Genetic control of wood quality and growth traits of *Pinus pinaster* Ait. in Portugal

Maria João Magalhães Gaspar

Supervisors

Professora Doutora Maria Helena R. N. Ribeiro de Almeida
Professora Associada do Departamento de Engenharia Florestal
Instituto Superior de Agronomia
Universidade Técnica de Lisboa

Doutor José Luis Penetra Cerveira Louzada
Investigador Auxilar do Departamento Florestal
Universidade de Trás-os-Montes e Alto Douro

Thesis presented to Universidade de Trás-os-Montes e
Alto Douro to obtain the PhD Degree in Forest Science

Universidade de Trás-os-Montes e Alto Douro
Vila Real, 2009

To my family

The research described in this thesis was financial supported by the Portuguese Foundation for Science and Technology (Doctoral grant SFRH/BD/27412/2006).

ABSTRACT

Pinus pinaster Ait. (Maritime pine) is one of the most important native species in Portugal, occupying 23% of the national forestry area. Due to its economic importance, a national tree improvement plan for this species has been in operation since the early 1980's. The maritime pine breeding programme is presently in its second generation of selection (the progeny trials reported in this study are part of this programme). The main objective of this thesis was to study the genetic control of wood properties promoting wood quality of *P. pinaster*, paying special attention to the genetic relationships between them and with growth traits. This work contributes to the Portuguese *P. pinaster* breeding programme by adding information on wood quality of 46 open-pollinated families that belong to its first generation, which were represented in a progeny test that was burned by a forest fire.

The results indicate that ring density is the most heritable (h_i^2=0.63) of the variables studied. Also, results pointed out that under Mediterranean climate conditions, *P. pinaster* earlywood characteristics are subject to a stronger genetic control ($h^2_{(MND)}$=0.54, $h^2_{(EWD)}$=0.60) than latewood components ($h^2_{i\,(MXD)}$=0.34; $h^2_{i\,(LWD)}$=0.26), which might be more depend on environmental conditions. Pith to bark trends showed that heritability values increase with age for most wood-density components and that age-age genetic correlations for wood density components are generally high, indicating the possibility of early selection. Also, the results showed that genetic correlation between all wood density components were higher than the corresponding phenotypic correlations, indicating that trait evaluation based on phenotypic correlations rather than genetic ones would result in an underestimation of potential gains from indirect selection. Furthermore, all wood density components were highly genetically correlated with ring density ($0.79<r_g<1$) and also closely associated among themselves, indicating that these traits are probably largely controlled by the same set of genes, and that selection for one characteristic should result in a simultaneous response of associated traits.

Data indicated that ring density has a negative genetic correlation with the lignin content (r_g=-0.16), which is desirable if the final purpose is pulp production.

Concerning the genetic control of radial modulus of elasticity and radial modulus of rupture, results showed that these traits are under a moderate genetic control (h_i^2=0.34 and h_i^2=0.31, respectively), suggesting possible genetic gains by selection for higher resistance and elasticity. These traits were positively correlated with ring density and spiral grain, while the correlations with lignin content were negative.

Genetic and phenotypic correlations between growth and wood quality traits were also studied, as well as the possible consequences of selection for growth at early ages on wood quality. The results showed that genetic selection based on growth will not result in a decrease of wood density and will not affect the spiral grain intensity. Furthermore, it is possible to select for growth improvement with an increase in the radial modulus of elasticity and with lower lignin proportion.

A major difficulty in estimating additive genetic parameters is the assumption of an average genetic relationship between the progeny in an open pollinated trial of 0.125, which can produce bias. In order to avoid bias in the genetic parameters estimations, the mean value of coancestry coefficient of the families was estimated using microsatellite markers. A mean coancestry coefficient of 0.130 was obtained, and it was concluded that the associated error in a heritability estimates was low.

In order to explore the possible effect that previous parameters estimates may have on *P. pinaster* improvement, expected genetic gains were estimated for several family based multi-trait selection indices for two possible final use scenarios, namely solid wood and other for pulp production. The results showed that several of the 10 best families are common for the two final use scenarios, and that it is possible to select a group of families that will give good results for solid wood end use or pulp production. These findings comprise an important contribution to a small country with limited financial resources like Portugal, since it addresses the optimization of resources with a multi-option use in a small breeding programme such as Portuguese *P. pinaster*

RESUMO

Pinus pinaster Ait. é uma das mais importantes espécies florestais Portuguesas, ocupando 23% da área florestal nacional. Dada a sua importância económica no país, foi implementado um programa de melhoramento no início dos anos 80. Este programa está presentemente na sua 2ª geração de selecção, sendo que o ensaio utilizado no presente estudo é parte desse programa. Esta tese teve como principal objectivo o estudo do controlo genético de algumas das mais importantes propriedades da madeira da *P. pinaster*, dando especial atenção às relações genéticas entre as características e entre estas e os parâmetros de crescimento. Pretendeu-se com este trabalho contribuir para o programa de melhoramento da *P. pinaster*, adicionando informação sobre a qualidade da madeira de 46 famílias de polinização livre pertencentes à 1ª geração deste programa, representadas num ensaio que foi alvo de um incêndio no ano de 2003.

Os resultados obtidos indicam que a densidade média do anel foi a característica estudada que apresentou valores mais elevados de heritabilidade (h_i^2=0.63). Os resultados obtidos indicam que nas condições mediterrâneas as características do lenho inicial estão sujeitas a maior controlo genético ($h_{i\,\mathrm{(MND)}}^2$=0.54, $h_{i\,\mathrm{(EWD)}}^2$=0.60) do que as do lenho final($h_{i\,\mathrm{(MXD)}}^2$=0.34; $h_{i\,\mathrm{(LWD)}}^2$=0.26), as quais estão mais dependentes de factores ambientais. Os valores de heritabilidade aumentaram da medula para o câmbio para a maioria das componentes da densidade, sendo que as correlações genéticas entre idades para estas características foram geralmente altas, indicando a possibilidade de selecção precoce. As correlações genéticas obtidas entre as diferentes componentes da densidade foram mais elevadas do que as correspondentes correlações fenotípicas, indicando que a realização de selecção indirecta com base nas correlações fenotípicas em lugar da utilização das correlações genéticas irá resultar numa subestima dos possíveis ganhos genéticos. As diversas componentes da densidade apresentaram correlações genéticas elevadas com a densidade média do anel ($0.79<r_g<1$), bem como entre elas, indicando que estas características podem ser controladas, pelo menos em larga medida, pelo mesmo conjunto de genes, e que a selecção para uma determinada característica irá resultar numa resposta simultânea das características associadas.

A densidade do anel apresentou correlações genéticas negativas com o teor de lenhina (r_g=-0.16), facto favorável quando o objectivo final é a produção de pasta. No que respeita ao controlo genético do módulo radial de elasticidade e do módulo radial de ruptura, os resultados indicam que estas características estão sob um controlo genético moderado (h_i^2=0.34 and h_i^2=0.31, respectivamente), o que sugere a possibilidade de obtenção de ganhos genéticos através da selecção para uma maior resistência e elasticidade. Estas características apresentaram correlações positivas com a densidade do anel e com o fio da madeira sendo, no entanto, negativas com o teor em lenhina.

Foram também estudadas as correlações genéticas e fenotípicas entre o crescimento e as características de qualidade da madeira, bem como as possíveis consequências da selecção para o crescimento em idades juvenis na qualidade da madeira. Os resultados mostram que a selecção baseada no crescimento não resulta num decréscimo da densidade da madeira e que não afecta a inclinação do fio da madeira. Para além disto é possível seleccionar para um maior crescimento com um aumento simultâneo do módulo de elasticidade radial e menor teor em lenhina. Os resultados indicam também que a selecção para maiores crescimentos aos 12 anos de idade não irá, provavelmente, afectar negativamente as propriedades da madeira aos 17 anos.

Um requisito para a estimativa das componentes de variância e outros parâmetros genéticos, para qualquer carácter quantitativo, é o conhecimento do coeficiente de parentesco. Normalmente em ensaios de polinização livre é assumido um valor de 0.125 entre as progénies, no entanto a utilização de coeficientes parentesco incorrectos pode implicar enviesamentos nas estimativas da heritabilidade. Neste trabalho, o valor médio do coeficiente de consanguinidade entre as famílias utilizadas, foi estimado recorrendo a microsatélites, tendo se obtido um valor médio de 0.13, o que sugere que o erro associado à estimativa de heritabilidade é pequeno.

De forma a explorar as o impacto que os parâmetros genéticos estimados anteriormente podem ter no melhoramento da *P. pinaster,* foram estimados os ganhos genéticos para as diversas famílias recorrendo a índices de selecção. Foram considerados dois cenários de utilização final da madeira, nomeadamente a utilização da madeira sólida e a produção de pasta. Os resultados mostraram que de entre o grupo das 10 melhores famílias existem várias que são comuns aos dois tipos de utilização final da madeira, e que é possível seleccionar um grupo de famílias que irão produzir bons resultados em ambas as situações. Num país pequeno e com recursos financeiros limitados como é Portugal, a optimização dos recursos através da utilização objectivos múltiplos, é de extrema importância num programa de melhoramento de pequenas dimensões, como é o caso do programa de melhoramento do pinheiro bravo.

Completing a thesis is a challenge, and thanking to all of those who contributed it is an even greater one. It is a pleasure to express my profound gratitude and recognition to the institutions and the people that made this thesis possible.

To the Portuguese Foundation for Science and Technology (FCT) for its financial support, without the grant the present work would be completely impossible.

To the University of Trás-os-Montes and Alto Douro for giving me permission to carry out this research work at the Forestry Department as well as for all the conditions given that allowed the accomplishment of this work.

To my Ph.D. supervisor Prof. Helena Almeida I would like to express my deep and sincere gratitude. Many thanks for all valuable knowledge's, encouragement and gentleness, and for all the cooperation established throughout all the thesis periods that hopefully will continue in the future.

It is difficult to overstate my appreciation to my co-supervisor Prof. Louzada. Thank you for your constant support, expertise, kindness, and most of all, for your patience. I believe that one of the main gains of this thesis was working with Prof. Louzada and gaining his trust and friendship.

To Engº Alexandre Aguiar, from the "Instituto Nacional de Recursos Biológicos" that manages the *Pinus pinaster* improvement program since it's starting point, for supporting and allowing me to perform this study.

I am deeply grateful to Doctor Ricardo Alia and Doctor Santiago Gonzales-Martinez, from the Departament of "Sistemas y Recursos, Grupo de Genética Florestal" INIA, Spain for having accepted to assist and supervise the molecular markers work, and for sharing all their expertise. It was a challenge for me that would not be possible without their assistance.

To Prof. Elena Hidalgo, from the Department of "Producción Vegetal y Recursos Forestales", E.T.S. Ingenierías Agrarias de Palencia, University of Valladolid, Spain, for warmly welcoming me to her laboratories facilities and allowing me to perform all the molecular analytical procedures. A special thanks to Ana and José for all friendship and help during the work in Palencia.

To the Prof. Pedro Fevereiro for receiving me in his lab and to all the Plant Cell Biotechnology Laboratory team, in particular to Jorge Paiva for helping me with the DNA extraction, and for all friendship and support.

To Doctor José Carlos Rodrigues, from the "Instituto Investigação Científica Tropical", for sharing his scientific knowledge and for receiving me in his lab, allowing me to perform the wood chemical analysis. I also want to thanks to Ana, for all the help and sympathy during our stays in Lisbon.

To Prof. José Morais, from the Engineering Department of UTAD, for allowing me to do the mechanical tests in his lab, and for all the support.

To Samantha Hughes, for her kindness in revising the English.

I also would like to express my appreciation to all the workers of the Forestry Department, especially to Sr. Rodrigues and Sr. César, for their help in the field work, often undertaken in very difficult conditions. A special word is due to Marla, Sr. Armindo not only for the help during all the practical work but also for their friendship and support.

I would like to express my thanks to all my colleagues of the Forestry Department, with who I share the everyday work. To Prof. Hermínio for the surrounding music that inspired me during the long afternoons of work. To all the lunch companions, the breaks are very important to renew energies, and to Frederico that saved my thesis cover. A special word of gratitude and friendship to Simone, Mimas, Roxo and Domingos, your friendship, support and encouragement was very important during all this years.

To Hugo and Carmen, a very special thanks. You made me feel at home during my stays in Lisbon and helped me going through difficult times away from the children.

I also would like to express my gratitude to my family in law, for all the support and friendship.

To my parents and sister, a very deep deep thank you. Even so far away, you were always here for me, always available, always supportive. Without you I would not be finishing this work.

Words fail me to express my appreciation to Luis. A most special thanks with all my heart and love for your unconditional support, encouragement and help. You always believed in me and gave me faith.

To my sons Bia and Pedro, my reason to live, my deep gratitude for your patience and love throughout this period. You were always my source of strength that kept me going.

Maria João ☺

TABLE OF CONTENTS

CHAPTER 1.

General Introduction

CHAPTER 2.

Age trends in genetic parameters of wood density components in 46 half-sibling families of *Pinus pinaster*.

CHAPTER 3.

Genetic correlations between wood quality traits of *Pinus pinaster* Ait.

CHAPTER 4.

Phenotypic and genetic correlations between lignin and wood density components in *Pinus pinaster* AIT.

CHAPTER 5.

Genetic variations of wood mechanical traits in 46 half-sib families of *Pinus pinaster* Ait.

CHAPTER 6.

Does selecting for improved growth affect wood quality of *Pinus pinaster* in Portugal?

CHAPTER 7.

Use of molecular markers for estimating breeding parameters: a case study in Maritime pine progeny trial.

CHAPTER 8.

General discussion, Conclusions and Perspectives

LIST OF FIGURES

CHAPTER 1.

General Introduction

CHAPTER 2.

Age trends in genetic parameters of wood density components in 46 half-sibling families of *Pinus pinaster*.

CHAPTER 3.

Genetic correlations between wood quality traits of *Pinus pinaster* Ait.

CHAPTER 5.

Genetic variations of wood mechanical traits in 46 half-sib families of *Pinus pinaster* Ait.

CHAPTER 7.

Use of molecular markers for estimating breeding parameters: a case study in Maritime pine progeny trial.

LIST OF TABLES

CHAPTER 1.

General Introduction

CHAPTER 2.

Age trends in genetic parameters of wood density components in 46 half-sibling families of *Pinus pinaster*

CHAPTER 3.

Genetic correlations between wood quality traits of *Pinus pinaster* Ait.

CHAPTER 4.

Phenotypic and genetic correlations between lignin and wood density components in *Pinus pinaster* AIT.

CHAPTER 5.

Genetic variations of wood mechanical traits in 46 half-sib families of *Pinus pinaster* AIT.

CHAPTER 6.

Does selecting for improved growth affect wood quality of *Pinus pinaster* in Portugal?

CHAPTER 8.

General discussion, Conclusions and Perspectives

CHAPTER 1

General Introduction

1.1. INTRODUCTION

The Portuguese forestry sector is strategic in providing important benefits to the environment and the national economy, contributing to nature conservation, environmental equilibrium, promoting biodiversity, protecting against erosion, and ensuring air and water quality. The Portuguese Sustainable Forest Development Plan (DGF 1999) states that forest areas should be managed to ensure optimisation and compatibility of production and use, concerning the multiple uses of forest resources and to respect the principles that support sustainability. Forested areas provide a vital service, acting as 'carbon pools' for increasing levels of atmospheric $CO_{2,}$ since they comprise a large proportion of terrestrial vegetal biomass (Pastor and Post 1988). Terrestrial plants annually fix approximately 15% of the atmospheric pool of carbon by photosynthesis, (Williams et al. 1997). The 21st century has brought new challenges for forest management and forest ecosystems, which represent a very promising tool for dealing climate change (Lopes et al 2009).

In Portugal, the forestry sector accounts for approximately 2% of the national gross domestic product (GDP) and represents 10% of national exports, employing 113,000 workers directly, equivalent to 2% of the national active population (AEP 2008). The economic value per hectare of Portuguese forest is considerably higher than in other Mediterranean countries (DGRF 2006). Despite the obvious importance of this sector, Portugal is the only European Mediterranean country where the average area of forest destroyed by fire per year has increased in the last two decades. In the 1960's the average annual area of forest destroyed by fire was only 10,000 hectares. Between 2001 and 2006 the average area destroyed annually by fire was close to 190,000 hectares, with 60% occurring in forested areas and 40% in areas covered by shrubs (DGRF 2006). The probability that a given area will burn in other South Europe countries is approximately 0.5%; in Portugal the probability is 4 to 5 times greater.

The climatic factors characteristic of Mediterranean areas, condition and determine the number and scale of forest fires whose origin is connected with diverse structural and socio-economic factors (Baptista 2000). About 85% of Portuguese forest is privately owned; with only 3% belong to the State, and the remaining 12% being communally owned lands. This structure of land ownership restricts government action on forest management, including forest fire prevention.

According to the National Forest Inventory (2005/2006), maritime pine forest is one of the largest productive ecosystems, occupying 23% of the forested area (Table 1.1).

Table 1.1 Forest surfaces occupied by dominant tree Species (DGRF 2007).

Dominant Species	Area percentage
Pinus pinaster	23%
Quercus suber	23%
Eucalyptus spp.	21%
Other Oaks	16%
Pinus pinea	3%
Castanea sativa	1%
Other hardwoods	3%
Other softwoods	1%
Young stands	9%

Maritime pine (*Pinus pinaster* Ait.) is a broadly distributed (Alía and Martín, 2003), highly valuable coniferous species (*Pinaceae*). Worldwide, *P. pinaster* is estimated to cover a total area of approximately 4.4 million ha, of which 4.2 million ha are located in the Mediterranean basin and Atlantic coast of Portugal, Spain and France, which corresponds to the natural area of distribution (Figure 1.1). In remaining areas it has settled as an exotic in other reforested areas such as Australia, South Africa, New Zealand, Chile, Argentina and Uruguay (Sanz et al. 2006).

Maritime pine wood has an array of uses such as carpentry, structural engineering material, chipboard, pulp and paper production, floor boards and palettes and as new source of energy. The increasingly higher demand for wood results in greater pressure on forest systems (Barnett and Jeronimidis 2003). Nevertheless, in recent years, society has developed greater environmental awareness, such as encouraging the purchase of products from sustainable forest management, thereby introducing a new variable into the production system. Certification of forest products has become a powerful marketing tool that can influence competitiveness and market access. One of the guidelines in the Portuguese Sustainable Forest Development Plan (DGF 1999) is to develop and ensure competitiveness in the forestry sector, in order to improve quality and productivity of existing forest areas. In our opinion, tree improvement represents an important tool since it allows an increase in forest quality and productivity, and increases awareness of gene resource conservation.

Figure 1.1 Distribution map of maritime pine (in Alia and Martin 2003).

Similar to other countries, Portugal implemented the first measures to improve maritime pine in the 1960s. However, it was only in the 80s that a structured plan was implemented to achieve higher quality individuals, a key component of a modern forestry (Roulund et al. 1988). The first actions consisted in the selection of plus trees in "Mata Nacional de Leiria" by the senior forester D. H. Perry in 1963-1964, followed by the establishment of seed orchards obtained by grafting of selected trees. The selection criteria used was based on volume, stem form, spiral grain and branch habits. Details on the plus tree selection and the scoring system that was employed, are described in Perry and Hopkins (1967). Currently, the Maritime pine breeding programme has reached the second selection generation, and like other improvement plans its deployment is supported through 10 ha clonal seed orchard and 60 ha seedling seed orchard. The importance of this tree improvement plan is well recognised with expected genetic gains of 21% for wood volume and 17% for stem form in newly planted material (Aguiar 2007).

Although there is some knowledge on the genetic variation of growth traits and tree form of *P. pinaster* (Kremer and Lascoux 1988; Alia et al. 1991; Butcher and Hopkins 1993; Hopkins and Butcher 1993, 1994; Danjon 1995; Harfouche et al*.* 1995; Aguiar et al. 2003; Correia et al. 2004), information concerning genetic control of wood quality is limited (Chaperon et al. 1989; Lousada and Fonseca 2002; Pot et al. 2002; Lousada 2003). Until now the market has valorised wood quantity and neglected wood quality. Wood quality is a multi-feature concept that can be studied at different levels (wood density, chemical composition, mechanical traits, and fibre dimension) making its evaluation complex and expensive process. Wood quality is a subjective term, that must be defined in terms of attributes that make it valuable relative to a given use (Jozsa and Middleton 1994).

