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Abstract. A *Pinus pinaster* Ait. progeny trial involving 46 open pollinated families was established at two locations in Portugal (Leiria and Escaroupim). Height, diameter, Pilodyn and stem characteristics (straightness, branch size and number of whorls) were assessed at age 5 and 12 and were used to estimate variance components, heritability, phenotypic and genetic correlations between traits.

Estimates varied considerably between the two sites. However, this study indicates that the heritability for height followed the general pattern of increasing with the age of the trees. A strong and favourable genetic correlation between the stem form and branching was also found. Heritability of Pilodyn was very low, suggesting that measurements taken at age 12 were not a reliable measure of wood density, probably due to the high proportion of juvenile wood.
Key words: progeny trial; variance components; heritability; genetic correlations; *Pinus pinaster* Ait.

Sumário. Na Mata Nacional de Leiria e na Mata Nacional do Escaroupim foi estabelecido um ensaio de descendências de *Pinus pinaster* Ait constituído por 46 famílias de meios irmãos. Foram feitas várias medições aos 5 e aos 12 anos entre as quais a altura das árvores, o diâmetro, a forma do tronco, hábitos de ramificação e número de verticilos. O Pilodyn foi utilizado como avaliador indirecto da densidade da madeira. Para todas estas características foram estimadas as componentes da variância, a heritabilidade e as correlações fenotípicas e genéticas.

As estimativas variaram consideravelmente entre os dois locais. Contudo, este estudo confirma que a heritabilidade para a altura total das árvores aumenta com a idade das mesmas. Por outro lado, foi estimada uma forte e positiva correlação genética entre a forma do tronco e os hábitos de ramificação. Em relação ao Pilodyn, a heritabildade calculada foi muito baixa, sugerindo que as medições efectuadas aos 12 anos são pouco fiáveis, provavelmente devido à alta proporção de lenho juvenil
Palavras-chave: ensaio de descendências; componentes da variância; heritabilidade; correlações genéticas; *Pinus pinaster* Ait.

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Résumé. Un essai sur 46 familles de demi-frères de *Pinus pinaster* Ait. a été installé en deux endroits au Portugal (Leiria et Escaroupim). La hauteur, le diamètre, le Pilodyn et les caractéristiques du tronc (rectitude, dimension des branches et nombre de verticilles), ont été mesurés à l'âge de 5 et 12 ans pour estimer les composantes de variance, l'héritabilité, et les corrélations phénotypiques et génétiques entre les caractères
Les estimatives ont considérablement varié entre les deux endroits. Cependant, cette étude indique que l'héritabilité pour la hauteur a suivi le modèle général d'accroissement avec l'âge des arbres. Une corrélation génétique forte et favorable entre la forme du tronc et la branchaison a aussi été repérée. L'héritabilité du Pilodyn était très réduite, suggérant que les mesures à l'âge de 12 ans ne permettent pas d'évaluer la densité du bois, probablement dû à la proportion élevée de bois jeune.
Mots clés: essais de descendance; composantes de la variance; héritabilité; corrélations génétiques; *Pinus pinaster* Ait.

Introduction

In Portugal, forest products represents an important economic asset, accounting for 34% of the national gross domestic product (CESE, 1998), and contributing substantially to a favourable trade balance. Overall, forest products exports represented 11% of total exports in 2000.

In Portugal, *Pinus pinaster* is one of the most important native species, covering around 1 000 000 ha (DGF, 2000), contributing over the years to the rural economy of the country and its people (RADICH and ALVES, 2000). A tree improvement plan has been developed since the early 80s (ROULUND *et al.*, 1988) with the aim of increasing volume per hectare and stem conformation, traditionally one of the species caveats. The progeny trials reported in the present study are part of such program.

Stem volume has been historically the principal objective of tree improvement programs (ZOBEL and KELLISON, 1978), and is generally accepted and justified in economic terms (ZOBEL and TALBERT, 1984). However, correlation of this trait with stem form and branch characteristics should be considered due to their influence on final product value, especially when the expected end use are sawn timber and veneer. Branch size, branch angle and the number of branches are known to affect negatively the size and number of knots formed. The presence of stem forking and crookedness, increases the amount of compression wood and the depreciation of timber as a valued timber product.

The exact influence of these characteristics in the economic value on the final crop is difficult to quantify as reported for *Pinus pinaster* by BUTCHER and HOPKINS (1993), and assumption will differ between different countries and markets. However, the degree of inheritance and the way it changes with age and sites for economically important traits remains an important issue. Information is still scarce for early and late selection traits in tree improvement programs and in *P. pinaster* in particular. COSTA and DUREL, (1996), sustain that is important to give more attention to diameter growth in maritime pine, because of its stronger correlation with total volume and its simplicity to measure, although height seems to be a better trait for early selection for growth. Published estimates in Portugal, France, Australia and Spain suggested the species have a relatively low heritability for growth, a characteristic common in many conifers (HOPKINS and BUTCHER, 1994).

DANJON (1995), found very different estimates of heritabilities for height in two sites of different ages, 0.09 e 0.30 for trees with 5 years old, and 0.20 e 0.67 for the same trials with 20 years old. KREMER (1981), also reported heritabilities of 0.17 and 0.20 for height for 8 years old maritime pine trees, which are also in general agreement with those found by COTTERILL *et al*. (1987). An heritability value of 0.28 for this trait was estimated by BUTCHER and HOPKINS (1993).

For other traits of more subjective nature such as some stem characteristics, heritabilities are reported to vary considerably. BUTCHER and HOPKINS (1993) reported 0.14 for stem straightness, while DANJON (1995), mentioned 0.45 for a straightness scoring (scale from 0 to 20), and 0.10 estimated for butt stem sweep (in cm). In the same study, heritabilities of 0.58 and 0.60 for polycyclism, 0.00, 0.34 for volume and 0.15 and 0.38 for diameter were reported. Also for diameter, Butcher and Hopkins (1993) reported 0.10, and a very low value of 0.04, was indicated in an eight years old trial, by COTTERILL *et al*. (1987). On the other hand, wood density has consistently been reported to have high heritability (e.g. 0.44 was reported by (CHAPERON *et al*., 1988).

This study reports estimates of genetic parameters for growth, stem and branch characteristics and Pilodyn as an estimator of wood density, at age 5 and 12 from two progeny trials in coastal Portugal, based on Portuguese progenies.

Materials and methods

Location

The progeny trials reported here were established by Estação Florestal Nacional (EFN) in 1987. Trial 1 is located at M. N do Escaroupim, central Portugal (lat 39º 05′ N, long 8º45'W, alt 10 m). The soil is constituted by Dystric Regosols (RG dy). The parent rock are Quaternary (Pleistocene) sandy deposits from fluvial origin. Mean annual rainfall is around 600 mm, with mean temperatures for January and July of 9.7ºC and 23.2ºC, respectively. Trial 2 and 3 are located at Pinhal de Leiria, central coastal Portugal (lat 39º50'N, long 8º55'W, alt 30m). The soil is, Spodic Podzols (PZ sd), derived from sand dunes of maritime origin. The site is characterized by a mediterranean climate, with mean annual rainfall of around 880 mm. Mean monthly temperatures for January and July are 9.4ºC and 19.4º C, respectively.

Silviculture

Seedlings were raised, in the nursery for 9 months, in plastic bags with 250 cm^3 of volume). The site was harrowing and ploughed before planting, in March 1987 (trials 1 and 2). Plants were established at a spacing of 2x2m. There was no fertilizer applied. (AGUIAR,1993).

Genetic material

The progeny trials includes 46 open pollinated families, originated from seed collected in the Escaroupim clonal seed orchard II (AGUIAR, 1993). This seed orchard includes 49 genotypes and was established by grafting in 1975-80, with the aim to produce improved seed for afforestation of coastal regions in Portugal. The ortets were obtained from plus-trees selected in Mata Nacional de Leiria by the senior Forester D. H. Perry in 1963/64. The selection criteria used was based on volume, stem form, spiral grain and branch habits. Details of plus phenotypes selected and of scoring system employed, are described in (PERRY and HOPKINS, 1967).