Wood properties result from the combination of 1) macroscopic morphology – the presence, extent and distribution of different types of wood tissue (e.g. reaction wood, heartwood, knots, growth rings); 2) anatomy - types of cells and biometry, and their proportion; 3) chemical composition - cell wall components and extraneous materials (Pereira et al. 2003). Wood quality characteristics may be inherent to a particular species, but can also be influenced by tree growth condition, as well as many of the traits that are genetically controlled. The wood properties of most conifers vary considerably as a result of variation in fibre morphology within each annual ring formed, horizontal and vertical positions in the stem, between trees and between stands (Zobel and van Buijtenen 1989).

Over time it has been recognised that wood quality and quantity cannot be treated as independent factors and that wood quality improvement should be considered in breeding programmes (Zobel and Talbert 1984; Vargas-Hernandez and Adams 1991; Borralho et al. 1993; Zhang and Morgenstern 1995; Zobel and Jett 1995). Genetic improvement and silviculture have resulted in the reduction of rotation age or an increase in growth rate, resulting in an increase in the proportion of juvenile wood (Kennedy 1995; Hylen 1999; Gapare et al. 2007). In *Pinus* species, juvenile wood is usually characterized by shorter tracheids and thinner cell walls compared to mature wood, which often produces wood with lower specific gravity, mechanical strength and more drying defects. Because of its low strength and instability upon drying, juvenile wood presents limitations for most solid wood products (Zobel and Sprague 1998). One way to reduce some of the negative effects of short-rotation trees on wood quality is to breed for improvement of juvenile-wood characteristics (Nichols et al. 1980; Vargas-Hernandez and Adams 1991; Zamudio et al. 2005).

A serious drawback to the advancement of knowledge of tree improvement in the Mediterranean region is the frequent occurrence of forest fires. Forest improvement research often faces partial or complete loss of the study material before results can be obtained as a result of forest fires, which limits research on adult trees and advances in this research area.

1.2. OBJECTIVES OF THE PRESENT INVESTIGATION

The main objective of this thesis was to study the genetic control of some of the most important wood properties for the wood quality of *P. pinaster* and the phenotypic and genotypic correlation patterns between the several traits. Intra-ring wood density components, wood chemical, mechanical and growth traits, were the traits in study.

With this work we intend to contribute to greater efficiency of Portuguese improvement programme of this specie, through the development of methodologies that allow a better accuracy in the evaluation of the genetic control of the characteristics of growth and quality of wood, using information produced from a test progeny, that was burned by a forest fire in August of 2003, killing most of the trees. Thus, data on wood quality traits and growth will be collected on these trees, and genetic parameters will be estimated.

1.2.1. Layout of this Thesis

This dissertation consists of 8 chapters. The scientific articles that compose the core of this thesis (six chapters) were published (2), in press (2) or submitted (2) to international journals belonging to the Journals Citation Report (JCR).

Chapter 2- Gaspar, M.J., Louzada, J.L., Silva, M.E., Aguiar, A., and Almeida, M.H. 2008. Age trends in genetic parameters of wood density components in 46 half-sibling families of *Pinus pinaster*. Canadian Journal Forest Research 38 (6): 1470-1477.

Chapter 3- Gaspar, M.J., Louzada, J.L., Aguiar, A., and Almeida, M.H. 2008. Genetic correlations between wood quality traits of *Pinus pinaster* Ait. Annals of Forest Science 65 (7): 703.

Chapter 4- Gaspar, M.J., Alves, A., Louzada, J.L., Santos, A., Simões, R., Fernandes, C., Almeida, M.H., and Rodrigues, J.C. Submitted. Phenotypic and genetic correlations between lignin and wood density components in *Pinus pinaster* Ait. Annals of Forest Science.

Chapter 5- Gaspar, M.J., Louzada, J.L., Morais, J., Fernandes, C., Rodrigues, J.C., and Almeida, M.H. Submitted. Genetic variations of wood mechanical traits in 46 half-sib families of *Pinus pinaster* Ait. Silvae Genetica.

Chapter 6- Gaspar, M.J., Louzada, J.L., Rodrigues, J., Aguiar, A., and Almeida, M.H. (in press). Does selecting for improved growth affect wood quality of *Pinus pinaster* in Portugal? Forest Ecology and Management. Doi:10.1016/j.foreco.2009.03.046

Chapter 7- Gaspar, M.J., de-Lucas, A., Alia, R, Paiva, J.A.P., Hidalgo, E., Louzada, J.L., Almeida, M.H., and González-Martínez, S.C. In Press. Use of molecular markers for estimating breeding parameters: a case study in maritime pine progeny trial. Tree Genetic and Genomes. DOI 10.1007/s11295-009-0213-1

1.3. REFERENCES

AEP. 2008. Sector Florestal. Associação Empresarial de Portugal.

Aguiar, A. 2007. Desenvolvimento do programa de melhoramento de Pinheiro bravo e sua contribuição para a maximização do potencial produtivo desta espécie. *In*: Jornadas técnicas: Investigação aplicada na gestão do pinhal, Coimbra.

Aguiar, A., Almeida, M.H., and Borralho, N. 2003. Genetic control of growth, wood density and stem characteristics of Pinus pinaster in Portugal. Silva Lusitana 11 (2): 131-139.

Alía, R., Gil, L., Pardos, J.A., and Catalan, G. 1991. Interacción procedencia-edad en 52 procedencias de *Pinus pinaster* ait. en España. Investigación Agraria- Sistemas y Recursos Forestales 0: 11-24.

Alía, R., and Martín, S. 2003. EUFORGEN Technical guidelines for genetic conservation and use for Maritime pine (*Pinus pinaster*) International Plant Genetic Resources Institute.

Baptista, M. 2000. Protecting forests against fire. In Forests of Portugal. Edited by J. N. Vieira, M. J. Pinto, and R. Pereira. Direcção Geral das Florestas, Lisboa.

Barnett, J.R., and Jeronimidis, G. 2003. Wood Quality and its Biological Basis. Blackwell Publishing, Oxford, 226 pp.

Borralho, N.M.G., Cotterill, P.P., and Kanowski I.P.J. 1993. Breeding objectivs for production of *Eucalyptus globulus* under different industrial cost structures. Can J Forest Res 23 (4): 648-656.

Butcher, T., and Hopkins, E. 1993. Realized gains from breeding *Pinus pinaster* Forest Ecol Manag 58 (3-4): 211-231.

Chaperon, H., Raoux, H., Siohan, A., and Alazard, P. 1989. Variabilité génetique des proprietés technologiques du bois de pin maritime. Annales Afocel, Annales de Recherches sylvicoles 174 (7): 327-345.

Correia, I., Almeida, M.H., and Aguiar, A. 2004. Variabilidade do Crescimento e da Forma de Proveniências de *Pinus pinaster* Aiton aos 8 anos, na Mata Nacional do Escaroupim. Silva Lusitana 12 (2): 151-182.

Danjon, F. 1995. Observed Selection Effects on Height Growth, Diameter and Stem Form in Maritime Pine. Silvae Genet 44 (1): 10-19.

DGF. 1999. Plano de Desenvolvimento Sustentável da Floresta Portuguesa. Direcção Geral das Florestas.

DGRF. 2006. Estratégia Nacional para as Florestas. Direcção Geral dos Recursos Florestais. p. 109.

DGRF. 2007. National Forest Inventory 2005/2006. Direcção Geral dos Recursos Florestais.

Gapare, W., Hathorn, A., Kain, D., Matheson, C., and Wu, H. 2007. Inheritance of spiral grain in the juvenile core of *Pinus radiata*. Can J Forest Res 37 (1): 116-127.

Harfouche, A., Baradat, P., and Durel, C. 1995. Intraspecific variability in Maritime pine (*Pinus pinaster* Ait.) in the South-East of France. 1. Variability in autochthonous populations and in the whole range of species. Ann For Sci 52 (4): 307-328.

Hopkins, E., and Butcher, T. 1993. Provenance comparisons of *Pinus pinaster* Ait. in western Australia. CALMScience 1 (1): 55-105.

Hopkins, E., and Butcher, T. 1994. Improvement of *Pinus pinaster* Ait. in Western Australia. CALMScience 1 (2): 159-242.

Hylen, G. 1999. Age trends in genetic parameters of wood density in young Norway spruce. Can J For Res 29: 135-143.

Jozsa, L.A., and Middleton, G.R. 1994. A discussion of wood quality attributes and their practical implications. Forintek Canada Corp

Kennedy, R.W. 1995. Coniferous wood quality in the future - Concerns and strategies. Wood Sci Technol 29 (5): 321-338.

Kremer, A., and Lascoux, D.M. 1988. Genetic architecture of height growth in maritime pine (*Pinus pinaster* Ait.). Silvae Genet 37 (1): 1-8.

Lopes, D., Aranha, J., Walford, N., e Lucas, N. 2009. Accuracy of remote sensing data *versus* other sources of information for estimating Net Primary Production in *Eucalyptus globulus* and *Pinus pinaster* ecosystems in Portugal. Can J Remo Sens. 35 (1): 1-17.

Lousada, J.L.P.C. 2003. Genetic correlations between wood density components in *Pinus pinaster* Ait. Ann For Sci 60: 285-294.

Lousada, J.L.P.C., and Fonseca, F.M.A. 2002. The heritability of wood density components in *Pinus pinaster* Ait. and implications for tree breeding. Ann For Sci 59: 867-873.

Nichols, J.W.P., Morris, J.D., and Pederick, L.A. 1980. Heritability estimates of density characteristics in juvenil *Pinus radiata* wood. Silvae Genet 29 (2): 54-61.

Pastor, J., and Post, W.M. 1988. Response of northern forest to CO_2-induced climate change. Nature 334 (6177): 55-58.

Pereira, H., Graça, J., and Rodrigues, J.C. 2003. Wood chemistry in relation to quality. In Wood Quality and its Biological Basis. Edited by J. R. Barnett, and G. Jeronimidis. Blackwell Publishing Ltd, Oxford. p. 226.

Perry, D., and Hopkins, E. 1967. Importation of breeding material of *Pinus pinaster* Ait. from Portugal. For. Dep. W. A. Bulletin 75.

Pot, D., Chantre, G., Rozenberg, P., Rodrigues, J.C., Jones, G.L., Pereira, H., Hannrup, B., Cahalan, C., and Plomion, C. 2002. Genetic control of pulp and timber properties in maritime pine (*Pinus pinaster* Ait.). Ann For Sci 59 (5-6): 563-575.

Roulund, H., Alpuim, M., Varela, M.C., and Aguiar, A. 1988. A Tree improvement plan for *Pinus pinaster* in Portugal. EFN.

Sanz, F., Latour, S., Neves, M., Bastet, E., Pischedda, D., Piñeiro, G., Gauthier, T., Lesbats, J., Plantier, C., Marques, A., Lanvin, J.N., Santos, J., Touza, M., Pedras, F., Parrot, J., Reuling, D., and Faria, C. 2006. Industrial Application of *Pinus pinaster*. ATLANWOOD - Industrial Applications from the *Pinus Pinaster.*

Vargas-Hernandez, J., and Adams, W.T. 1991. Genetic-variation of wood density components in young coastal Douglas-Fir - Implications for tree breeding. Can J Forest Res 21 (12): 1801-1807.

Williams, M., Rastetter, E.B., Fernandes, D.N., Goulden, M.L., Shaver, G.R., and Johnson, L.C. 1997. Predicting gross primary in terrestrial ecosystems. Ecological Applications 7 (3): 882-894.

Zamudio, F., Rozenberg, P., Baettig, R., Vergara, A., Yanez, M., and Gantz, C. 2005. Genetic variation of wood density components in a radiata pine progeny test located in the south of Chile. Ann For Sci 62 (2): 105-114.

Zhang, S.Y., and Morgenstern, E.K. 1995. Genetic-variation and inheritance of wood density in black spruce (*Picea mariana*) and its relationship with growth - implications for tree breeding. Wood Sci Technol 30 (1): 63-75.

Zobel, B.J., and Jett, J.B. 1995. Genetics of Wood Production. Springer-Verlag ed, Berlin, Heidelberg, and New York, 377 pp.

Zobel, B.J., and Sprague, J.R. 1998. Juvenil Wood in Forest Trees. Springer-Verlag ed, Berlin, Heidelberg, and New York, 300 pp.

Zobel, B.J., and Talbert, J. 1984. Applied Forest Tree Improvement. John Wiley & Sons, New York, 500 pp.

Zobel, B.J., and van Buijtenen, J.P. 1989. Wood Variation - Its causes and control. Springer-Verlag ed, Berlin, 363 pp.

CHAPTER 2

Age trends in genetic parameters of wood density components in 46 half-sibling families of *Pinus pinaster* Ait.

Published as: Gaspar, M.J.; Louzada, J. L.; Silva, M. E.; Aguiar A. and Almeida, M. H. 2008. Age trends in genetic parameters of wood density components in 46 half-sibling families of *Pinus pinaster* Ait. Canadian Journal of Forestry Research 38: 1470-1477.

2.1. ABSTRACT

This study contributes to the *Pinus pinaster* Ait. breeding programme, which is reaching the third generation, by adding information on wood quality of 46 open-pollinated families from a progeny trial located in Leiria, Portugal, that originated from seed collected in a clonal seed orchard. A total number of 552 seventeen-year-old trees were sampled at 2m height. Trends were studied from the pith outward in variance components and heritability (h_i^2) of wood density components and ring-width characteristics as well as genetic correlations between cambial ages. Mean ring density (RD), minimum density (MND), maximum density (MXD), earlywood density (EWD), latewood density (LWD), earlywood width (EWW), latewood width (LWW), ring width (RW), latewood percentage (LWP) and heterogeneity index (HI),were determined using X-ray densitometry procedures. RD had higher genetic control (h_i^2=0.63), and heritability values of earlywood components ($h_{i\,(\mathrm{MND})}^2$ = 0.54, $h_{i\,(\mathrm{EWD})}^2$=0.60) exceeded those of latewood components ($h_{i\,(\mathrm{MXD})}^2$=0.34; $h_{i\,(\mathrm{LWD})}^2$=0.26). Heritabilities increased with ring number from pith for almost all wood-density components, and there were high age-age genetic correlations for wood density traits (r_g> 0.98).

2.2. INTRODUCTION

Pinus pinaster Ait. (Maritime pine) is one of the most important native species in Portugal, occupying 23% of the national forestry area (DGRF, 2007) and representing 11% of the total value of forest product exports in 2000 (Correia et al. 2004). The economic importance of this species has led to the development of genetic improvement initiatives. A national tree improvement plan has been in operation since the early 1980s (Roulund et al. 1988) with the aim of increasing volume per hectare and improving stem straightness, which has traditionally been one of the species drawbacks. Currently, the maritime pine breeding programme has reached two generations of selection; the progeny trial reported in this study is part of this programme. This trial contains plants produced 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, 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 about the plus phenotypes selected and of scoring system employed, are described in (Perry and Hopkins 1967).

It is widely recognised that wood quality and quantity cannot be treated in isolation from each other, and that wood quality improvement should be integral to breeding programmes (Zobel and Jett 1995; Zobel and Talbert 1984). Furthermore, reducing rotation age or increasing growth rate typically incurs the risk of increasing the proportion of juvenile wood (Hylen 1999; Kennedy 1995). In *Pinus* species, juvenile wood is usually characterized by shorter tracheids and thinner cell walls than mature wood, which often produces wood of lower specific gravity. Because of its low strength and instability upon drying, juvenile wood still presents a problem for most solid wood products (Zobel and Sprague 1998). One way of reducing some of the negative effects of short-rotation trees on wood quality is to breed to increase juvenile-wood density (Gapare et al. 2006; Zamudio et al. 2005). Further, a trait in mature wood that is highly correlated with its value in juvenile material also presents advantages in the breeding programme, because the overall length of the programme will be reduced; hence, forest tree breeding will be more effective. Early selection would speed up the breeding process and increase the genetic gain achieved per unit time (Lambeth 1980; Mckeand 1988). A prerequisite of early tests is a high correlation between the expression of the juvenile and mature traits (Nanson 1970).

Among the desirable wood quality properties to breed for, wood density is considered the most significant for improvement (Zobel and van Buijtenen 1989) because of its close relationship with important properties related to technological traits, and because it is relatively easy to measure. However, given the complex nature of this trait, variations in wood density can be difficult to interpret, because a particular wood density value can be derived from various combinations of components. Overall ring density depends on earlywood (EW) and latewood (LW) densities, and the relative proportions of each (Vargas-Hernandez and Adams 1991).

Wood uniformity is another characteristic benefit to be expected from the application of genetics to wood properties (Zobel and van Buijtenen 1989). Wood heterogeneity is an important defect, and uniformity of juvenile wood is usually lower than that of mature wood (Zobel and Jett 1995; Zobel and van Buijtenen 1989). Thus, reducing variability is usually a main objective of a breeding program involving wood properties. The knowledge of genetic control of these characteristics would contribute to a better understanding of the genetics of overall wood density, permitting manipulation through the alteration of one or more components and, ultimately, leading to a possible increase in the efficiency of selection for this trait (Louzada and Fonseca 2002). Studies on different species have found that wood density is usually a trait with strong genetic control (for review see Zobel and van Buijtenen 1989). However, there are contradictory results concerning density components.

The aim of this study is to evaluate the inheritance of mean ring density (RD) and its components at different cambial ages. The work contributes to the *Pinus pinaster* breeding programme by adding information on wood quality of 46 open-pollinated families that belong to the first generation of the maritime pine breeding programme.

2.3. MATERIALS AND METHODS

The study trial site was Pinhal de Leiria (established in 1987), located in central coastal Portugal (lat 39°50’, long 8°55’, alt 30m). The site has a Mediterranean climate, with a mean air temperature of 14.2°C, a mean annual rainfall of 880 mm, and Spodic Podzols soils derived from costal sand dunes. Seedlings were raised for 9 months in the nursery in plastic bags (250cm^3 volume). Seedlings were planted at 2m×2m spacing in March 1987 after harrowing and ploughing the site; no fertilizer was applied. The trial included 46 open-pollinated families that were 17 years old, which originated from seed collected in the Escaroupim clonal seed orchard II (Aguiar 1993).

The 46 families were replicated in 3 blocks with 4 trees per plot, giving a total of 552 trees for analysis. Trees were sampled at 2m height. One internodal wood disc, 10cm thick, was collected and sawn into a 2mm thick radial strip segment from the pith to bark. The strips were conditioned at 12% moisture content. Radial samples were X-rayed perpendicular to the transverse section and their images scanned by microdensitometric analysis (Hughes and Sardinha 1975; Polge 1978). The time of exposure to radiation was 300s, at an intensity of 18mA and an accelerating tension of 12kV, with a 2.5m distance between X-ray source and film. The data comprising the radial density profiles were recorded every 100μm with a slit height (tangential direction) of 455μm.

The first and last annual rings of each sample were excluded since they were usually incomplete. Growth-ring boundaries were identified on the radial profiles by locating the sharp density variations through visual observation of the macroscopic anatomical features. For each ring, average (RD), minimum density (MND), maximum density (MXD), earlywood density (EWD), latewood density (LWD), earlywood width (EWW), latewood width (LWW), ring width (RW) and latewood percentage (LWP) were determined. The EW-LW boundary in each growth ring was assigned a fixed density of 500Kg m^{-3}. This density has been used for several conifer species (Barbour et al. 1994; Fujimoto et al. 2006), even though some researchers have been using the average of minimum and maximum ring density to define the transition point for EW/LW (Nicholls et al. 1980; Vargas-Hernandez and Adams 1991; Zamudio et al. 2005). The advantages of using a fixed density to determine the EW-LW boundary have been well explained by Rudman (1968) and Jozsa et al. (1987). This value of 500Kg m^{-3} was a result of an adjustment to the value proposed by Louzada (2000) who compared different criteria and indicated that this is the best density for 18 years old *Pinus pinaster*. Although this criterion does not allow identifying the beginning of the LW, it can be used to designate the portions of the ring with a density higher than the threshold as LW. Intra-ring density variation was quantified using the heterogeneity index (HI) proposed by Ferrand (1982), defined as the standard deviation of all density values across the annual ring. Because rings close to the pith have less volume than those near the bark, they contribute less to the whole disc volume. To compensate for this effect, wood density components were weighed for each ring by its respective cross-sectional area.

2.3.1. Data analysis

All traits, for every ring, were analysed using the following model:

$$Y_{ijk}=\mu + B_j + F_i + B\times F_{ij} + \varepsilon_{ijk} \qquad [2.1]$$

where Y represents the phenotypic individual observation, μ is the overall mean, B_j is the effect of the jth block (fixed), F_i is the effect of the ith family (random), $B\times F_{ij}$ is the effect of the statistical interaction between the ith family and the jth block (random); and ε is the residual error. Variance components for family (σ_f^2), family-block interaction ($\sigma_{f\times b}^2$) and residual errors (σ_ε^2), with their standard errors, were estimated by restricted maximum likelihood, using the average information REML algorithm implemented in the ASREML program (Gilmour et al. 1998).

Narrow sense heritability (h_i^2) was estimated for each trait measured at each cambial age (ring number) as:

$$h_i^2 = \frac{\sigma_a^2}{\sigma_P^2} \qquad [2.2]$$

where σ_a^2 represents the additive genetic variance and σ_P^2 represents the total phenotypic variance. Families were assumed to be maternal half-siblings, therefore the $\sigma_a^2 = 4\sigma_f^2$ relationship was assumed to estimate the genetic parameters. It should be noted that the half-sib relation is an assumption that can be violated to some degree and an inherent upward bias in estimates of additive genetic variance can be expected.

Phenotypic variance was estimated as $\sigma_P^2 = \sigma_f^2 + \sigma_{f\times b}^2 + \sigma_\varepsilon^2$. Standard errors for heritability were estimated by ASREML using a Taylor series approximation (Gilmour et al. 1998).

Genetic correlations within the same trait measured at two different cambial ages (*X* and *Y*) were evaluated as follows using a multivariate extension of equation (2.1).

$$r_a = \frac{Cov_a(x,y)}{\sqrt{\sigma^2_{a_x}.\sigma^2_{a_y}}} \qquad [2.3]$$

where $cov_a(x,y)$ is the additive genetic covariance between two different cambial ages, and $\sigma^2_{a_x}$ and $\sigma^2_{a_y}$ are the additive variance components for cambial ages *X* and *Y*, respectively.

2.4. RESULTS AND DISCUSSION

Table 2.1 lists summary descriptive statistics for different wood density components and respective heritability estimates. The RD (474Kgm^{-3}), LWD (618Kgm^{-3}), and EWD (386Kgm^{-3}) are similar to values reported elsewhere (Louzada and Fonseca 2002; Markussen et al. 2003) for young *P. pinaster* trees.

Table 2.1 Descriptive statistics table for different wood density components weighted for the last ring and respective heritability estimates (standard errors given in brackets).

Trait	Mean	Std. Dev.	Coeff. Var.	Min	Max	h^2_i (se)
RD (kgm^{-3})	474	0.051	10.68	336	655	0.63 (0.190)
MND (kgm^{-3})	308	0.037	12.11	207	445	0.54 (0.184)
MXD (kgm^{-3})	707	0.060	8.51	558	917	0.34 (0.164)
EWD (kgm^{-3})	386	0.026	6.69	304	463	0.60 (0.191)
LWD (kgm^{-3})	618	0.034	5.43	535	732	0.26 (0.141)
LWP (%)	38.196	16.927	44.31	6.420	82.240	0.46 (0.168)
EWW (mm)	2.674	0.876	32.74	0.900	6.100	0.32 (0.137)
LWW (mm)	1.556	0.907	58.29	0.300	6.400	0.31 (0.156)
RW (mm)	4.224	0.901	21.33	2.500	8.600	0.10 (0.111)a
HI (kgm^{-3})	120	0.019	16.05	7	196	----- a

RD = Average Ring Density, MND = Minimum Density, MXD = Maximum Density, EWD = Earlywood Density, LWD = Latewood Density, LWP = Latewood Percentage, EWW= Earlywood Width, LWW= Latewood Width, RW = Ring Width, HI = Heterogeneity Index; a: in the analysis of variance the differences among families were not significant ($p > 0.05$); ---- the heritability value was quantified with the null value

Considering these values (LWD 1.6 times higher than the EWD), we can conclude that this wood can be considered homogeneous when compared with *Pseudotsuga menziesii* (Mirb.) Franco, where the LW is 3.5 to 4 times as dense as EW (Polge 1963). For the ring width characteristics EWW is larger (2.67mm) than LWW (1.56mm), which also has the larger coefficient of variation (CV). All the growth variables (RW, EEW, LWW and LWP) have CVs > 21%, whereas wood density variables tend to have coefficients <12%. Mean values of individual rings for the traits LWD and MXD increased throughout the core (Fig. 2.1). For the other density traits (RD, MND, EWD) the density values decreased over the first successive rings, then increased. Both RW and EWW increased remarkably in the first three rings, followed by a gradual decline, while LWW was much more constant (Fig. 2.1). Latewood proportion started with a significant decrease but, at age 4 began to increase gradually to a mean of 40% at ring number 11 (Fig. 2.1). The pronounced decline in LWP was due to an enlargement in EWW.

RD was the trait under strongest genetic control (h_i^2 = 0.63) (Table 2.1), which is consistent with results from previous studies on this species. Chaperon et al. (1989) estimated an h_i^2 value of 0.44 for specific density, whereas Louzada and Fonseca (2002) obtained higher heritability values, varying from 0.53 to 0.74, for average ring density. However, on 15-year-old trees of the same species in France Pot et al. (2002) obtained considerably lower values (h_i^2=0.29) for the same trait. Differences may result from distinct environmental conditions since the heritability of a certain trait can vary with the population, age and test location, or from random estimation errors.

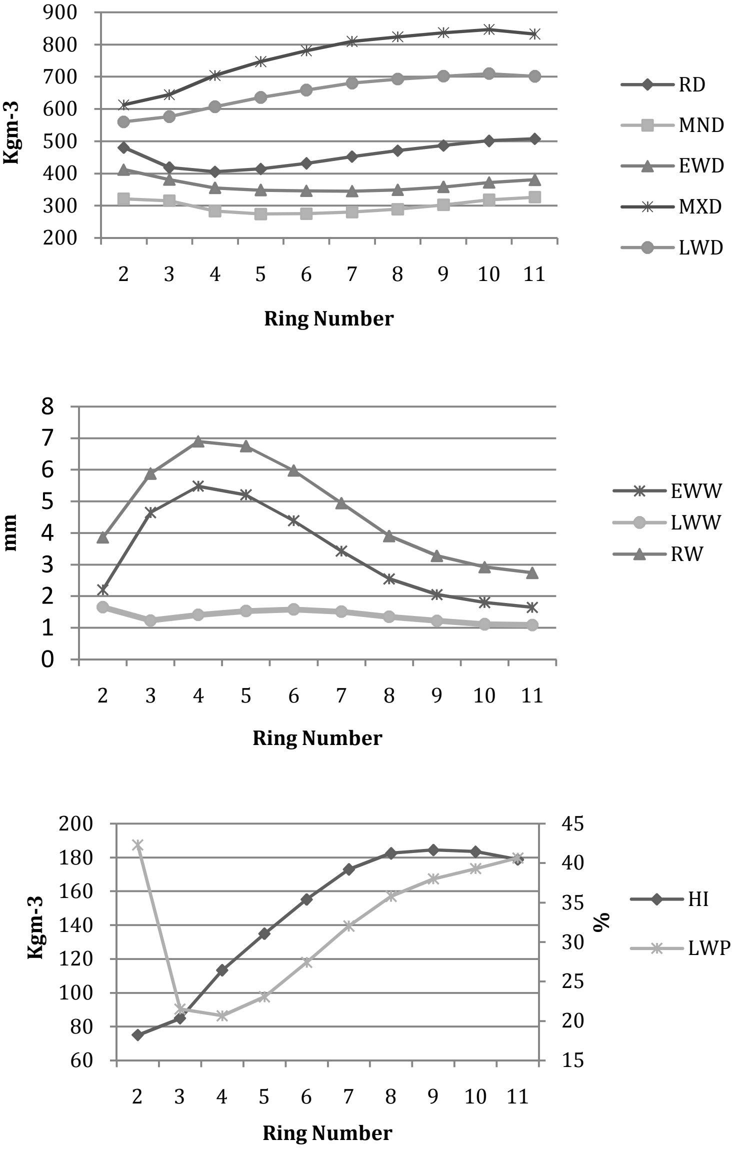


Figure 2.1 Average values of individual rings for all density components until ring 11.

Studies on other *Pinus* species reveal a multiplicity of results with heritability estimates ranging from 0.43 to 0.85 (Kumar 2002, 2004; Nicholls et al. 1980; Zamudio et al. 2002) for *P. radiata*, and (Talbert et al. 1983) for *P. taeda*. Heritability estimates for EW components ($h^2_{i\,(\mathrm{MND})}$=0.54, $h^2_{i\,(\mathrm{EWD})}$=0.60) are superior to those obtained for LW components ($h^2_{i\,(\mathrm{MXD})}$=0.34; $h^2_{i\,(\mathrm{LWD})}$=0.26), confirming that for *P. pinaster*, EW characteristics are subject to stronger genetic control and that LW components are almost entirely subject to environmental factors. In fact, LWD appears to be more sensitive to climatic fluctuations (Lebourgeois 2000). Xylogenesis (wood formation) is controlled by both exogenous (ex: photoperiod, water availability and temperature) and endogenous (phytohormones) (Paiva 2006). According to Plomion et al. (2001) in temperate zones EW is formed early in the growing season when temperature, water availability and photoperiod are optimal for active growth. EW has thin walled tracheids or fibers with a large radial diameter. The LW, which is formed in summer or autumn when cambial cell division and expansion rates decline, has narrower lumens and thicker cell walls. Spring provides optimal conditions for tree growth and it is during this period that genetic effects stand out. In summer, growth conditions are less stable due to high temperatures and water stress, which may increase the phenotypic variance, leading to reduced heritability.

In this study, density components varied significantly among families and displayed high genetic control; however, none of them presented a higher heritability than RD, even though there were slightly higher levels for the EW components (MND and EWD). These results are consistent with those obtained for *Pinus radiata* (Nicholls et al. 1980), *Pseudotsuga menziesii* (Vargas-Hernandez and Adams 1991; 1992) and *Cryptomeria japonica* (Fujisawa et al. 1993). In contrast, other studies on *P. pinaster* (Louzada and Fonseca 2002), *P. radiata* (Kumar 2002) and *Picea mariana* (Zhang 1998; Zhang and Jiang 1998; Zhang and Morgenstern 1995) indicate that EWD is the component most dependent on genetic effects.

In theory, these components are of limited value in improving selection efficiency for wood density (Vargas-Hernandez and Adams 1991). Several authors (Vargas-Hernandez and Adams 1992; Zhang 1998; Zhang and Jiang 1998; Zhang and Morgenstern 1995) observed no significant gain in the target trait when using the EW components, compared to those obtained using RD, meaning that inclusion of these components would not augment wood density. In contrast, the results of Louzada and Fonseca (2002), indicate that selection on the basis of EW components could result in increased wood density and a decrease in ring heterogeneity. Indeed, wood uniformity (see Zobel and Jett 1995) could be cited as one of the wood properties most closely associated with profitability (being much sought after by product managers). Nevertheless, the inconsistency of results between studies highlights the importance in identifying the genetic control of these traits.

For other ring characteristics, variation among families did not produce statistically significant differences ($p>0.05$) for RW and HI. Variation of these two components is most likely due to environmental factors, not genetic ones. The low estimates values for heritability are easily understandable since growth characteristics (diameter) often exhibit relatively modest heritabilities (Cotterill et al. 1987; Harfouche et al. 1995; Hopkins and Butcher 1994). In a study by Aguiar et al. (2003) carried out after 12 years in this progeny trial, heritability of diameter was estimated at 0.18. Remaining ring characteristics (LWP, LWW and EWW) displayed intermediate heritability. Our estimated heritabilities for these traits are similar to those from previous studies of *Pinus pinaster* (Louzada and Fonseca 2002) and other conifer species (Fujisawa et al. 1993; Kumar 2002; Nicholls et al. 1980; Vargas-Hernandez and Adams 1991, 1992; Zhang and Jiang 1998; Zhang and Morgenstern 1995). Although we observed low heritabilities for RW, the moderate values obtained for EWW and LWW provide a basis for the genetic manipulation of the wood quantity and quality of this species, in the absence of adverse genetic correlations with other density components.

2.4.1. Trends in heritability with ring number

This study allows the assessment of temporal change in the genetic control of these characteristics, because the heritabilities of the different wood properties were estimated ring by ring. This is important, because genetic control of wood frequently changes with ring number from the pith (Zobel and Jett 1995). Only density components showed statistically significant family variation (RW and HI were excluded) in all rings (Table 2.2 and Figure 2.2), the evolution of estimated heritabilities and of additive and phenotypic variances with ring numbers.

Table 2.2 Estimated heritability values (with standard errors given in brackets) of wood density components for rings 2-11.

RING	RD	MND	MXD	EWD	LWD		EWW	LWW	RW		LWP	HI	
2	0.49	0.20	0.37	0.28	0.40		0.29	0.32	0.08	a	0.40	0.078	a
	(0.169)	(0.138)	(0.148)	(0.138)	(0.147)		(0.133)	(0.154)	(0.107)		(0.157)	(0.131)	
3	0.45	0.21	0.37	0.31	0.40		0.29	0.33	0.08	a	0.40	0.077	a
	(0.170)	(0.139)	(0.148)	(0.143)	(0.147)		(0.135)	(0.155)	(0.108)		(0.157)	(0.132)	
4	0.45	0.23	0.39	0.33	0.41		0.28	0.32	0.08	a	0.40	0.092	a
	(0.1712)	(0.142)	(0.150)	(0.148)	(0.148)		(0.133)	(0.154)	(0.109)		(0.158)	(0.133)	
5	0.51	0.26	0.38	0.35	0.40		0.29	0.31	0.06	a	0.40	0.094	a
	(0.173)	(0.146)	(0.150)	(0.153)	(0.148)		(0.132)	(0.154)	(0.109)		(0.158)	(0.134)	
6	0.52	0.29	0.40	0.37	0.41		0.29	0.32	0.06	a	0.41	0.098	a
	(0.176)	(0.151)	(0.154)	(0.158)	(0.149)		(0.132)	(0.157)	(0.112)		(0.160)	(0.135)	
7	0.54	0.33	0.41	0.40	0.40		0.31	0.33	0.06	a	0.42	0.092	a
	(0.179)	(0.158)	(0.155)	(0.166)	(0.149)		(0.136)	(0.158)	(0.113)		(0.161)	(0.135)	
8	0.57	0.38	0.42	0.44	0.38		0.33	0.32	0.10	a	0.43	0.065	a
	(0.181)	(0.166)	(0.157)	(0.172)	(0.145)		(0.139)	(0.157)	(0.117)		(0.163)	(0.130)	
9	0.60	0.42	0.38	0.50	0.33		0.33	0.34	0.10	a	0.45	---	a
	(0.184)	(0.171)	(0.156)	(0.179)	(0.141)		(0.140)	(0.158)	(0.115)		(0.164)		
10	0.60	0.46	0.31	0.55	----	a	0.32	0.32	0.11	a	0.45	---	a
	(0.186)	(0.176)	(0.156)	(0.185)			(0.138)	(0.158)	(0.116)		(0.166)		
11	0.60	0.50	0.30	0.56	0.13	a	0.31	0.32	0.11	a	0.45	---	a
	(0.187)	(0.179)	(0.156)	(0.187)			(0.137)	(0.157)	(0.113)		(0.167)		

Note: See table 2.1 for abbreviations
a: the differences between Families were not significant ($P > 0.05$).
---- the heritability value was quantified with the null value.

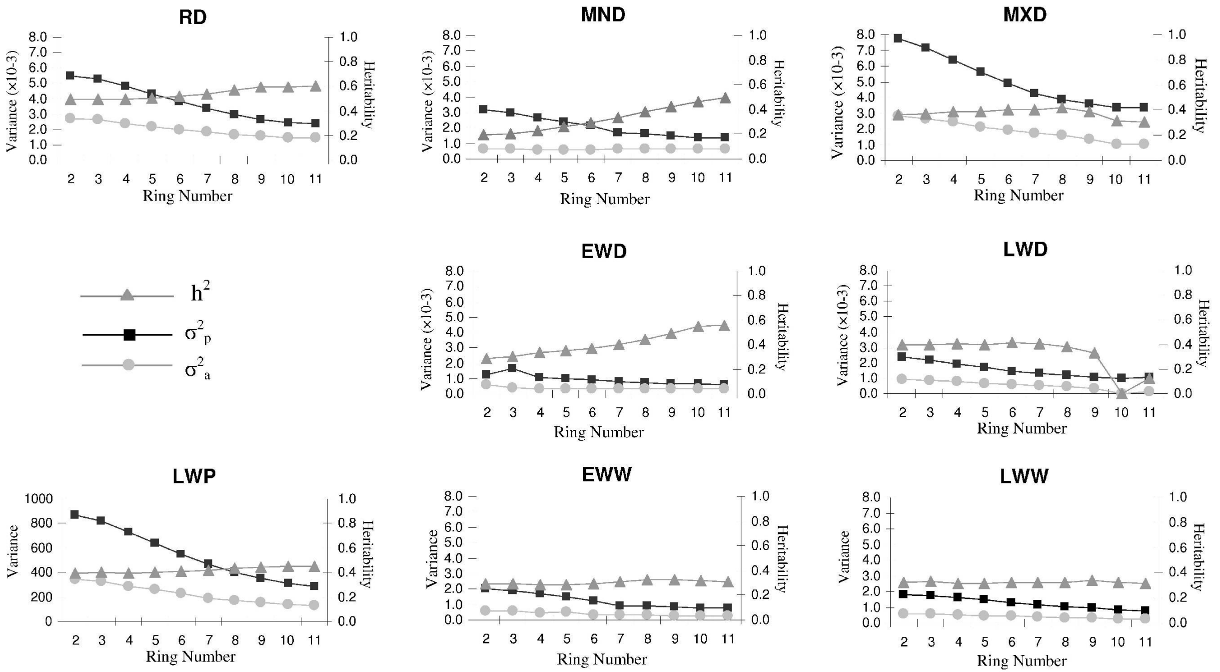


Figure 2.2 Age trends in phenotypic (σ_P^2) and additive (σ_a^2) variance components, and individual heritability, for average ring density and its componets

Heritability estimates increase with ring number for most density components except for LWD and MXD, which showed decreases from ring 7. Phenotypic variance decreased with age across all measured density components. This initial period of high phenotypic variance could be due to juvenile trees sensitivity to climate fluctuations, land preparation, establishment history (Louzada and Fonseca 2002), meaning that genetic potential can only be expressed from the 7th to 9th year of cambial age. The observed increase of the estimated heritabilities in the components analysed was never attributable to enhanced additive variance. Additive variance is higher in components: RD, MXD and LWP. However, only RD presents high heritability whereas MXD and LWP exhibit both high additive variance and phenotypic variance, resulting in low heritabilities. This indicates that LW formed during the summer months is subject to strong environmental influences that largely override the genetic factors.

Results from other species concur that wood density is subject to strong genetic control. However, conflicting results have been reported concerning changes in genetic control for wood density and its components with ring number. Previous studies have found similar increases in the heritability of RD and its components. For example, in *Pseudotsuga menziesii*, Vargas-Hernandez and Adams (1992) reported that heritability estimates increased with age for all density traits.