Field design

The trials were established in eight randomized complete blocks of 2 x 4 tree plots. The total number of trees per family established in each trial was therefore 64, giving a total number of 2944 trees planted. Trial 1 was thinned at age 7, after the first height assessment, culling the worst 4 trees in each plot.

Measurements

Traits measured were diameter, height, ramification, stem straightness and number of whorls. Diameter was measured at breast height over bark. Height was assessed with a telescopic rod. Ramification and straightness was scored according to a 1-6 scale (with 1 =worst to 6=best). The total number of whorls was also recorded. Wood density was evaluated through Pilodyn measurements done on all trees at breast height, after bark removed.

Height was measured at age 2, 5 and 12, diameter was measured at age 5 and 12, and the remaining traits at age 12 only.

Data analysis

Analyses were carried out using a multivariate mixed model REML analysis using the program ASREML (GILMOUR *el al.*, 1998). Each trial was analysed separately, using the following model:

$$y = u + rep + a + f.\ rep + e \qquad (1)$$

where y represents an individual tree observation for all the traits and ages, *u* is the overall trial mean, *rep* is the effect due to replicates, *a* is the additive genetic effect, *f. rep* is the interaction between family and replicate and *e* is the residual. Variance components and associated standard errors were estimated directly from ASREML.

Heritabilities were calculated as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{f.rep}^2 + \sigma_e^2} \qquad (2)$$

The heritability assumed a coefficient of relation amongst open pollinated sibs of ¼, hence assuming self incompatibility. Genetic correlations between traits measured in the same trial were calculated using the multi-site extension of model (1) above, with the same trait measured in different trials taken as different traits. Standard errors for heritability and genetic correlations were estimated by ASREML a Taylor series approximation (GILMOUR *et al.*, 1998).

In trial 1, the selective thinning at age 7, is expected to have affected the variance estimates at later ages. To correct for this effect, the analysis pooled traits measured before and after the thinning in the multivariate analysis. The use of growth measurements taken prior to thinning when analysing growth after thinning was shown to be effective in correcting potential biases due to selective thinning (WEI and BORRALHO, 1998).

Results and Discussion

Means

The two trials showed significantly different rates of growth (Table 1). At age 12, trial 1 had faster growth rate than Trial 2. Mean height in site 1 (Escaroupim) was 9.3 m, compared with 6.6 m at site 2 (Leiria). Diameters at age 12 were 12.7 and 9.2 cm for site 1 and 2, respectively.

Variances and genetic parameters

Estimates of variance components, heritabilities and genetic and phenotypic correlations are listed in Table 1, for growth traits (height and diameter) at ages 2, 5 and 12. Heritabilities were generally low, but the values varied considerably between the two trials. Site 1 had very low heritabilities (between 0.05 and 0.07) for height and diameter. In Site 2, on the other hand, heritabilities were higher, ranging between 0.17 and 0.33.

There was relatively little change in heritability with age. In site 2, the heritabilities for height dropped from an initial 0.22 at age 2 to 0. 17 at age 5, increased thereof to a maximum of 0.33 at age 12. Given the associated standard errors of around 0.04 for each estimate, this suggests a significant improvement of heritability with age. In site 1, estimates of heritability for height and diameter remained low between age 2 and 12, at around 0.05. Genetic correlations between growth traits were generally high (Table 1), although the only reliable estimate between diameter and height were obtained in site 2, with r_G = 0.91. Phenotypic correlations between diameter and height were consistently high in both trials.

Stem characteristics

Estimates of genetic parameters for stem characteristics (straightness, branch size and number of whorls), and their relationship with growth (estimated from measurements of height and diameter) and Pilodyn are given in Table 2.