Hylen (1999) observed a similar pattern in young *P. abies* (L.) Karst. More recently, Li and Wu (2005) observed an increase of heritability values to 0.4 in *P. radiata*, for area-weighted density over 14 rings from the pith, a value that remained stable in older trees. In contrast, in *P. pinaster*, Louzada and Fonseca (2002) found an initial increase in the heritability values of the density features followed by a slight decrease. They also found a tendency for increased heritability up until sixth to eighth ring followed by stabilization in the genetic control of the evolution of LWP and RW characteristics. In *Pinus elliottii* Engelm., Hodge and Purnell (1993) observed that heritabilities for density components in the rings near the pith were slightly higher than those nearer the bark. Nicholls (1980) reported that the heritability of basic density in *P. radiata* decreased from the pith outward, reaching a minimum at approximately the ninth growth ring from the pith, followed by an increase in heritability with further increase in age. It is possible that the considerable variability of results in the estimates of genetic parameters in previous studies could have been affected by several factors such as site conditions, genetic material, experimental and measurement procedures for wood density, as well as other components (Wright 1976; Zobel and Jett 1995). Moreover, limiting environmental factors can occur in the spring (EW) in some regions and summer (LW) in other regions. RD, MND and EWD are shown to be highly heritable traits, suggesting that large gains can be obtained for these characters.

2.4.2. Age-age genetic correlations

In this context, it is essential to know not only the heritability value of a specific character, but also how that characteristic is correlated age by age and with others factors in order to fully assess the implications of its selection.

Results in Table 2.3 express the age-age genetic correlations between three different ring ages. Age-age genotypic correlations between the third and sixth rings were close to 1 for most wood density components. Correlations between wood density traits at sixth and eleventh ring were always higher than those between the third and the eleventh in all traits. For most characteristics the high correlation values (> 0.9) clearly indicate that it does not compensate to wait 6 or 11 years for evaluation since the third ring produces identical information. This is a very important result, regarding the advantages of early selection. According to Wu et al. (1998), there are three main advantages early selection in tree breeding: (i) increased election intensity or reduced field testing size; (ii) a shortened generation interval; and (iii) genetic information from early testing can be used to enhance selection efficiency at mature age. However, in the traits EWD, LWD, and EWW it may be preferable to wait until the sixth year in order to carry out a more accurate evaluation of individuals.

Table 2.3 Age-age genetic correlation (SEs in parentheses) among three different ring numbers (3, 6 and 11) for each wood density components.

TRAIT	Ring Ring	6	11
RD	3	0.990 (0.005)	0.933 (0.031)
	6	-	0.973 (0.014)
MND	3	0.989 (0.008)	0.932 (0.046)
	6	--	0.978 (0.019)
MXD	3	0.979 (0.012)	0.900 (0.061)
	6	-	0.937 (0.037)
EWD	3	0.981 (0.012)	0.855 (0.073)
	6	-	0.940 (0.033)
LWD	3	0.980 (0.011)	0.874 (0.140)
	6	-	0.922 (0.124)
EWW	3	0.972 (0.016)	0.880 (0.065)
	6	--	0.968 (0.022)
LWW	3	0.995 (0.003)	0.986 (0.008)
	6	-	0.996 (0.003)
LWP	3	0.997 (0.002)	0.980 (0.011)
	6	-	0.992 (0.005)
RW	3	0.900 (0.090)	0.652 (0.269)
	6	-	0.915 (0.077)

Note: See Table 2.1 for abbreviations

According to Hylen (1999) this can indicate that accumulation over rings smoothes out environmental effects and that genetic control of traits is more strongly manifested. RW correlations values between the third and eleventh ring (r_g=0.65) are considerably inferior to those of the remaining characteristics. However, between the sixth and eleventh ring, the value is 0.92; thus, it can be assumed that families with larger growth rates at sixth ring retain this tendency in subsequent ages. This indicates that growth characteristics are not genetically controlled to the same extent as the remaining traits, suggesting that early tests for *P. pinaster* should increase the efficiency of the tree-breeding programme, for at least some characteristics. Zobell and Jett (1995) state that even when this age-age correlation is fairly weak, such early evaluations are valuable in eliminating families with undesirable wood density properties.

Our results are consistent with previous studies on *P. pinaster* (Louzada 2003) namely that for RD, MND and EWD traits, the high genetic correlation between young and older ages makes it possible to select at a very young cambial age. Studies on other species also indicate that age-age genetic correlations for wood density traits tend to be high (Hylen 1999; Kumar 2002; Loo et al. 1984; Talbert et al. 1983; Vargas-Hernandez and Adams 1992; Williams and Megraw 1994; Zobel and van Buijtenen 1989). An extreme example is the finding of Nepveu and Birot (1979, *cit in* Zobel and van Buijtenen 1989) in *P. abies*, where they found that adult specific gravity could be predicted even from a single ring at the juvenile stage.

2.5. CONCLUSIONS

This study indicates that in *P. pinaster* RD is the most heritable of the variables studied, suggesting that large gains can be obtained from selecting on the basis of this variable. Estimated heritabilities for the EW components (MND, EWD) were superior to those obtained from LW components (MXD, LWD), indicating that, in this species, earlywood characteristics are subject to stronger genetic control than latewood components which depend more on environmental factors. Pith-to-bark trends show that heritability values increase with age for almost all wood-density components and that age-age genetic correlations for wood density components are generally high. The genetic correlation between ring 2 and 10 were always greater than 0.97, except for EWW, EWD and LWD, allowing early selection for these traits.

Acknowledgements

M.J. Gaspar was funded by the Portuguese Foundation for Science and Technology (Doctoral grant SFRH/BD/27412/2006).

2.6. REFERENCES

Aguiar, A. 1993. Análise da variação do crescimento em altura de descendências maternais de pinheiro bravo. Silva Lusitana 1 (2): 267-274.

Aguiar, A., Almeida, M.H., and Borralho, N. 2003. Genetic control of growth, wood density and stem characteristics of *Pinus pinaster* in Portugal. Silva Lusitana 11 (2): 131-139.

Barbour, R.J., Fayle, D.C.F., Chauret, G., Cook, J., Karsh, M.B., and Ran, S.K. 1994. Breast-height relative density and radial growth in mature Jack pine (*Pinus banksiana*) for 38 years after thinning. Can. J. For. Res. 24 (12): 2439-2447.

Chaperon, H., Raoux, H., Siohan, A., and Alazard, P. 1989. Variabilité génetique des proprietés technologiques du bois de Pin Maritime. Annales Afocel, Ann. Rech. Sylvic 174 (7): 327-345.

Correia, I., Almeida, M.H., and Aguiar, A. 2004. Variabilidade do crescimento e da forma de proveniências de *Pinus pinaster* Aiton aos 8 anos, na Mata Nacional do Escaroupim. Silva Lusitana 12 (2): 151-182.

Cotterill, P.P., Dean, C.A., and Vanwyk, G. 1987. Additive and dominance genetic effects in *Pinus pinaster*, *Pinus radiata* and *Pinus elliottii* and some implications for breeding strategy. Silvae Genet. 36 (5-6): 221-232.

DGRF. 2007. National Forest Inventory 2005/2006. Direcção Geral dos Recursos Florestais.

Ferrand, J.C. 1982. Réflexions sur la densité du bois. 2ère Partie: Calcule de la densité et de son hétérogénéité. Holzforschung 36 (3): 153-157.

Fujimoto, T., Akutsu, H., Kita, K., Uchiyama, K., Kuromaru, M., and Oda, K. 2006. Age trends of genetic parameters of spiral grain in hybrid larch F-1 and implications for efficiency of early selection. J. Wood. Sci. 52 (2): 101-106.

Fujisawa, Y., Ohta, S., and Tajima, M. 1993. Wood characteristics and genetic variations in sugi (*Cryptomeria japonica*) 2. Variation in growth ring components among plus-trees clones and test stands. Mokuzai Gakkaishi 39 (8): 875-882.

Gapare, W.J., Wu, H.X., and Abarquez, A. 2006. Genetic control of the time of transition from juvenile to mature wood in *Pinus radiata* D. Don. Ann For Sci 63 (8): 871-878.

Gilmour, A., Gogel, B., Cullis, B., Welham, S., and Thompson, R. 1998. ASREML Users Manual. *In* VSN International. New south Wales Agriculture, Orange.

Harfouche, A., Baradat, P., and Durel, C. 1995. Intraspecific variability in Maritime pine (*Pinus pinaster* Ait.) in the South-East of France. 1. Variability in autochthonous populations and in the whole range of species. Ann For Sci 52 (4): 307-328.

Hodge, G.R., and Purnell, R.C. 1993. Genetic parameter estimates for wood density, transition age, and radial growth in Slash pine. Can. J. For. Res. 23 (9): 1881-1891.

Hopkins, E., and Butcher, T. 1994. Improvement of *Pinus pinaster* Ait. in Western Australia. CALM Science 1 (2): 159-242.

Hughes, J.F., and Sardinha, R.M.A. 1975. The application of optical densitometry in the study of wood structure and properties. J Microsc 104.

Hylen, G. 1999. Age trends in genetic parameters of wood density in young Norway spruce. Can J For Res 29: 135-143.

Jozsa, L., Richards, J., and Johnson, S. 1987. Calibration of Forintek's direct reading x-ray densitometer. Report No 36a. Forintek Canada Corp., Vancover, p.16.

Kennedy, R.W. 1995. Coniferous wood quality in the future - Concerns and strategies. Wood Sci Technol 29 (5): 321-338.

Kumar, S. 2002. Earlywood-latewood demarcation criteria and their effect on genetic parameters of growth ring density components and efficiency of selection for end-of-rotation density of Radiata pine. Silvae Genet 51 (5-6): 241-246.

Kumar, S. 2004. Genetic parameter estimates for wood stiffness, strength, internal checking, and resin bleeding for Radiata pine. Can J For Res 34 (12): 2601-2610.

Lambeth, C.C. 1980. Juvenile-mature correlations in Pinaceae and implications for early selection. Forest Sci 26 (4): 571-580.

Lebourgeois, F. 2000. Climatic signals in earlywood, latewood and total ring width of Corsican pine from western France. Ann For Sci 57: 155-164.

Li, L., and Wu, H.X. 2005. Efficiency of early selection for rotation-aged growth and wood density traits in *Pinus radiata*. Can J For Res 35 (8): 2019-2029.

Loo, J.A., Tauer, C.G., and van Buijtenen, J.P. 1984. Juvenil mature relationships and heritability estimates of several traits in loblolly pine (*Pinus taeda*). Can J For Res 14: 822-825.

Louzada, J.L.P.C. 2000. Variação fenotípica e genética em características estruturais na madeira de *Pinus pinaster* Ait. *In* Departamento Florestal. Universidade de Trás-os-Montes e Alto Douro, Vila Real. 293 pp.

Louzada, J.L.P.C. 2003. Genetic correlations between wood density components in *Pinus pinaster* Ait. Ann For Sci 60: 285-294.

Louzada, J.L.P.C., and Fonseca, F.M.A. 2002. The heritability of wood density components in *Pinus pinaster* Ait. and implications for tree breeding. Ann For Sci 59: 867-873.

Markussen, T., Fladung, M., Achere, V., Favre, J.M., Faivre-Rampant, P., Aragones, A., Perez, D.D., Harvengt, L., Espinel, S., and Ritter, E. 2003. Identification of QTLs controlling growth, chemical and physical wood property traits in *Pinus pinaster* (Ait.). Silvae Genet 52 (1): 8-15.

Mckeand, S.E. 1988. Optimum age for family selection for growth in genetic tests of Loblolly-pine. Forest Sci 34 (2): 400-411.

Nanson, A. 1970. Juvenile and correlated trait selection and its effect on selection programs. *In* 2nd Meeting of Working Group on Quantitative Genetics IUFRO, Louisiana.

Nicholls, J.W.P., Morris, J.D., and Pederick, L.A. 1980. Heritability estimates of density characteristics in juvenile *Pinus radiata* wood. Silvae Genet 29 (2): 54-61.

Paiva, J. 2006. Phenotipic and molecular plasticity of wood forming tissues in Maritime pine (*Pinus pinaster* Ait.). *In* ITQB. Universidade Nova de Lisboa, Lisbon. p. 196.

Perry, D., and Hopkins, E. 1967. Importation of breeding material of *Pinus pinaster* Ait. from Portugal. For. Dep. W. A. Bulletin 75.

Plomion, C., Leprovost, G., and Stokes, A. 2001. Wood formation in trees. Plant Physiol 127 (4): 1513-1523.

Polge, H. 1963. Structural analysis of wood by densitometric studies of radiographs. *In* Pre-prited paper for JOYCE_LOEBEL Review. p. 4.

Polge, H. 1978. Fifteen years of wood radiation densitometry. Wood Sci. Technol. 12: 187-196.

Pot, D., Chantre, G., Rozenberg, P., Rodrigues, J.C., Jones, G.L., Pereira, H., Hannrup, B., Cahalan, C., and Plomion, C. 2002. Genetic control of pulp and timber properties in maritime pine (*Pinus pinaster* Ait.). Ann For Sci 59 (5-6): 563-575.

Roulund, H., Alpuim, M., Varela, M.C., and Aguiar, A. 1988. A Tree Improvement plan for *Pinus pinaster* in Portugal. EFN.

Rudman, P. 1968. Growth Ring Analysis. J I Wood Sci 4: 58-63.

Talbert, J.T., Jett, J.B., and Bryant, R.L. 1983. Inheritance of wood specific-gravity in an unimproved Loblolly-pine population - 20 years of results. Silvae Genet 32 (1-2): 33-37.

Vargas-Hernandez, J., and Adams, W.T. 1991. Genetic-variation of wood density components in young coastal Douglas-fir - Implications for tree breeding. Can J For Res 21 (12): 1801-1807.

Vargas-Hernandez, J., and Adams, W.T. 1992. Age-age correlations and early selection for wood density in young coastal Douglas-fir. Forest Sci 38 (2): 467-478.

Williams, C.G., and Megraw, R.A. 1994. Juvenil-mature relationships for wood density in *Pinus taeda*. Can J For Res 24: 714-722.

Wright, J.W. 1976. Introduction to Forest Genetics. Academic Press, New York, 463pp.

Wu, H.X., Matheson, A.C., and Spencer, D. 1998. Inbreeding in *Pinus radiata*. I. The effect of inbreeding on growth, survival and variance. Theor Appl Genet 97 (8): 1256-1268.

Zamudio, F., Baettyg, R., Vergara, A., Guerra, F., and Rozenberg, P. 2002. Genetic trends in wood density and radial growth with cambial age in a Radiata pine progeny test. Ann For Sci 59 (5-6): 541-549.

Zamudio, F., Rozenbergb, P., Baettig, R., Vergara, A., Yanez, M., and Gantz, C. 2005. Genetic variation of wood density components in a Radiata pine progeny test located in the south of Chile. Ann For Sci 62 (2): 105-114.

Zhang, S.Y. 1998. Effect of age on the variation, correlations and inheritance of selected wood characteristics in Black spruce (*Picea mariana*). Wood Sci Technol 32 (3): 197-204.

Zhang, S.Y., and Jiang, Z.H. 1998. Variability of selected wood characteristics in 40 half-sib families of Black spruce (*Picea mariana*). Wood Sci Technol 32 (1): 71-82.

Zhang, S.Y., and Morgenstern, E.K. 1995. Genetic-variation and inheritance of wood density in Black spruce (*Picea mariana*) and its relationship with growth - implications for tree breeding. Wood Sci Technol 30 (1): 63-75.

Zobel, B.J., and Jett, J.B. 1995. Genetics of Wood Production. Springer-Verlag ed, Berlin, Heidelberg, and New York, 377 pp.

Zobel, B.J., and Sprague, J.R. 1998. Juvenile Wood in Forest Trees. Springer-Verlag ed, Berlin, Heidelberg, and New York, 300 pp.

Zobel, B.J., and Talbert, J. 1984. Applied Forest Tree Improvement. John Wiley & Sons, New York, 511 pp.

Zobel, B.J., and van Buijtenen, J.P. 1989. Wood Variation - Its causes and control. Springer-Verlag ed, Berlin, 363 pp.

CHAPTER 3

Genetic correlations between wood quality traits of *Pinus pinaster* Ait.

Published as: Gaspar, M.J.; Louzada, J.L.; Aguiar, A. and Almeida, M.H. 2008. Genetic correlations between wood quality traits of *Pinus pinaster* Ait. Annals of Forest Science 65 (7): 703..

3.1. ABSTRACT

It is essential to understand how characteristics are related to each other in breeding programmes to select wood properties, in order to avoid that, in selecting for one trait, we are negatively affecting another. Moreover, measuring wood properties is time consuming and expensive. This study assesses genetic and phenotypic correlations between wood density components and spiral grain of 46 half-sib families of *Pinus pinaster* in seventeen-year-old trees. Results showed that genetic correlations for all wood density components were higher than corresponding phenotypic correlations. Furthermore, all wood density components were highly genetically correlated with ring density, and also closely associated among themselves. Results showed a higher genetic correlation of ring density with earlywood density (r_g=0.96) than with latewood density (r_g=0.79). A moderate to high positive genetic correlation was found between spiral grain and wood density characteristics (0.29-0.61). We conclude that ring density (overall wood density) can be improved by increasing either earlywood density, latewood percent, or both of these traits, and spiral grain can be modify in future plantations.

3.2. INTRODUCTION

The 2005-2006 National Forest Inventory estimates the total forest area in Portugal at 3.4 million hectares. The volume of standing wood of the main species that supply industry is estimated at 67 million m^3 for *Pinus* wood and 41 million m^3 for *Eucalyptus* wood. Due to the economic importance of *P. pinaster* (Roulund et al. 1988), a national tree improvement plan for this species has been in operation since the early 1980's. The Maritime pine breeding programme is presently in its second generation of selection (the progeny trials reported in this study are part of this programme). Selection of genotypes for seed orchards and breeding populations is usually based solely upon diameter, height and stem traits. Such selection criteria may have an indirect effect on different wood properties (Zobell and Jett 1995), but there is little genetic information available on the relation between these selection traits and wood properties.

A consequence of future plantings is an increase in the proportion of juvenile wood due to the reduction of rotation age because of an increase in growth rate (Hylen 1999; Zamudio et al. 2005). Despite some exceptions depending on the type of end product, juvenile wood is generally considered to be of lower quality than mature wood (Zamudio et al. 2005). An increase in the proportion of juvenile wood will represent a problem for most solid wood products due to its low strength and instability following drying (Zobel and Sprague 1998). Juvenile wood in *Pinus* species is usually characterized by a shorter tracheid, and cell walls thinner than in mature wood, which often produces lower specific gravity, and also by a severe spiral grain (Zobel and Spargue 1998).

Wood density is closely associated to the most important wood quality properties and technological traits, providing a vital index that confers important information in this area (Zobel and Jett 1995; Zobel and van Buijtenen 1989). Nevertheless, variations in wood density can be difficult to understand, since this complex trait is not a single property but a complex of several intra-ring components, including minimum and maximum wood densities, early and late wood densities, proportion of latewood etc (Pliura et al. 2006). Another important wood property is the spiral grain, a term applied to the helical orientation of the tracheids in a tree stem. This property confers a twisted appearance to the trunk after the bark has been removed, causing twisting in dried sawn timber through anisotropic shrinkage (Harris 1989), and is recognised as one of the key properties determining the suitability of wood for use as sawn timber (Raymond 2002), one of the main applications for *P. pinaster* wood in Portugal.

One way to reduce some of the negative effects of short rotations on wood quality is by breeding to improve juvenile wood characteristics (Nichols et al. 1980; Zamudio et al. 2005) as a result of improved knowledge of the genetic control of wood characteristics, wood growth, and their interrelationship. This process will result in increased selection efficiency for a selected trait through the modification of one or more characteristics (Louzada and Fonseca 2002).

In a breeding programme that selects for wood properties, it is essential to understand how the characteristics are related to one another. Knowledge of additive genetic correlations is extremely important in any selection programme, since the measurement of wood properties is time consuming and expensive. Genetic correlations play an important role in the prediction of correlated responses and the development of effective selection indices.

The aim of this study was to estimate genetic and phenotypic correlations between intra-ring wood characteristics and spiral grain, and to evaluate the implications of these relationships in tree breeding for wood quality in *P. pinaster*. These findings are of great importance for the continued development of maritime pine breeding programmes to guide future generation's selection.

3.3. MATERIALS AND METHODS

3.3.1. Trial and measurements

3.3.1.1. Location

The field trial location was at Pinhal de Leiria situated in central coastal Portugal (lat 39°50', long 8°55', alt 30m). The site is characterized by a Mediterranean climate, with a mean air temperature between 12.5°C and 15°C, relative humidity between 80 and 85% and yearly rainfall values of 700mm to 800mm, with a 4 month period of summer drought. The Spodic Podzols (PZ sd) soils are derived from sand dunes of maritime origin. Seedlings were raised for 9 months in the nursery in plastic bags (250cm^3 volume). Planting (2m×2m spacing) took place in March 1987 after harrowing and ploughing the site; no fertilizer was applied.

3.3.1.2. Genetic material

The trial included 46 open pollinated families, derived from seed collected in the Escaroupim clonal seed orchard II (Aguiar 1993). This seed orchard includes 49 genotypes; it was established by grafting in 1975-80 and belongs to the first generation of the breeding program. Only 46 families were considered in order to avoid poor seed production. The families were replicated in 8 blocks with 2×4 tree plots. In the year of 2003, wood samples were collected from a subset of 4 trees per family and block in the same 3 blocks, giving a total number of 552 trees. The sampled trees within each plot were randomly selected. A single wood disc with a thickness of 10cm was collected at a predetermined sampling height of 2m. The disk was sawn into a 2mm-thick radial strip segment from the pith to the bark, and then conditioned at 12% moisture content. These radial samples were X-rayed perpendicular to the transverse section and their image scanned by microdensitometric analysis (Louzada 2000). Growth ring boundaries were identified on the radial profiles by locating sharp density variations through cross-examination using visual observation of the macroscopic anatomical features. Annual rings were numbered from pith-to-bark, and the first and the last annual rings of each sample were excluded since they were usually incomplete.

For each ring, average ring density (RD), minimum density (MND), maximum density (MXD), earlywood density (EWD), latewood density (LWD), earlywood width (EWW), latewood width (LWW), ring width (RW) and latewood percentage (LWP) were determined for each ring. The earlywood-latewood boundary or earlywood/latewood limit in each growth ring was assigned a fixed density 0.5gcm^3. Similar to several authors in the study of other conifer species (Fujimoto et al. 2006; Hodge and Purnell 1993), we used a single fixed value of density; even though some researchers have used the average of minimum and maximum ring density to define the EW/LW transition point (Vargas-Hernandez and Adams 1991; Zamudio et al. 2005). The advantage of the former criterion for determining the EW/LW boundary is explained in detail by Jozsa et al. (1987) and Rudman (1968). This criterion does not allow identification of the beginning of latewood, but does allow identification of ring portions with a higher density over a certain threshold, which we define here as LW. The applied value of 0.5gcm^{-3} was derived from an adjustment to the value proposed by Louzada (2000) for *P. pinaster* in the comparison of different criteria, and was considered the most correct value for 18 years old specimens of this species. Intra-ring density variation was quantified using the heterogeneity index (HI), defined by the standard deviation of all density values across the annual ring. Rings close to the pith have less volume than those closer to the bark, thereby contributing less to overall disc volume. In order to compensate for this effect, wood density components were weighted for each ring density component by its respective cross-sectional area (Ferrand 1982).

In each disc, the grain angle was measured at the most recent formed latewood of the last ring, using a grain slope detector (Harris 1989) and the pith as a reference (Hannrup et al. 2002). To define this reference, nails were hammered into the pith from both sides of the disc and a string was tied between them so that the cord ran through a vertical line. The spiral grain angle (SPG) was calculated as the arctangent of the ratio of the distance from the reference line (a) to the line traced with the needle of the slope of the grain detector and the thickness of the disc (b) (Fig 3.1).

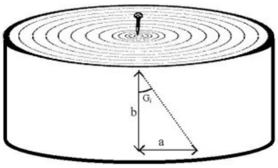


Figure 3.1 Procedure for measuring grain angle on wood discs (G_i: Spiral Grain Angle; G_i=arct(a/b); a: distance from the reference line to the line traced with the needle; b: thickness of the disc).

Following the protocol given by Harris (1989), spirality direction was indicated by assigning a positive sign to the left handed angles. Measurements in each disc were taken in two opposite radii, and the mean grain angle was calculated as the mean of the absolute value of the angles, ignoring differences in sign.

3.3.2. Data analysis

The mixed linear model associated with the data for a given trait was:

$$Y_{ijk}=\mu + B_j + F_i + B\times F_{ij} + \varepsilon_{ijk} \qquad [3.1]$$

where Y represents the phenotypic individual observation; μ is the overall mean; B_j - the effect of the jth block (fixed); F_i - the effect of the ith family (random); $B\times F_{ij}$ - the effect of the interaction between the ith family and the jth block (random) and ε- the residual error. Variance components for family (σ_f^2), family-block interaction ($\sigma_{f\times b}^2$) and residual errors (σ_ε^2), with the respective associated standard errors, were estimated by restricted maximum likelihood, using the average information REML algorithm implemented in the ASREML programme (Gilmour et al. 1998).

Narrow sense heritability (h^2_i) was calculated for each trait measured as:

$$h_i^2 = \frac{\sigma_a^2}{\sigma_P^2} \qquad [3.2]$$

where σ_a^2 represents the additive genetic variance and σ_P^2 represents the total phenotypic variance. Families were considered to be maternal half-siblings, therefore the $\sigma_a^2 = 4\times\sigma_f^2$ relationship was assumed to estimate the genetic parameters. Phenotypic variance was estimated as $\sigma_P^2 = \sigma_f^2 + \sigma_{f\times b}^2 + \sigma_\varepsilon^2$. Standard errors for heritability were estimated by ASREML using a Taylor series approximation (Gilmour et al. 1998).

The genetic and phenotypic correlations between all wood density components were calculated using a multivariate extension of model (3.1). When the family effect was found to be null for any of the traits analysed in the univariate analysis, this effect was not included in the multivariate analysis. Genetic correlations between traits (X and Y) measured at 17 years-old were evaluated as follows:

$$r_a = \frac{Cov_a(x,y)}{\sqrt{\sigma_{a_x}^2 \cdot \sigma_{a_y}^2}} \qquad [3.3]$$

where $\mathrm{cov}_a(x,y)$ is the additive genetic covariance, and $\sigma_{a_x}^2$ and $\sigma_{a_y}^2$ are the additive variance components for traits *X* and *Y*, respectively.

The phenotypic correlation between traits (*X* and *Y*), was estimated as:

$$r_P = \frac{Cov_P(x,y)}{\sqrt{\sigma_{P_x}^2 \cdot \sigma_{P_y}^2}} \qquad [3.4]$$

where $\mathrm{cov}_P(x,y)$ is the phenotypic covariance between traits X and Y, estimated as $\mathrm{cov}_P(x,y) = \mathrm{cov}_f(x,y) + \mathrm{cov}_{fb}(x,y) + \mathrm{cov}_\varepsilon(x,y)$, which is the sum of the family, interaction, and residual covariance components. $\sigma^2_{P_x}$ and $\sigma^2_{P_y}$ are the phenotypic variances for traits X and Y, respectively. Standard errors for genetic and phenotypic correlations were estimated by ASREML a Taylor series approximation (Gilmour et al. 1998).

3.4. RESULTS AND DISCUSSION

Summary statistics for each characteristic and the respective heritability estimation are shown in Table 3.1. The intraring wood characteristics values were previously reported by (Gaspar et al. 2008).

Table 3.1 Descriptive statistics table for different wood traits and respective heritability estimates (standard errors given in brackets).

Trait	Mean	Std. Dev.	Coeff. Var.	Min	Max	h^2_i (se)
RD (gcm^{-3})	0.474	0.051	10.68	0.336	0.655	0.63 (0.190)
MND (gcm^{-3})	0.308	0.037	12.11	0.207	0.445	0.54 (0.184)
MXD (gcm^{-3})	0.707	0.060	8.51	0.558	0.917	0.34 (0.164)
EWD (gcm^{-3})	0.386	0.026	6.69	0.304	0.463	0.60 (0.191)
LWD (gcm^{-3})	0.618	0.034	5.43	0.535	0.732	0.26 (0.141)
LWP (%)	38.196	16.927	44.31	6.420	82.240	0.46 (0.168)
EWW (mm)	2.674	0.876	32.74	0.900	6.100	0.32 (0.137)
LWW (mm)	1.556	0.907	58.29	0.300	6.400	0.31 (0.156)
RW (mm)	4.224	0.901	21.33	2.500	8.600	0.10 (0.111)a
HI (gcm^{-3})	0.120	0.019	16.05	0.07	0.196	----- a
SPG	4.2	2.259	53.40	-1.30	13.11	0.47 (0.150)

RD = Average Ring Density, MND = Minimum Density, MXD = Maximum Density, EWD=Earlywood Density, LWD = Latewood Density, LWP = Latewood Percentage, EWW= Earlywood Width, LWW= Latewood Width, RW = Ring Width; SPG=Spiral Grain; a: in the analysis of variance the differences among Families were not significant ($P > 0.05$); ---- the heritability value was quantified with the null value

The trait under strongest genetic control was average ring density (RD, $h_i^2 = 0.63$) among the heritability values, whilst RW showed the lowest heritability estimation ($h_i^2 = 0.10$). No significant differences ($p > 0.05$) were observed between families for this trait, indicating that the variation in growth characteristics most likely derives from environmental factors rather than genetic ones (Gaspar et al. 2008). Growth characteristics (diameter) frequently exhibit relatively low values of heritabilty (Bouffier et al. 2008; Cotterill et al. 1987; Harfouche et al. 1995; Hopkins and Butcher 1994; Zas et al. 2004). In a study carried out after 12 years in this progeny trial, Aguiar et al. (2003) estimated a heritability value for diameter of 0.18. The grain angle presented a coefficient of variation (CV) of 53 with an average value of 4.2°. Spirality was predominantly left-handed (98%), with a few trees displaying a weak level of right-handed spirality. This conforms to the general pattern of spirality in conifers, where the grain angle is usually left handed in early years, followed by a gradual change towards right-handed spirality with increasing age (Harris 1989). Heritability estimates for SPG were 0.47, indicating moderate to strong genetic control of this characteristic. Our estimated heritability for this trait is higher than that derived by Arbez et al. (1978) from a previous study on maritime pine ($h_i^2 = 0.16$) but similar to values reported for other coniferous species. Hansen and Roulund (1997) observed that individual narrow-sense heritability of the spiral grain in Sitka spruce was 0.63 and 0.78. In *Picea abies*, Hannrup et al. (2002) reported a broad-sense heritability of 0.42, whilst Hallingback et al. (2008) and Silva et al. (2000) reported narrow-sense heritability for this species from (0.32-0.44) and (0.29-0.47) respectively. Heritability for spiral grain in *Pinus sylvestris* has been found to be 0.4 (Hannrup et al. 2003). In *Pinus radiata* Gapare et al. (2007) estimated a narrow-sense heritability of 0.44 and 0.46. (Zobel and Jett 1995) pointed out that much of the variation in spiral grain within a species derives from the different methods used to assess this characteristic. Nevertheless, our findings indicated that potentially, tree breeders can modify spiral grain in future plantations.

3.4.1. Genetic correlations between traits

Phenotypic and additive genetic correlations between all traits are presented in the Table 3.2.

Data shows that genetic correlations for all traits (except for SPG) were higher than the corresponding phenotypic correlations, indicating that the use of phenotypic correlations, rather than genetic ones, to evaluate traits will result in the underestimation of potential gains from indirect selection.

Table 3.2 Phenotypic (upper triangle) and genetic (lower triangle) correlations coefficients (with standard errors given in brackets) between all traits.

	RD	MND	MXD	EWD	LWD	LWP	EWW	LWW	SPG
RD		0.66 (0.027)	0.80 (0.017)	0.67 (0.026)	0.72 (0.026)	0.90 (0.008)	-0.72 (0.021)	0.80 (0.016)	0.18 (0.046)
MND	1.02 (0.078)		0.42 (0.039)	0.79 (0.018)	0.33 (0.041)	0.54 (0.034)	-0.52 (0.034)	0.42 (0.039)	0.16 (0.046)
MXD	0.90 (0.084)	0.95 (0.194)		0.34 (0.164)	0.93 (0.063)	0.69 (0.024)	-0.57 (0.030)	0.60 (0.029)	0.10 (0.046)
EWD	0.96 (0.065)	1.06 (0.044)	0.88 (0.193)		0.19 (0.045)	0.49 (0.036)	-0.37 (0.040)	0.46 (0.037)	0.18 (0.046)
LWD	0.79 (0.134)	0.72 (0.248)	0.97 (0.024)	0.72 (0.256)		0.59 (0.029)	-0.54 (0.032)	0.46 (0.036)	0.05 (0.045)
LWP	0.96 (0.027)	0.95 (0.124)	0.91 (0.102)	0.86 (0.128)	0.80 (0.158)		-0.76 (0.019)	0.87 (0.011)	0.15 (0.197)
EWW	-0.85 (0.095)	-0.91 (0.135)	-0.59 (0.208)	-0.85 (0.163)	-0.39 (0.276)	-0.89 (0.086)		-0.49 (0.034)	-0.04 (0.046)
LWW	0.99 (0.055)	1.00 (0.196)	0.89 (0.148)	0.88 (0.161)	0.83 (0.208)	1.00 (0.039)	-0.83 (0.179)		0.19 (0.044)
SPG	0.55 (0.181)	0.61 (0.177)	0.31 (0.233)	0.57 (0.179)	0.29 (0.258)	0.55 (0.197)	-0.59 (0.239)	0.51 (0.210)	

Note: See Table 3.1 for trait abbreviations.

All wood characteristics studied showed a strong genetic correlation with RD, and between themselves, indicating that these traits are probably controlled, to a large extent, by the same set of genes (Vargas-Hernandez and Adams 1991), and that selection for one characteristic should result in a simultaneous response of associated traits. The strong genetic relationship between density and each one of its components highlights the fact that overall density is a complex trait resulting from different combinations of the fraction, and relative density of each component (Hylen 1997). All correlations with RD were positive, with the exception of EWW where strong negative genetic correlations were detected, due to the fact that, contrary to latewood, earlywood is characterized by lower density, larger lumens, and thinner cell walls (Hylen 1999; Kumar 2002; Loo et al. 1984; Talbert et al. 1983; Vargas-Hernandez and Adams 1992; Williams and Megraw 1994; Zobel and van Buijtenen 1989). These results are consistent with those obtained in studies on *Pseudotsuga menziesii* (Hodge and Purnell 1993) and *Picea mariana* (Abdel-Gadir et al. 1993). Zhang (1998) suggested that such trends were most likely due to a direct reaction of differentiating cells in the cambial zone due to the relative availability of photosynthates and growth regulators at different times during the growing season.

The present study showed a higher genetic correlation of RD with EWD than with LWD, and a higher phenotypic correlation of RD with LWD than with EWD. Louzada (2003) reported that *P. pinaster* earlywood density has a higher genetic and phenotypic correlation with overall wood density than latewood density. Similar results were obtained in *P. menziesii* by Vargas-Hernandez and Adams (1991). Also Abdel-Gadir et al. (1993) studied *Abies balsamea* and observed higher phenotypic correlation between wood density and earlywood than with latewood density. Conversely, although similar results for the genetic correlation among overall wood density and earlywood density were obtained, Zhang and Morgstern (1995) also encountered strong negative genetic correlation (-0.73) between overall wood density and latewood density. Louzada (2003) reported the same conclusions concerning *P. pinaster* namely *"EWD not only reflects a better tree genetic potential but also is controlled over years, in a large way, by the same genes".* This study verified that EWD manipulation resulted in a significant genetically correlated response in other components (RD and LWP), whilst latewood density appeared to be more sensitive to climate variation. Latewood is formed later in the growing season, when cambial cell division and expansion declines. In this period, conditions in Mediterranean regions are suboptimal due to constraining factors such as high temperatures and water stress which mask genetic effects, leading to reduced heritability ($h^2_{i\ (MND)}$ = 0.54, $h^2_{i\ (EWD)}$ = 0.60, $h^2_{i\ (MXD)}$ = 0.34; $h^2_{i\ (LWD)}$ = 0.26), confirming that, for *P. pinaster*, earlywood characteristics are subject to stronger genetic control, and that latewood components are more susceptible to environmental factors (Gaspar et al. 2008). Our findings indicate that earlywood density is most likely a result of genetic factors, but that latewood density depends mostly on environmental factors.

Most authors emphasize the importance of percentage of latewood as the source of variation in pine wood density (Zobell and Jett 1995). Occasionally, the relationship between specific gravity and latewood percentage in conifers is not as high as could be expected (Zobell and Jett 1995). This study revealed a very high genetic and phenotypic correlation between RD and LWP (0.96 and 0.90, respectively). Significant genetic correlations between these two traits have been reported elsewhere (Fujisawa et al. 1993; Hodge and Purnell 1993) although Vargas-Hernandez and Adams (1991) and Zhang and Morgenstern (1995) obtained only moderate genetic correlation values between these traits. We conclude that the reported differences related with LWP possibly result from different methodologies used to define the threshold value between EW and LW. Some researchers used the average of minimum and maximum ring density (Cown and Parker 1978; Fujimoto et al. 2006), whilst others use a fixed density value (Nichols et al. 1980; Vargas-Hernandez and Adams 1991). This study adopted the latter approach to define the EW/LW threshold and the results suggest that ring density can be improved by increasing earlywood density, latewood percent, or both of these traits.

A moderate to high positive genetic correlation was observed between spiral grain and wood density characteristics (0.29-0.61). However, other studies have obtained lower genetic correlations between spiral grain and wood density (Eisemann et al. 1990; Hannrup et al. 2004). SPG presented a higher dependency on earlywood components (MND and EWD), and had high genetic correlation with RD (0.55), being more dependent on earlywood ($r_{g(SG,EWD)}$=0.57) than on latewood ($r_{g(SG,LWD)}$=0.29). Also, genetic correlations between SPG and intra-ring width characteristics (EWW and LWW) were high but presented contradictory signs (-0.59 and 0.51 respectively). The opposite signs were expectable as EWW and LWW are highly negatively correlated (r_g=-0.83), consequently if SPG is positively correlated with LWW, it will be negatively correlated with EWW. Generally correlations between grain angle and growth have been week (Eisemann et al. 1990; Hannrup et al. 2004; Hannrup et al. 2003; Hallingback 2008), indicating that there won´t be genetic loss in growth when breeding for low SPG, or the other way around. Unfortunately in this study we weren't able to evaluate the magnitude of this correlation, but it should be investigated further.

3.5. CONCLUSIONS

The results of this study showed that genetic correlations for all wood density components were higher and more stable than the corresponding phenotypic correlations. Furthermore, all wood density components were strongly genetically correlated with RD, and were also strongly associated among themselves.

The present study also showed that EWD has a higher genetic correlation with RD than LWD, while RD presented higher phenotypic correlation with LWD than with EWD. These results indicate that, in *P. pinaster*, earlywood characteristics are more subject to genetic control whereas latewood components are more strongly influenced by environmental factors. Results also suggest that ring density (overall wood density) can be improved by increasing either earlywood density, latewood percent, or both of these traits. In addition, our results indicate that, potentially, tree breeders can modify spiral grain in future plantations.

Acknowledgements

Acknowledgements are due to two referees who with their comments greatly improved this paper. M.J. Gaspar was funded by the Portuguese Foundation for Science and Technology (Doctoral grant SFRH/BD/27412/2006).

3.6. REFERENCES

Abdel-Gadir, A.Y., Krahmer, R.L., and Mckimmy, M.D. 1993. Intra-ring variations in mature Douglas fir trees from provenance plantations. Wood Fiber Sci 25 (2): 170-181.