Table 1 - Overall trial mean, variance components, heritability, phenotypic (below diagonal) and genetic correlations (above diagonal) for height (m) at age 2 (ht2), 5 (ht5) and 12 (ht12), and diameter (cm) at age 5 (d5) and 12 (d12), in trials 1 and 2. Correlation estimates with very large standard errors are shaded. Some correlations were not estimated from the data since no convergence was found

Trial	Trait	Mean	Add	Rep.Fam	Error	h^2 (s.e.)	ht2	ht5	ht12	d5	d12
1	ht2	1.40	0.006	0.015	0.067	0.067(0.04)		0.93(0.25)	0.91(0.52)	-	-
	ht5	5.48	0.026	0.068	0.408	0.053(0.04)	0.55		1.26(0.41)	0.68(0.26)	-
	ht12	9.33	0.061	0.246	0.704	0.060(0.03)	0.37	0.64			0.48(0.43)
	d5	7.3	0.098	0.199	0.688	0.049(0.03)	-	0.68	1.10(0.39)		
	d12	12.7	0.331	1.031	4.680	0.056(0.06)	0.57	-	0.72	-	
2	ht2	0.45	0.006	0.004	0.016	0.228(0.07)		0.96(0.05)	0.79(0.11)	-	-
	ht5	1.64	0.060	0.068	0.219	0.170(0.07)	0.75		0.85(0.09)	-	-
	ht12	6.59	0.413	0.283	0.568	0.327(0.10)	0.60	0.77		-	0.91(0.04)
	d12	9.15	1.199	0.335	5.146	0.180(0.06)	-	-	0.84		

Table 2 - Overall trial mean, variance components, heritability, phenotypic (below diagonal) and genetic correlations (above diagonal) for stem straightness (STR), branch size (BRCH), number of whorls (WHRL) volume (VOL) and pilodyn (PIL) at age 12 in trials 1 and 2. Correlation estimates with very large standard errors are shaded

Trial	Trait	Mean	Add	R.Fam	Error	h2 (s.e.)	STR	BRCH	WHRL	VOL	PIL
1	STR	3.11	0.166	0.057	0.635	0.194(0.07)		0.95(0.26)	0.34(0.29)	-0.59(0.49)	-0.07(0.62)
	BRCH	3.11	0.042	0.040	0.649	0.057(0.06)	0.34		-0.01(0.06)	-1.16(0.64)	0.92(1.08)
	WHRL	5.8	0.251	0.236	1.503	0.126(0.06)	0.07	0.08		0.89(0.69)	1.01(1.05)
	VOL	42.2	25.78	102.89	322.2	0.057(0.06)	-0.03	-0.27	-0.20		-0.65(0.92)
	PIL	30.6	0.056	0.235	2.043	0.024(0.04)	-0.02	0.11	-0.03	-0.21	
2	STR	2.82	0.090	0.021	0.663	0.116(0.04)		0.90(0.16)	-0.17(0.23)	0.13(0.25)	0.14(0.30)
	BRCH	3.12	0.085	0.037	0.743	0.098(0.04)	0.34		-0.31(0.23)	0.18(0.27)	0.00(0.33)
	WHRL	7.44	0.922	0.202	2.043	0.291(0.08)	0.00	-0.23		0.21(0.20)	0.00(0.27)
	VOL	16.9	30.60	10.91	105.2	0.209(0.07)	-0.02	-0.33	0.33		-0.76(0.16)
	PIL	32.0	0.356	0.350	2.848	0.100(0.05)	0.03	0.26	-0.25	-0.48	

Straightness and branching were scored according to a 1 to 6 scale, with trees being classified in order to reproduce a normal distribution between the 6 classes (COTTERILL and DEAN, 1990). In reality, the data showed a tendency to down score both traits in the two sites, with an average score below 3.5, and a variance below 1.0. This was more apparent for stem straightness in site 1.

The mean number of whorls (counted from the ground to the top) differed considerably between sites, being 5.8 and 7.4 for site 1 and 2, respectively. Typically, there is only one whorl per year, but the number is expected to increase due to occasional disruption in typical season patterns. The large differences between the two sites however, reflect the smaller size of trees in site 2, hence having more whorls per meter of stem.