Aguiar, A. 1993. Análise da variação do crescimento em altura de descendências maternais de pinheiro bravo. Silva Lusitana 1 (2): 267-274.

Arbez, M., Baradat, P., Birot, Y., Azoeuf, P., and Hoslin, R. 1978. Variability and heredity of spiral grain measured by radioisotopes in *Pinus pinaster* Ait. and *Pinus nigra* Arn Ssp Laricio Var Calabrica. Can J Forest Res 8 (3): 280-289.

Bouffier, L., Charlot, C., Raffin, A., Rozenberg, P., and Kremer, A. 2008. Can wood density be efficiently selected at early stage in Maritime pine (*Pinus pinaster* Ait.)? Ann for Sci 65: 106.

Cotterill, P.P., Dean, C.A., and Vanwyk, G. 1987. Additive and dominance genetic effects in *Pinus pinaster*, *Pinus radiata* and *Pinus elliottii* and some implications for breeding strategy. Silvae Genet 36 (5-6): 221-232.

Cown, D.J., and Parker, M.L. 1978. Comparison of annual ring density profiles in hardwoods and softwoods by x-ray densitometry. Can J Forest Res 8 (4): 442-449.

Eisemann, R.L., Harding, K.J., and Eccles, D.B. 1990. Genetic parameters and predicted selection responses for growth and wood properties in a population of *Araucaria cunninghamii*. Silvae Genet 39 (5-6): 206-216.

Ferrand, J.C. 1982. Réflexions sur la Densité du Bois. 2ère Partie: Calcul de la densité et de son hétérogénéité. Holzforschung 36 (3): 153-157.

Fujimoto, T., Akutsu, H., Kita, K., Uchiyama, K., Kuromaru, M., and Oda, K. 2006. Age trends of genetic parameters of spiral grain in hybrid larch F-1 and implications for efficiency of early selection. J Wood Sci 52 (2): 101-106.

Fujisawa, Y., Ohta, S., and Tajima, M. 1993. Wood characteristics and genetic variations insugi (*Cryptomeria japonica*) 2. Variation in growth ring components among plus-trees clones and test stands. Mokuzai Gakkaishi 39 (8): 875-882.

Gapare, W., Hathorn, A., Kain, D., Matheson, C., and Wu, H. 2007. Inheritance of spiral grain in the juvenile core of *Pinus radiata*. Can J Forest Res 37 (1): 116-127.

Gaspar, M.J., Louzada, J.L.P.C., Silva, M.E., Aguiar, A., and Almeida, M.H. 2008. Age trends in genetic parameters of wood density components in 46 half-sibling families of *Pinus pinaster* Ait. Can J Forest Res 38 (6): 1470-1477.

Gilmour, A., Gogel, B., Cullis, B., Welham, S., and Thompson, R. 1998. ASREML Users Manual. *In* VSN International. New south Wales Agriculture, Orange.

Hallingback, H.R., Jansson, G., and Hannrup, B. 2008. Genetic parameters for grain angle in 28-year-old Norway spruce progeny trials and their parent seed orchard. Ann For Sci 65 (3): 301.

Hannrup, B., Cahalan, C., Chantre, G., Grabner, M., Karlsson, B., Le Bayon, I., Jones, G.L., Muller, U., Pereira, H., Rodrigues, J.C., Rosner, S., Rozenberg, P., Wilhelmsson, L., and Wimmer, R. 2004. Genetic parameters of growth and wood quality traits in *Picea abies*. Scand J Forest Res 19 (1): 14-29.

Hannrup, B., Grabner, M., Karlsson, B., Müller, U., Rosner, S., Wilhelmsson, L., and Wimmer, R. 2002. Genetic parameters for spiral-grain angle in two 19-year-old clonal Norway spruce trials. Ann For Sci 59: 551-556.

Hannrup, B., Säll, H., and Jansson, G. 2003. Genetic parameters for spiral grain in Scots pine and Norway spruce. Silvae Genet 52: 215-220.

Hansen, J.K., and Roulund, H. 1997. Genetic parameters for spiral grain, stem form, pilodyn and growth in 13 years old clones of Sitka spruce (*Picea sitchensis* (Bong.) Carr.). Silvae Genet 46 (2-3): 107-113.

Harfouche, A., Baradat, P., and Durel, C. 1995. Intraspecific variability in Maritime pine (*Pinus pinaster* Ait.) in the South-East of France. 1. Variability in autochthonous populations nd in the whole range of species. Ann For Sci 52 (4): 307-328.

Harris, J.M. 1989. Spiral grain and wave phenomena in wood formation, Berlin, Heidelberg, and New York, 230 pp.

Hodge, G.R., and Purnell, R.C. 1993. Genetic parameter estimates for wood density, transition age, and radial growth in Slash pine. Can J Forest Res 23 (9): 1881-1891.

Hopkins, E., and Butcher, T. 1994. Improvement of *Pinus pinaster* Ait. in Western Australia. CALM Science 1 (2): 159-242.

Hylen, G. 1997. Genetic variation of wood density and its relationship with growth traits in young Norway spruce. Silvae Genet 46 (1): 55-60

Hylen, G. 1999. Age trends in genetic parameters of wood density in young Norway spruce. Can J For Res 29: 135-143.

Jozsa, L., Richards, J. and Johnson, S. 1987. Calibration of Forintek's direct reading x-ray densitometer. Report No 36a. Forintek Canada Corp., Vancover, p.16.

Kumar, S. 2002. Earlywood-latewood demarcation criteria and their effect on genetic parameters of growth ring density components and efficiency of selection for end-of-rotation density of Radiata pine. Silvae Genet 51 (5-6): 241-246.

Loo, J.A., Tauer, C.G., and van Buijtenen, J.P. 1984. Juvenil mature relationships and heritability estimates of several traits in Loblolly pine (*Pinus taeda*). Can J Forest Res 14: 822-825.

Louzada, J.L.P.C. 2000. Variação fenotípica e genética em características estruturais na madeira de *Pinus pinaster* Ait. *In* Departamento Florestal. Universidade de Trás-os-Montes e Alto Douro, Vila Real, 293pp.

Louzada, J.L.P.C. 2003. Genetic correlations between wood density components in *Pinus pinaster* Ait. Ann For Sci 60: 285-294.

Louzada, J.L.P.C., and Fonseca, F.M.A. 2002. The heritability of wood density components in *Pinus pinaster* Ait. and implications for tree breeding. Ann For Sci 59: 867-873.

Nichols, J.W.P., Morris, J.D., and Pederick, L.A. 1980. Heritability estimates of density characteristics in juvenil *Pinus radiata* wood. Silvae Genet 29 (2): 54-61.

Pliura, A., Zhang, S.Y., Bousquet, J., and MacKay, J. 2006. Age trends in genotypic variation of wood density and its intra-ring components in young poplar hybrid crosses. Ann For Sci 63 (7): 673-685.

Raymond, C.A. 2002. Genetics of Eucalyptus wood properties. Ann For Sci 59 (5-6): 525-531.

Roulund, H., Alpuim, M., Varela, M.C., and Aguiar, A. 1988. A tree improvement plan for *Pinus pinaster* in Portugal. EFN.

Rudman, P. 1968. Growth ring analysis. Journal of the Institute of Wood Science 4: 58-63.

Silva, J., Borralho, N., and Wellendorf, H. 2000. Genetic parameter estimates for diameter growth, pilodyn penetration and spiral grain in *Picea abies* (L.) KARST. Silvae Genet 49 (1): 29-36.

Talbert, J.T., Jett, J.B., and Bryant, R.L. 1983. Inheritance of wood specific-gravity in an unimproved Loblolly-pine population - 20 years of results. Silvae Genet 32 (1-2): 33-37.

Vargas-Hernandez, J., and Adams, W.T. 1991. Genetic-variation of wood density components in young coastal Douglas-Fir - Implications for tree breeding. Can J Forest Res 21 (12): 1801-1807.

Vargas-Hernandez, J., and Adams, W.T. 1992. Age-age correlations and early selection for wood density in young coastal Douglas-fir. Forest Sci 38 (2): 467-478.

Williams, C.G., and Megraw, R.A. 1994. Juvenil-mature relationships for wood density in *Pinus taeda*. Can J Forest Res 24: 714-722.

Zamudio, F., Rozenberg, P., Baettig, R., Vergara, A., Yanez, M., and Gantz, C. 2005. Genetic variation of wood density components in a Radiata pine progeny test located in the south of Chile. Ann For Sci 62 (2): 105-114.

Zas, R., Merlo, E., and Fernandez-López, J. 2004. Genetic parameter estimates for Maritime pine in the Atlantic coast of North-West Spain. Forest Genetics 11 (1): 45-53.

Zhang, S.Y. 1998. Effect of age on the variation, correlations and inheritance of selected wood characteristics in Black spruce (*Picea mariana*). Wood Sci Technol 32 (3): 197-204.

Zhang, S.Y., and Morgenstern, E.K. 1995. Genetic-variation and inheritance of wood density in Black spruce (*Picea mariana*) and its relationship with growth - implications for tree breeding. Wood Sci Technol 30 (1): 63-75.

Zobel, B.J., and Jett, J.B. 1995. Genetics of Wood Production. Springer-Verlag ed, Berlin, Heidelberg, and New York, 377 pp.

Zobel, B.J., and Sprague, J.R. 1998. Juvenil Wood in Forest Trees. Springer-Verlag ed, Berlin, Heidelberg, and New York, 300 pp.

Zobel, B.J., and van Buijtenen, J.P. 1989. Wood Variation - Its causes and control. Springer-Verlag ed, Berlin, 363 pp.

CHAPTER 4

Phenotypic and genetic correlations between lignin and wood density components in *Pinus pinaster* Ait.

Submitted as*:* Gaspar, M.J.; Alves, A.; Louzada, J.L.; Santos, A.; Simões, R.; Fernandes, C., Almeida, M.H. and Rodrigues, J.C. S. Phenotypic and genetic correlations between lignin and wood density components in *Pinus pinaster* AIT. Annals of Forest Science.

4.1. ABSTRACT

Genetic selection for one trait can have a significant impact on other traits that are also important for final use; thus it is important to know how characteristics are related to each other. Genetic control and relationships among *Pinus pinaster* lignin content and density traits were assessed by estimating heritability and phenotypic and genetic correlations using 552 trees representing 46 half-sib families. Results showed very low or even null phenotypic correlations of lignin content with all the other traits, indicating that the phenotypic correlation should not be used to perform mass selection of traits. Ring density presented a low but negative genetic correlation with lignin. Furthermore, results showed that lignin content was more dependent on the proportion of latewood present in the ring. We conclude that it seems possible for breeders to adjust the course of selection depending on their objective. If the purpose is pulp production, the improvement of density with a reduction of lignin is desirable, so the increase of LWP appears to be the correct way to obtain higher wood density.

4.2. INTRODUCTION

Maritime pine (*Pinus pinaster* Ait.) is a highly valuable coniferous species (*Pinaceae*), broadly distributed across the western Mediterranean Basin, Southern Europe and Africa and the Atlantic coast of Portugal, Spain and France (Alía and Martín 2003). In Portugal it is one of the most productive forest ecosystems, occupying approximately 23% of the total Portuguese forest area (DGRF 2007). Its wood is used as timber and as raw material for pulp, being the only source of long fibre for pulp, with an annual consumption around 600 000 ton (CELPA 2007).

The economic importance of this species has led to the development of genetic improvement initiatives. A national tree improvement plan has been in operation since the early 1980s (Roulund et al. 1988) with the aim of increasing volume per hectare and improving stem straightness, a well known drawback of this species.

Currently, the maritime pine breeding program has reached the second generation of selection; the progeny trial reported in this study is part of this program.

Among the desirable wood-quality properties to breed for, wood density is the most frequently studied (Zobel and van Buijtenen 1989), because it is widely recognize that this is an important, easy to measure trait, that largely defines the appropriateness of wood for a variety of end products (Zobel and Jett 1995). However, due to the complex nature of this trait, variations in wood density can be difficult to interpret, since wood density is not a single property, but a complex of characteristics (i.e. density components). Overall ring density depends on earlywood (EW) and latewood (LW) densities, and the relative proportions of each (Vargas-Hernandez and Adams 1991). Previous studies performed on the genetic control of the wood density components of this species, found high heritability and genetic correlations of wood density and its components (Bouffier et al. 2008; Bouffier et al 2009; Gaspar et al. 2008a, Gaspar et al. 2008b; Louzada 2003; Louzada and Fonseca 2002). Although single trait genetic gains are possible, improvement of the average of one trait may have consequences for other traits (Zobel and Talbert 1984), which could in turn have significant impacts on pulp and timber production and quality. Sound knowledge of genetic correlations is important for both defining breeding goals and selecting the right genotypes (Kumar et al. 2008). If improving one desired characteristic results in a reduced value for a second desirable property, very careful consideration must be made concerning the most effective breeding plan (Zobel and van Buijtenen 1989). Thus, it is essential to evaluate the extent to which selection for density affects other wood quality properties in maritime pine such as chemical traits (Pot et al. 2002).

The existence of genetic variation in cellulose and lignin content for *P. pinaster* has been reported by Perez et al. (2007) and Pot et al. (2002). However, there is little information on how these traits interact with others traits, particularly with intra-ring characters. The objective of the present work was to estimate the phenotypic and genetic correlations of lignin content with intra-ring wood density components of *P. pinaster* wood, in order to evaluate how selection for density affects lignin content.

4.3. MATERIAL AND METHODS

The progeny test used in this study belongs to a series of three sites (Aguiar et al. 2003). The trial includes 46 open-pollinated families from plus-trees originally selected in Mata Nacional de Leiria based on volume, stem form, spiral grain and branch habits (Perry and Hopkins 1967).

The field trial location was at Pinhal de Leiria (established in 1987), located in central coastal Portugal (lat 39°50’, long 8°55’, alt 30m). The site is characterized by a Mediterranean climate, with a mean air temperature between 12.5°C and 15°C, relative humidity between 80 and 85% and yearly rainfall values of 700mm to 800mm, with a 4 month period of summer drought. The Spodic Podzols (PZ sd) soils are derived from sand dunes of maritime origin. Seedlings were raised for 9 months in the nursery in plastic bags (250cm^3 volume). Planting (2m×2m spacing) took place in March 1987 after harrowing and ploughing the site; no fertilizer was applied. The trial included 46 open-pollinated families 17 years old, which originated from seed collected in the Escaroupim clonal seed orchard II (Aguiar 1993).

A total of 552 trees from the 46 open-pollinated families at age 17 were used in this study. The trial was established following a complete block design with 3 replicates and 4 tree-plots. In all trees one internodal wood disc, 10cm thick was collected at 2m height.

For chemical analysis only the last four years of each disk were used in order to avoid compression wood. The lignin content (LG) was predicted using Near Infrared Spectroscopy (NIRS) on extractive-free wood meal samples according to Rodrigues et al. (2006) and Perez et al. (2007). Other traits assessed were average ring density (RD), minimum density (MND), maximum density (MXD), earlywood density (EWD), latewood density (LWD), earlywood width (EWW), latewood width (LWW), latewood percentage (LWP), ring width (RW) and heterogeneity index (HI) were determined for all trees, using microdensitometric analysis as described in Gaspar et al. (2008a).

4.3.1. Data analysis

The mixed linear model associated with the data for a given trait was:

$$Y_{ijk}=\mu + B_j + F_i + B\times F_{ij} + \varepsilon_{ijk} \qquad [4.1]$$

where Y represents the phenotypic individual observation; μ is the overall mean; B_j is the effect of the jth block (fixed); F_i is the effect of the ith family (random); $B\times F_{ij}$ is the effect of the interaction between the ith family and the jth block (random) and ε is the residual error. Variance components for family (σ_f^2), family-block interaction σ_{f*b}^2), and residual errors (σ_ε^2), with the respective associated standard errors, were estimated by restricted maximum likelihood, using the average information REML algorithm implemented in the ASREML programme (Gilmour et al. 1998).

Narrow sense heritability (h_i^2) was calculated for each trait measured as:

$$h_i^2 = \frac{\sigma_a^2}{\sigma_P^2} \qquad [4.2]$$

where σ_a^2 represents the additive genetic variance and σ_P^2 the total phenotypic variance. Total phenotypic variance was estimated as:

$$\sigma_P^2=\sigma_f^2+\sigma_{f*b}^2+\sigma_\varepsilon^2 \qquad [4.3]$$

and adjusted additive variances as:

$$\sigma_a^2=\frac{1}{0.26}\times\sigma_f^2 \qquad [4.4]$$

where the coancestry coefficient (0.26) was obtained from correlated paternity, estimated by molecular markers as described by Gaspar et al. (Accepted). Standard errors for heritability were estimated by ASREML using a Taylor series approximation (Gilmour et al. 1998).

The genetic and phenotypic correlations between all wood density components were calculated using a multivariate extension of model (4.1). When the family effect was found to be null for any of the traits analysed in the univariate analysis, this effect was not included in the multivariate analysis. Genetic correlations between traits (*X* and *Y*) were evaluated as follows:

$$\mathrm{r_a} = \frac{\mathrm{Cov_a(x,y)}}{\sqrt{\sigma^2_{\mathrm{a_x}} \cdot \sigma^2_{\mathrm{a_y}}}} \qquad [4.5]$$

where $\mathrm{cov}_a(x,y)$ is the additive genetic covariance, and $\sigma^2_{a_x}$ and $\sigma^2_{a_y}$ are the additive variance components for traits *X* and *Y*, respectively.

The phenotypic correlation between traits (*X* and *Y*), was estimated as:

$$\mathrm{r_P} = \frac{\mathrm{Cov_P(x,y)}}{\sqrt{\sigma^2_{\mathrm{P_x}} \cdot \sigma^2_{\mathrm{P_y}}}} \qquad [4.6]$$

where $\mathrm{cov}_P(x,y)$ is the phenotypic covariance between traits *X* and *Y*, estimated as $\mathrm{cov}_P(x,y) = \mathrm{cov}_f(x,y) + \mathrm{cov}_{fb}(x,y) + \mathrm{cov}_\varepsilon(x,y)$, which is the sum of the family, interaction, and residual covariance components. $\sigma^2_{P_x}$ and $\sigma^2_{P_y}$ are the phenotypic variances for traits X and Y, respectively. Standard errors for genetic and phenotypic correlations were estimated by ASREML a Taylor series approximation (Gilmour et al. 1998).

4.4. RESULTS

Table 4.1 gives descriptive statistics and respective heritability estimation for each characteristic. Average lignin content (LG) was 28%, with a coefficient of variation (CV) of 2%. Highly significant ($p<0.001$) differences between families were observed for this trait. The heritability value estimated for this trait was 0.34, suggesting that it is under a moderate genetic control. All within-ring components showed significant ($p<0.05$) differences between families, with the exception of RW. Within all traits studied, earlywood components and average ring density (MND, h^2_i=0.62; EWD, h^2_i=0.61; RD, h^2_i=0.53) were under strongest genetic control.

Table 4.2 lists phenotypic and additive genetic correlations between lignin and all the other traits. For most characteristics low or even no phenotypic and genetic correlation values were observed.

Table 4.1 Descriptive statistics for the different wood traits (pondered for the last 4 rings) and respective heritability estimates (with SEs given in parentheses).

Trait[a]	Mean	SD	Individual level			Family Level			h
			Max	Min	CV	Max	Min	CV	
LG (%)	28.1	0.83	32.0	26.4	3.0	29.0	27.6	1.2	0.34 (0.147)
RD (g cm-3)	0.50	0.055	0.70	0.37	10.93	0.56	0.45	5.21	0.53 (0.181)
MND (g cm-3)	0.32	0.048	0.46	0.19	15.12	0.36	0.27	7.24	0.62 (0.189)
MXD (g cm-3)	0.83	0.066	1.03	0.64	7.95	0.90	0.76	3.66	0.31 (0.191)
EWD (g cm-3)	0.37	0.037	0.47	0.28	9.86	0.40	0.34	4.74	0.61 (0.187)
LWD (g cm-3)	0.70	0.043	0.83	0.57	6.12	0.74	0.66	2.56	0.26 (0.164)
LWP (%)	39.58	10.03	82.16	16.15	25.33	49.56	30.05	11.69	0.46 (0.176)
EWW (mm)	28.13	0.83	3.80	0.40	2.96	2.28	1.28	12.69	0.26 (0.145)
LWW (mm)	1.80	1.43	2.60	0.40	79.20	1.43	0.83	12.04	0.15 (0.152)
RW (mm)	2.90	0.69	5.60	1.50	23.66	3.35	2.32	8.89	0.08[b] (0.139)
HI (g cm-3)	0.18	0.02	0.26	0.08	14.82	0.20	0.14	6.62	0.41 (0.190)

a LG = Lignin content; RD = Average Ring Density, MND = Minimum Density, MXD = Maximum Density, EWD = Earlywood Density, LWD = Latewood Density, LWP = Latewood Percentage, EWW= Earlywood Width, LWW= Latewood Width; RW=Ring Width; HI=Heterogeneity index.

b In the analysis of variance, the difference between families were not significant (p>0.05).

All the phenotypic correlations were negative with the exception of growth traits (i.e., EWW and LWW) where moderate-low positive correlations were detected. Latewood density components (MXD and LWD) and HI had low but negative correlations. The stronger genetic correlations observed were moderately negative and were found in LWP and LWW (r_g=-0.31; r_g=-0.23). Correlations with RD were also negative but low (-0.16) while all other traits presented nearly no correlation.

Table 4.2 Phenotypic (r_p) and additive genetic (r_g) correlation coefficients (with standard errors given in brackets) between lignin content and the other wood traits

	r_p	r_g
RD	-0.1 (0.046)	-0.16 (0.237)
MND	-0.07 (0.047)	-0.08 (0.236)
MXD	-0.12 (0.047)	-0.00 (0.247)
EWD	-0.05 (0.047)	-0.08 (0.236)
LWD	-0.16 (0.045)	0.04 (0.264)
LWP	-0.04 (0.046)	-0.23 (0.234)
EWW	0.15 (0.044)	0.03 (0.276)
LWW	0.13 (0.045)	-0.31 (0.28)
IH	-0.13 (0.046)	0.03 (0.245)

Note: See Table 4.1 for trait abbreviations

4.5. DISCUSSION

In our study the estimated heritability for lignin content (h_i^2=0.34) was lower than that obtained by Pot et al. (2002) in 15-year-old trees of the same species in France with an estimated heritability values for LG of 0.47. Even higher heritability estimates for this trait (h_i^2=0.75) where reported by Perez et al. (2007) also in France, in 15-year-old trial of the same species. These differences may result from the distinct environmental conditions, as heritability of a certain trait can vary with the population, age, and test location or from random estimation errors. Moreover, this range of results may be due to variations in the percentage of lignin associated with the existence of reaction wood (compression wood in softwoods). Compression wood (CW) contains more lignin and less cellulose than normal wood (Tsoumis 1991), thus, the presence of this kind of wood can influence estimates of the genetic effect of lignin content in our study CW was carefully avoided.

Hannrup et al. (2004) obtained a low narrow sense heritability value (h_i^2=0.10), but a high value for the broad sense heritability (h_i^2=0.54) for *Picea abies*. According to this author, non additive effects may contribute to the high broad-sense heritabilities for LG. However, Pot et al. (2002) found indications that lignin content is predominantly additively inherited. A study performed in *Pinus taeda* by Sykes et al. (2006) also gave individual-tree heritability estimates of 0.12 and 0.23, while Ukrainetz et al. (2008) did not find significant statistical differences between families of *Pseudotsuga menziesii*. Due to weak heritabilities, selection against lignin content through breeding could yield modest genetic gains. Moreover, selection of the family with lower LG would cause less than 2% reduction in lignin content when compared to the selection of the family with highest lignin content. Similar conclusions were drawn by Sykes et al. (2003) who concluded that lowering LG through breeding will involve considerable effort and cost. However, the relationship between lignin content in wood and pulp yield and quality variables could be a starting point to evaluate changes in this character in terms of economic profits for a given pulp production system (Costa e Silva et al. 1998). The chemical removal of lignin from wood during the pulping process makes use of large amounts of concentrated chemicals, so reducing lignin content in wood would be advantageous in decreasing energy and chemical consumption.

The results obtained in this study for the intra-ring density traits are generally higher that the heritability values estimated in a previous performed with the same samples (Gaspar et al. 2008a). In the preceding work all rings were taken into account for the determination of the wood density components, while the present study considered only the last four rings. The heritabilities obtained for earlywood components were higher than those obtained in the previous study. Results could be easily explained by the fact that in the first years the heritability increases with ring number for most density components (Gaspar et al. 2008a). Consequently, it was obtained estimations for the years with higher heritability values. Other studies performed on *P. pinaster* (Louzada and Fonseca 2002), *Pinus radiata* (Kumar 2002), *Picea mariana* (Zhang 1998; Zhang and Jiang 1998; Zhang and Morgenstern 1995) and *P. menziesii* (Ukrainetz et al. 2008) also indicate that EWD is the component more dependent on genetic effects. When transition wood is formed, the wood properties become more regular which may contribute to more precise estimates of genetic parameters (Sykes et al. 2003). Even though the material used in this study is relatively young (17 years), heritabilities determined using just the last 4 rings may be more meaningful than those where the juvenile wood is included, since these results can be closer to the adult behaviour of these trees.

Understanding how characteristics are related to one another is relevant to tree improvement since it allows the assessment of the degree to which one trait will change as a result of a change in another trait (Zobel and Talbert 1984). Lignin is a wood property with potential importance for pulp production, therefore the correlated response in LG should be considered when deciding on a selection strategy (Costa e Silva et al. 1998). The present study showed very low or even no phenotypic correlations of lignin content with all other traits, indicating that phenotypic correlation should not be used to perform mass selection of traits. Our findings concerning genetic correlations indicated that RD had negative but low genetic relationship with lignin, suggesting that selection based on wood density will not result in an increase of LG. Previous studies carried out with *P. Pinaster* (Pot et al. 2002), *P. abies* (Hannrup et al. 2004) and *P. menziesii* (Chantre et al. 2002) indicated the existence of a strong negative genetic correlation between these two traits. The correlations between RD and lignin content have important industrial implications, since selection for increase density will increase pup yield per hectare and decrease production costs. Perez et al. (1995) in a work performed with *P. pinaster* obtained negative correlations between pulp yield and lignin content, and a positive correlation between pulp yields and cellulose content.

Our results also showed that lignin content was more dependent on proportion of latewood present on the ring (LWP and LWW), suggesting that these traits may be controlled, at least partially, by the same set of genes, and that increasing the existence of more latewood would decrease the lignin content. These findings are in agreement with those of Hannrup et al (2004) who also obtained a negative genetic correlation between LWP and LG (-0.49). Sykes et al. (2006) concluded that latewood within a growth ring of loblolly pine had more desirable wood properties than earlywood, because cellulose content was higher and lignin content was lower in latewood. In fact, results of a previous study performed in the same samples (Gaspar et al. 2008b), showed that RD is genetically highly positively correlated with LWW and presented a high negative genetic correlation with EWW. Thus, it is expected that the negative genetic correlation of LG with RD also implies higher negative correlations with latewood intra-ring traits, and positive correlations with EWW. Results from this previous study also showed that selection for higher density could be performed by increasing either LWP or EWD (Gaspar et al. 2008a). Additionally, as previously stated, the results showed a negative genetic correlation between lignin content and the proportion of latewood present on the ring and a almost null correlation with EWD was obtained in the current work.

4.6. CONCLUSIONS

The results of this study showed that due to weak heritabilities and low CV observed for LG, selection against lignin content through breeding could yield modest gains. The wood density traits showed moderated to high heritabilities, suggesting that large gains can be obtained from selecting on basis of most of these variables. This work also allows us to conclude that selection based on density will not result in an increase of LG, and that the lignin content was more dependent on the proportion of latewood present on the ring.

Based on these results it seems possible for breeders to adjust the course of selection depending on the type of end product. For pulp production, the improvement of density with a reduction of lignin is desirable, so the increase of LWP should be the path to obtain higher wood density. Tree improvement programs should decide which traits will be of most importance in the future before incorporating them into their breeding programs.

Acknowledgements

The work was financially supported by Fundação para a Ciência e Tecnologia (Portugal), research project PTDC/AGR-CFL/72606/2006 and grants of first, second and fourth authors SFRH/BD/27412/2006, SFRH/BD/28679/2006 and SFRH/BD/42073/2007 respectively. This research is included in the activities of CITAB (Centro de Investigação e de Tecnologias Agro-Ambientais e Biológicas) and BIOPOL in Centro de Estudos Florestais (Portugal).

4.7. REFERENCES

Aguiar, A. 1993. Análise da variação do crescimento em altura de descendências maternais de pinheiro bravo. Silva Lusitana 1 (2): 267-274.

Alía, R., and Martín, S. 2003. Euforgen, Technical Guidelines for genetic conservation and use for Maritime pine (*Pinus pinaster*). International Plant Genetic Resources Institute.

CELPA. 2007. Boletim Estatístico da Indústria Papeleira Portuguesa.

Chantre, G., Rozenberg, P., Baonza, V., Macchioni, N., Le Turcq, A., Rueff, M., Petit-Conil, M., and Heois, B. 2002. Genetic selection within Douglas fir (*Pseudotsuga menziesii*) in Europe for papermaking uses. Ann For Sci 59 (5-6): 583-593.

DGRF. 2007. National Forest Inventory 2005/2006. Direcção Geral dos Recursos Florestais.

Gaspar, M.J., de-Lucas, A., Alia, R., Paiva, J.A.P., Hidalgo, E., Louzada, J.L., Almeida, M.H., and González-Martínez, S.C. In Press Use of molecular markers for estimating breeding parameters: a case study in Maritime pine progeny trial. Tree Gen Genom. DOI 10.1007/s11295-009-0213-1

Gaspar, M.J., Louzada, J.L., Silva, M.E., Aguiar, A., and Almeida, M.H. 2008a. Age trends in genetic parameters of wood density components in 46 half-sibling families of *Pinus pinaster*. Can J Forest Res 38 (6): 1470-1477.

Gaspar, M.J., Louzada, J.L., Aguiar, A., and Almeida, M.H. 2008b. Genetic correlations between wood quality traits of *Pinus pinaster* Ait. Ann For Sci 65 (7): 703.

Gilmour, A., Gogel, B., Cullis, B., Welham, S., and Thompson, R. 1998. ASREML Users Manual. *In* VSN International. New south Wales Agriculture, Orange.

Hannrup, B., Cahalan, C., Chantre, G., Grabner, M., Karlsson, B., Le Bayon, I., Jones, G.L., Muller, U., Pereira, H., Rodrigues, J.C., Rosner, S., Rozenberg, P., Wilhelmsson, L., and Wimmer, R. 2004. Genetic parameters of growth and wood quality traits in *Picea abies*. Scand J of Forest Res 19 (1): 14-29.

Kumar, S. 2002. Earlywood-latewood demarcation criteria and their effect on genetic parameters of growth ring density components and efficiency of selection for end-of-rotation density of radiata pine. Silvae Genet 51 (5-6): 241-246.

Kumar, S., Burdon, R.D., Stovold, G.T., and Gea, L.D. 2008. Implications of selection history on genetic architecture of growth, form, and wood-quality traits in *Pinus radiata*. Can J Forest Res 38 (9): 2372-2381.

Louzada, J.L.P.C. 2003. Genetic correlations between wood density components in *Pinus pinaster* Ait. Ann For Sci 60: 285-294.

Louzada, J.L.P.C., and Fonseca, F.M.A. 2002. The heritability of wood density components in *Pinus pinaster* Ait. and implications for tree breeding. Ann For Sci 59: 867-873.

Perez, D.D., Guillemain, A., Alazard, P., Plomion, C., Rozenberg, P., Rodrigues, J.C., Alvess, A., and Chantre, G. 2007. Improvement of *Pinus pinaster* Ait. elite trees selection by combining near infrared spectroscopy and genetic tools. Holzforschung 61 (6): 611-622.

Perez, D.S., Guillemain, A., Chantre, G., Alazard, P., Alves, A., Rodrigues, J., Rozenberg, P., Plomion, C., and Robin, E. 1995. Improvement of wood, pulp and paper quallity of maritime pine (*Pinus pinaster* Ait.) by combining rapid assessment techniques and genetics. *In* 13th International Symposium on Wood, Fibres and Pulping Chemistry, Oakland, New Zealand. pp. 101-108.

Perry, D., and Hopkins, E. 1967. Importation of breeding material of *Pinus pinaster* Ait. from Portugal. For. Dep. W. A. Bulletin 75.

Pot, D., Chantre, G., Rozenberg, P., Rodrigues, J.C., Jones, G.L., Pereira, H., Hannrup, B., Cahalan, C., and Plomion, C. 2002. Genetic control of pulp and timber properties in Maritime pine (*Pinus pinaster* Ait.). Ann For Sci 59 (5-6): 563-575.

Rodrigues, J., Alves, A., Pereira, H., Perez, D.D.S., Chantre, G., and Schwanninger, M. 2006. NIR PLSR results obtained by calibration with noisy, low-precision reference values: Are the results acceptable? Holzforschung 60 (4): 402-408.

Roulund, H., Alpuim, M., Varela, M.C., and Aguiar, A. 1988. A tree improvement plan for *Pinus pinaster* in Portugal. EFN.

Sykes, R., Li, B.L., Isik, F., Kadla, J., and Chang, H.M. 2006. Genetic variation and genotype by environment interactions of juvenile wood chemical properties in *Pinus taeda* L. Ann For Sci 63 (8): 897-904.

Tsoumis, G. 1991. Science and Technology of Wood: Structure, Properties, Utilization. Chapman & Hall New York, 494 pp.

Ukrainetz, N.K., Kang, K.Y., Aitken, S.N., Stoehr, M., and Mansfield, S.D. 2008. Heritability and phenotypic and genetic correlations of coastal Douglas-fir (*Pseudotsuga menziesii*) wood quality traits. Can J Forest Res 38 (6): 1536-1546.

Vargas-Hernandez, J., and Adams, W.T. 1991. Genetic-variation of wood density components in young coastal Douglas-Fir - Implications for tree breeding. Can J Forest Res 21 (12): 1801-1807.

Zhang, S.Y. 1998. Effect of age on the variation, correlations and inheritance of selected wood characteristics in Black spruce (*Picea mariana*). Wood Sci Technol 32 (3): 197-204.

Zhang, S.Y., and Jiang, Z.H. 1998. Variability of selected wood characteristics in 40 half-sib families of Black spruce (*Picea mariana*). Wood Sci Technol 32 (1): 71-82.

Zhang, S.Y., and Morgenstern, E.K. 1995. Genetic-variation and inheritance of wood density in Black spruce (*Picea mariana*) and its relationship with growth - implications for tree breeding. Wood Sci Technol 30(1): 63-75.

Zobel, B.J., and Jett, J.B. 1995. Genetics of Wood Production. Springer-Verlag ed, Berlin, Heidelberg, and New York, 377 pp.

Zobel, B.J., and Talbert, J. 1984. Applied Forest Tree Improvement. John Wiley & Sons, New York, 511 pp.

Zobel, B.J., and van Buijtenen, J.P. 1989. Wood Variation - Its causes and control. Springer-Verlag ed, Berlin, 363 pp.

CHAPTER 5

Genetic variations of wood mechanical traits in 46 half-sib families of *Pinus pinaster* Ait.

Submitted as: Gaspar, M.J.; Louzada, J.L.; Morais, J.; Fernandes, C.; Rodrigues, J.C. and Almeida, M.H. Genetic variations of wood mechanical traits in 46 half-sib families of *Pinus pinaster* AIT. Silvae Genetica.

5.1. ABSTRACT

Genetic parameters for radial modulus of elasticity (MOE_{Rad}) and the radial modulus of rupture (MOR_{Rad}), as well as its correlations with wood density components, spiral grain and lignin content were estimated in a seventeen-year-old *Pinus pinaster* progeny trial. The trial was located in Leiria, Portugal, included of 46 half sib families originated from seed collected in a clonal seed orchard. A total of 552 trees were sampled at 2m height. The results show that MOE_{Rad} and MOR_{Rad} are under moderate genetic control (h^2=0.34 and h^2=0.30, respectively), suggesting possible genetic gains by selection for higher resistance and elasticity. MOE_{Rad} had higher correlations with the all other traits, presenting higher correlation with latewood components than with earlywood components. Negative correlations were observed between mechanical traits and lignin content, while the correlations with spiral grain were positive.

5.2. INTRODUCTION

Maritime pine (*Pinus pinaster* Ait.) is an important commercial species in southwest Europe. It is the principal conifer in Portugal, covering 710.6×10^3ha, approximately 23% of the total Portuguese forestry area (DGRF 2007). The wood of this species is remarkably versatile and it is used in both timber and pulp industries, involving different stakeholders (forest owners, the timber and pulp industries) for whom different traits may be of interest. The quality of wood used for structural purposes may be defined in terms of strength, stiffness and stability (Perstorper et al. 1995). Due to efforts to improve the growth rate of the species and the consequent decrease of harvest age, a greater proportion of juvenile wood is present (Burdon et al. 2004; Kennedy 1995; Kumar et al. 2002). In *Pinus* species, juvenile wood is characterized by a shorter tracheid and thinner cell walls than mature wood, which often produces wood with a lower specific gravity. Due to its low strength and instability on drying, juvenile wood presents a problem for most solid wood products (Zobel and Sprague 1998). Wood with lower mechanical properties and higher heterogeneity would be probable consequences of future plantings comprising a greater proportion of juvenile wood (Zamudio et al. 2005). A way of mitigating the negative effects of short rotations on wood quality is to breed for improved juvenile wood properties (Gapare et al. 2006).

The knowledge of wood mechanical properties is of primordial importance for selection and application in the various areas of engineering (Santos and Pinho 2004). Mechanical stiffness and strength of wood are combinations of physical, chemical and anatomical properties of wood (Steffenrem et al. 2007). Nevertheless, density is regarded as the single most important predictor of the wood mechanical properties (Sharp 2003), although density is not a single property, but it is a complex of characteristics (density components).

Wood density has been the principal wood quality trait used for selection, due to the close relation with other important characteristics for different end uses (West 2006) and also the high heritability (Zobel and Jett 1995). Consequently, it is essential to understand how the different characteristics are related to one another, and this requires knowledge about the variation and the genetic correlations. Other key property influencing the mechanical traits is spiral grain (Sharp 2003). According to this author the existence of a deviation of longitudinal orientation of the cells always reduces the longitudinal strength of wood. Wood chemical composition can also influence the physical properties of different types of wood (Bodig 1981).

For structural applications it is necessary to characterize not only the longitudinal mechanical properties of wood but also the properties related with radial and tangential directions of wood anatomy (Xavier et al. 2007; Xavier et al. 2004). However, the majority of published works about the variability of mechanical properties of maritime pine have focused on the longitudinal properties (Machado and Cruz 2005). According to Ohbayashi et al. (2001) one purpose for studying characteristics such as the modulus of rupture in the radial orientation (MOR_{Rad}) is that low values of this trait might increase the potential for within-ring internal checking. These checks form occasionally during kiln drying, either softwood as in hardwood (Ilic 1995).

The aim of this study was to characterize the genetic control of radial modulus of rupture and radial elasticity module and the correlation patterns between these traits and intra-ring wood characteristics, spiral grain and lignin content.

5.3. MATERIAL AND METHODS

The progeny test used in this study included 46 open pollinated families, originated from seed collected in the Escaroupim clonal seed orchard II (Aguiar 1993). Established in 1987, the field trial location was Pinhal de Leiria, situated central coastal Portugal. The site is characterized by a Mediterranean climate, with a mean air temperature between 12.5°C and 15°C, relative humidity between 80 and 85% and yearly rainfall values usually between 700mm and 800mm, with a 4 month period of summer drought, and by Spodic Podzols (PZ sd) soils derived from sand dunes of maritime origin. Seedlings were raised for 9 months in the nursery in plastic bags (250cm^3 volume). Planting (2m×2m spacing) took place in March 1987 after harrowing and ploughing the site; no fertilizer was applied.