Of the three traits (stem straightness, branching and number of whorls) only branching had a low heritability. Stem straightness was moderately heritable (0.19 and 0.12 for sites 1 and 2, respectively). Similarly, the number of whorls had moderate heritability (0. 13 and 0. 29).

Pilodyn measures the penetration in the wood of a 2.5 mm pin shot with a fixed strength. It has been shown in a number of species including pines, it provides a reliable indirect measure of wood density, although its accuracy depends on the pin diameter and age. Notivol et al (1992) found good correlations between Pilodyn and wood density (-0,73) for *P. pinaster* provenances in a sample of 60 trees of 34 years of age. In our study, Pilodyn showed a near zero heritability in both trials (0.02 and 0.03), values which are not typical of Pilodyn and certainly not of wood density in conifers. Likely explanations for the failure to pick reasonable heritabilities with Pilodyn are the small size of trees (stem diameters were on average 12 and 9 cm in site 1 and 2). Small diameter trees are likely to shake or bounce back in response to the Pilodyn shot, hence giving potentially wrong measures. Also, at 1.3 meters, rings being sampled by the Pilodyn are of juvenile wood and will have very low density. This is consistent with the high mean penetration observed in both trials (around 32 mm), which is amongst the highest ever reported for

Pilodyn (and at the limit of its scale and resolution). Finally, in small size trees, the sampling height (1.3m) was taken still inside the live crown, in an area of high variation. The fixed height, rather than a proportional height, may have contributed to a large tree to tree variation in Pilodyn values. As a whole, the results suggest nevertheless those Pilodyn measurements taken at breast height as early as 12 years may be inappropriate to screen for wood density in *P. pinaster*.

Correlation between traits at each site

Only a few correlations between stem characteristics, volume and Pilodyn were able to be estimated from the data (Table 2). Of these, the most consistent was the strong genetic correlation between stem straightness and branching (r_G =0.95 and 0.90 in sites 1 and 2). Straighter trees were therefore genetically associated with thinner branches. Interestingly, in both trials, the phenotypic correlation, although of the same sign was considerably weaker (r_p =0.34), as a result of a negative residual correlation between the two traits.

Association between other traits are hard to interpret given the low accuracy of the estimates (in most cases a consequence of the low heritabilities of the traits in the first place). Estimated volume seems to be mostly independent of other traits; correlations were generally positive (favourable) between volume and stem straightness or branching, but unfavourable with number of whorls. As expected, the number of whorls was unfavourably correlated with branching (r_G =-0.31).

An interesting result was the strongly negative (hence favourable) phenotypic and genetic correlation between volume and Pilodyn, a result which contradicts a well established negative relation between the two traits. This result further suggests that the Pilodyn reading taken at fixed height in small size trees will tend to sample different zones of density depending on their specific size. Small trees will be sampled at a lower density section of the stem (towards the tip), whereas big trees will be sampled at a higher density section (towards the butt).

Final comments

The results from this study provide important basic information to manage *Pinus pinaster* breeding populations, particularly for growth and stem characteristics. As other authors pointed out (e.g. GERBER *et al*., 1997, COSTA and DUREL, 1996) we observed that after age 12, total height seems to be a good indirect measurement of volume. This study also indicates that heritability of height tends to increase with age of the trees, a result also reported by other authors (KREMER, 1982, 1992; DANJON, 1995) However, the high financial resources involved in field trials measurements make this activity hardly possible, thus the high genetic correlation between height and diameter at age 12 is an interesting result since this trait is cheaper to assess.

A surprising result was the strong and favourable genetic correlation between the stem form and branching, both with important impact on the wood end use, although this needs to be tempered by a large standard error. This feature will allow management of the breeding population towards production of good quality wood.

In what concerns wood properties, Pilodyn measurements taken at age 12 were not a reliable putative measure of wood density, probably due to the high proportion of juvenile wood and small size of the trees. So, this tool is with no interest for breeding purposes at least during the tree juvenile period.

There is the need to monitor field trials for a longer period of time to confirm the high and favourable correlations between height and diameter, and stem form and branching, as well as to determine from which age measurements with Pilodyn provide a reliable indirect assessment of wood density.

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