The 46 families were replicated in 3 blocks with 4 trees per plot, giving a total number of 552 trees for analysis. One wood disc with a thickness of 10cm was collected at a sampling height of 2m. The disk was sawn into a 2mm-thick radial strip segment from the pith to the bark, and then conditioned at 12% moisture content. This strips were used to determine ring density (RD), minimum density (MND), maximum density (MXD), earlywood density (EWD), latewood density (LWD), earlywood width (EWW), latewood width (LWW), latewood percentage (LWP), ring width (RW) using X-ray densitometry procedures as described by Gaspar (2008a) The same strips were used to evaluate MOE_{Rad} and MOR_{Rad}, through three-point bending tests (Brancheriau et al. 2002). Before testing the specimen dimensions (thickness and width) were measured with a digital calliper (0.01mm resolution). Bending tests were carried out in an electro-mechanical testing machine (Instron 5848 MicroTester®). The experiments were carried out at room temperature under displacement control (0.5mm/min) and with a span of 40mm.

The other traits assessed were spiral grain (SPG) determined in the last ring of all trees, as explained in (Gaspar 2008b) and the proportion of lignin content (LG) predicted using near infrared spectroscopy (NIR) on extractive-free wood meal samples according to Rodrigues et al. (2006) and Perez et al. (2007).

5.3.1. Data analysis

The mixed linear model associated with the data for a given trait was:

$$Y_{ijk}=\mu + B_j + F_i + B\times F_{ij} + \varepsilon_{ijk} \quad [5.1]$$

where Y represents the phenotypic individual observation; μ is the overall mean; B_j is the effect of the jth block (fixed); F_i is the effect of the ith family (random); $B\times F_{ij}$ is the effect of the interaction between the ith family and the jth block (random) and ε is the residual error. Variance components for family (σ_f^2), family-block interaction (σ_{f*b}^2) and residual errors (σ_ε^2), with the respective associated standard errors, were estimated by restricted maximum likelihood, using the average information REML algorithm implemented in the ASREML programme (Gilmour et al. 1998). Narrow sense heritability (h_i^2) was calculated for each measured trait as:

$$h_i^2=\frac{\sigma_a^2}{\sigma_P^2} \quad [5.2]$$

where σ_a^2 represents the additive genetic variance and σ_P^2 the total phenotypic variance. Total phenotypic variance was estimated as:

$$\sigma_P^2=\sigma_f^2+\sigma_{f*b}^2+\sigma_\varepsilon^2 \quad [5.3]$$

and adjusted additive variances as:

$$\sigma_a^2=\frac{1}{0.26}\times\sigma_f^2 \quad [5.4]$$

where the coancestry coefficient (0.26) was obtained from correlated paternity, estimated by molecular markers as described by Gaspar et al. (In Press). Standard errors for heritability were estimated by ASREML using a Taylor series approximation (Gilmour et al. 1998).

The genetic and phenotypic correlations between all wood density components were calculated using a multivariate extension of model (5.1). When the family effect was found to be null for any of the traits analysed in the univariate analysis, this effect was not included in the multivariate analysis. Genetic correlations between traits (*X* and *Y*) were evaluated as follows:

$$r_a = \frac{Cov_a(x,y)}{\sqrt{\sigma^2_{a_x} . \sigma^2_{a_y}}} \qquad [5.5]$$

where $\mathrm{cov}_a(x,y)$ is the additive genetic covariance, and $\sigma^2_{a_x}$ and $\sigma^2_{a_y}$ are the additive variance components for traits *X* and *Y*, respectively.

The phenotypic correlation between traits (*X* and *Y*), was estimated as:

$$r_P = \frac{Cov_P(x,y)}{\sqrt{\sigma^2_{P_x} . \sigma^2_{P_y}}} \qquad [5.6]$$

where $\mathrm{cov}_P(x,y)$ is the phenotypic covariance between traits *X* and *Y*, estimated as $\mathrm{cov}_P(x,y) = \mathrm{cov}_f(x,y) + \mathrm{cov}_{fb}(x,y) + \mathrm{cov}_\varepsilon(x,y)$, which is the sum of the family, interaction, and residual covariance components. $\sigma^2_{P_x}$ and $\sigma^2_{P_y}$ are the phenotypic variances for traits X and Y, respectively. Standard errors for genetic and phenotypic correlations were estimated by ASREML using a Taylor series approximation (Gilmour et al. 1998).

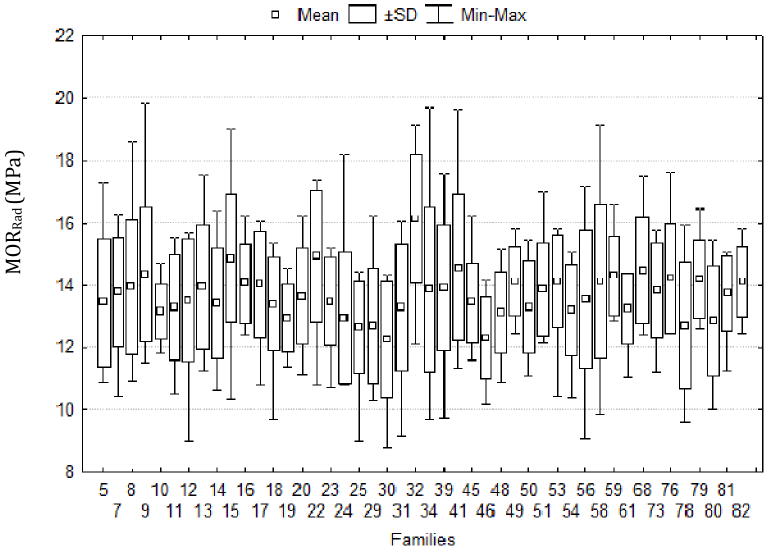
5.4. RESULTS AND DISCUSSION

Descriptive statistics table for radial modulus of rupture ($\mathrm{MOR_{Rad}}$) and radial modulus of elasticity ($\mathrm{MOE_{Rad}}$) are given in Table 5.1. Figure 5.1 show graphs of family mean values for mechanical traits, and highlight the differences that were found between families. About 50% of families had a mean value higher than the average value of $\mathrm{MOR_{Rad}}$ and 47% exceeded the mean value of $\mathrm{MOE_{Rad}}$. Family 32 had the highest mean values for both traits (MOE_{Rad}=1.32GPa, MOR_{Rad}=16.13MPa) and minimum value obtained for MOE_{Rad} and MOR_{Rad} were 1.042 GPa and 12.27 MPa for families 78 and 30, respectively. Ohbayashi et al. (2001) in a work performed in radiate pine obtained also for MOR_{Rad} a mean value of 7.8 MPa, slightly lower than the ones obtained by us.

Table 5.1 Descriptive statistics table for Radial Modulus of Rupture (MOR_{Rad}) and Radial Elasticity Module (MOE_{Rad}), respective additive and phenotypic variance components and individual heritability estimates (standard errors given in brackets)

	MOR_{Rad} (MPa)	MOE_{Rad} (GPa)
Mean	13. 8	1.1
Max	19.80	1.72
Min	8.76	0.78
SD	1.72	0.15
Cv (%)	12.5	13.2
V_a	0.983	0.638×10^{-2}
V_p	2.882	0.213×10^{-1}
h^2	0.34 (0.131)	0.30 (0.143)

The mean value obtained for MOR_{Rad} and MOE_{Rad} was 13.8 MPa and 1.1 GPa respectively, with a coefficient of variation of approximately 13% for both traits. Both traits had moderate heritability values 0.34±0.131 and 0.30±0.143 for MOR_{Rad} and MOE_{Rad}, respectively, indicating that these traits are subject to only modest genetic control in maritime pine. Genetic control of mechanical properties has not been well documented compared with growth traits and wood density. As far as the author's knowledge, no other studies have evaluated the genetic control of mechanical traits on radial direction. Nevertheless, our estimated heritabilities for this trait correspond well with results found in previous studies performed in other coniferous species, for MOE and MOR measured in the longitudinal direction. These studies also report moderate to high genetic control for these characteristics (Baltunis et al. 2007; Fujimoto et al. 2006; Johnson and Gartner 2006; Kumar 2004; Kumar et al. 2006). The differences between families with highest and lowest MOE_{Rad} and MOR_{Rad}, allied with the values of heritability estimates, indicate that selection for higher resistance and elasticity through breeding could yield some genetic gains. However, it is necessary to understand how characteristics are related to one another in order to be able to assess the degree to which one trait will change as a result of improving another trait (Zobel and Talbert 1984).



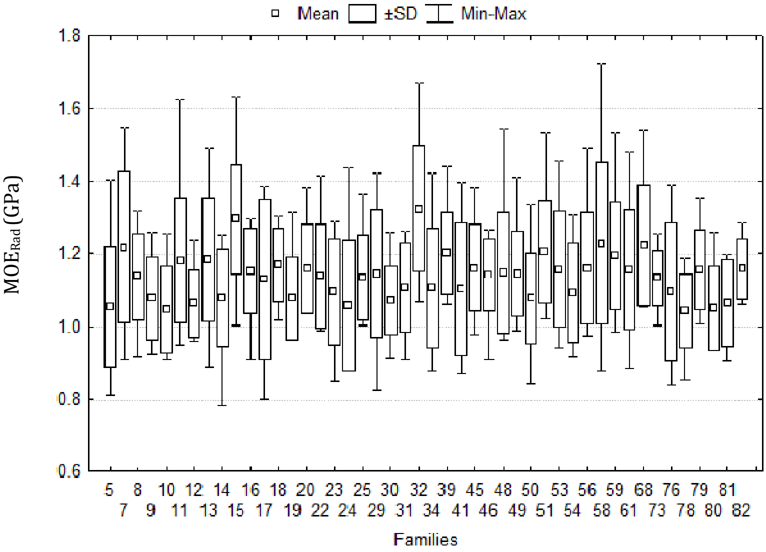


Figure 5.1 Mean family values, respective standard deviations and minim and maxim values of radial modulus of rupture (MOR_{Rad}) and radial modulus of elasticity (MOE_{Rad}).

Tables 5.2 and 5.3 present phenotypic and genetic correlations observed between MOR_{Rad} and MOE_{Rad} and the other traits in study. Results showed a high phenotypic and genetic correlation between the two mechanical traits (r_p=0.61 and r_g=0.56, respectively), indicating that these traits are at least for a large part controlled by the same set of genes and that indirect selection procedures could be carried out on these characters. Both mechanical traits presented positive phenotypic or genetic correlations with RD.

Table 5.2 Estimates of phenotypic correlation coefficients (with standard errors given in brackets) between all traits.

	MOE_{Rad}	MOR_{Rad}
MOR_{Rad}	0.61 (0.029)	-
RD	0.33 (0.042)	0.18 (0.046)
MND	0.21 (0.045)	0.15 (0.046)
MXD	0.28 (0.043)	0.17 (0.046)
EWD	0.26 (0.045)	0.17 (0.046)
LWD	0.25 (0.043)	0.15 (0.045)
LWP	0.27 (0.043)	0.13 (0.046)
EWW	-0.13 (0.045)	-0.09 (0.046)
LWW	0.30 (0.043)	0.13 (0.045)
SPG	0.18 (0.045)	0.13 (0.045)
LG	-0.16 (0.045)	-0.11 (0.045)

MOR_{Rad} = Radial Modulus of Rupture, MOE_{Rad}= Radial Elasticity Module, RD = Average Ring Density, MND = Minimum Density, MXD = Maximum Density, EWD = Earlywood Density, LWD = Latewood Density, LWP = Latewood Percentage, EWW= Earlywood Width, LWW= Latewood Width, SPG=Spiral Grain, LG= Proportion of Lignin Content.

These findings coincide with those obtained by Burdon et al. (2004); Dungey et al. (2006); Hannrup et al. (2004) and Steffenrem et al. (2007), although the mechanical traits were evaluated longitudinally. Our findings also indicate that MOE_{Rad} was more genetically dependent on latewood components, both density (MXD and LWD) and growth ones (LWP and LWW). The highest positive genetic correlation observed in this work was between MOE_{Rad} and LWW, indicating that selection for greater quantities of latewood would increase wood stiffness.

Table5.3 Estimates of genotypic correlation coefficients (with standard errors given in brackets) between all traits.

	MOE_{Rad}	MOR_{Rad}
MOR_{Rad}	0.56 (0.193)	-
RD	0.33 (0.2146)	0.11 (0.243)
MND	0.11 (0.241)	0.23 (0.242)
MXD	0.35 (0.237)	0.08 (0.270)
EWD	0.16 (0.234)	0.16 (0.242)
LWD	0.36 (0.257)	-0.01 (0.296)
LWP	0.37 (0.226)	0.03 (0.262)
EWW	-0.02 (0.293)	0.02 (0.302)
LWW	0.48 (0.218)	0.09 (0.273)
SPG	0.42 (0.231)	0.19 (0.260)
LG	-0.53 (0.229)	-0.09 (0.279)

Note: See Table 5.2 for trait abbreviations

Results from a previous study performed in the same samples showed that RD was genetically highly positively correlated with LWW and negatively correlated with EEW (Gaspar et al. 2008b). Thus, as MOE_{Rad} has a high genetic correlation with RD, it would be expected that MOE_{Rad} also presented high positive correlations with latewood intra-ring traits, and negative with EWW. This findings are in agreement with the "*weak band theory*" that was proposed by Bodig (1965) and Kennedy (1967) *cit. by* Liu and Ross (2005). According to this author, in the radial direction earlywood and latewood bands are arranged in series, perpendicular to the applied load. Both bands carry the same load, but latewood deforms much less than earlywood. The first failure occurs in the weakest earlywood band, with subsequent failures occurring in other earlywood bands and then latewood bands as compression progresses. Thus, if improvement of earlywood density is possible, the loading capacity in the radial direction can be superior. With the exception of MND and EWD, lower correlations of MOR_{Rad} with all the intra-ring traits were obtained. These results suggest that MOR_{Rad} could be enhanced trough the improvement of the earlywood density, even though this trait is less affected by intra-ring variations that MOE_{Rad}.

Phenotypic and genetic correlations between SPG and mechanical traits were positive but low, with the exception of the moderate genetic correlation value with MOE_{Rad} (r_g=0.42). A converse relation between MOE_{Rad} measured in the longitudinal direction and spiral grain or microfibrill angle is usually reported in literature (Hannrup et al. 2004). One possible explanation of our results may be due to the high genetic correlation observed between SPG with RD and LWW in the same samples (Gaspar et al., 2008b), since MOE_{Rad} also presents high genetic correlations with RD and LWW. Spiral grain does not have an overwhelming influence on the mechanical performance in the radial direction, so it is possible that this correlation is a result of an indirect relation with other trait. Thus, it is expected that this tendency of increasing MOE_{Rad} with SPG is a reflection of a higher incidence of juvenile wood and/or occurrence of reaction wood, which confounds the relation between these traits. For example, it is probable that a tree with a high incidence of reaction wood presents higher values of SPG (Harris 1989), since reaction wood in softwoods is characterized by high values of LWP, LWW and RD (Timell 1986; Zobel and van Buijtenen 1989), high MOE_{Rad} values are expectable. Thus, it is possible that SPG is positively correlated with MOE_{Rad} due to the indirect effect of reaction wood.

Phenotypic correlations between mechanical traits and LN were weak and negative ($r_{g(MOE)}$=-0.16 and $r_{g(MOR)}$=-0.11). The genetic correlation value obtained between MOE_{Rad} and LG was also negative but considerably higher (-0.53), while the correlation with MOR_{Rad} was also negative but very weak (-0.094). Similar genetic correlations between these traits were obtained by Chantre et al. (2002), Hannrup et al. (2004) and Steffenrem et al. (2007). According to Steffenrem et al. (2007) it is difficult to find support for these results in the literature "*since the cell structure in earlywood contains higher proportions of lignin – rich middle lamella than latewood, the effect of lignin on MOE and MOR could be confounded with other correlated traits* *such as RW, LWP and density*". Unpublished results of analyses performed in the same samples used in this work, indicates a negative genetic correlation between LWW and LG. The high correlation between LWW and MOE_{Rad} (0.48) can explain the negative relation of MOE_{Rad} and LN. These genetic correlations indicate that selecting trees with lower LG, for example for pulp production, will decrease the radial modulus of elasticity of the wood, so attention should be given to the final use of the material.

Destructive methods are usually used to evaluate mechanical properties. However a large number of living trees need to be evaluated using non-destructive methods in order to create breeding or production populations with trees that posses the best attributes, or simply because the genetic trial need to be maintained for further evaluations. With this work the genetic control of mechanical traits was evaluated using samples that could be obtained through core samples, what is a non destructive method.

5.5. CONCLUSIONS

Based on a non destructive method, using samples cores our heritability results indicates that MOE_{Rad} and MOR_{Rad} are under a moderate genetic control. Also the differences between families with the highest and the lowest MOR_{Rad} and MOE_{Rad} indicate that selection for higher resistance and elasticity through breeding could yield some genetic gains. The positive correlations observed between both mechanical traits and RD indicates that selecting for RD will not result in a decrease of MOR_{Rad} and, is possible to obtain an increase in MOE_{Rad}. The high genetic correlations observed between the MOE_{Rad} and latewood components make it a strong trait for indirect genetic improvement. Results suggest that MOR_{Rad} could be improved through the enhancement of earlywood density, even though this trait is less affected by intra-ring variations that MOE_{rad}.

Acknowledgements

Acknowledgements are due to two referees who with their comments greatly improved this paper. M.J. Gaspar was funded by the Portuguese Foundation for Science and Technology (Doctoral grant SFRH/BD/27412/2006).

5.6. REFERENCES

Aguiar, A. 1993. Análise da variação do crescimento em altura de descendências maternais de pinheiro bravo. Silva Lusitana 1 (2): 267-274.

Baltunis, B.S., Wu, H.X., and Powell, M.B. 2007. Inheritance of density, microfibril angle, and modulus of elasticity in juvenile wood of *Pinus radiata* at two locations in Australia. Can J Forest Res 37 (11): 2164-2174.

Bodig, J. 1981. Mechanics of wood and wood composits. Van Nostrand Reinhold Company, New York, 712 pp.

Brancheriau, L., Bailleres, H., and Guitard, D. 2002. Comparison between modulus of elasticity values calculated using 3 and 4 point bending tests on wooden samples. Wood Sci Technol 36 (5): 367-383.

Burdon, R.D., Kibblewhite, R.P., Walker, J.C.F., Megraw, R.A., Evans, R., and Cown, D.J. 2004. Juvenile versus mature wood: A new concept, orthogonal to corewood versus outerwood, with special reference to *Pinus radiata* and *Pinus taeda*. Forest Sci 50 (4): 399-415.

DGRF. 2007. National Forest Inventory 2005/2006. Direcção Geral dos Recursos Florestais.

Dungey, H.S., Matheson, A.C., Kain, D., and Evans, R. 2006. Genetics of wood stiffness and its component traits in *Pinus radiata*. Can J Forest Res 36 (5): 1165-1178.

Fujimoto, T., Akutsu, H., Nei, M., Kita, K., Kuromaru, M., and Oda, K. 2006. Genetic variation in wood stiffness and strength properties of hybrid larch (*Larix gmelinii* var. japonica x L kaempferi). J Forest Res-Jpn 11 (5): 343-349.

Gapare, W.J., Wu, H.X., and Abarquez, A. 2006. Genetic control of the time of transition from juvenile to mature wood in *Pinus radiata* D. Don. Ann For Sci 63 (8): 871-878.

Gaspar, M.J., de-Lucas, A., Alia, R., Paiva, J.A.P., Hidalgo, E., Louzada, J.L., Almeida, M.H., and González-Martínez, S.C. In press. Use of molecular markers for estimating breeding parameters: a case study in Maritime pine progeny trial. Tree Gen Genom. DOI 10.1007/s11295-009-0213-1

Gaspar, M.J., Louzada, J.L., Aguiar, A., and Almeida, M.H. 2008b. Genetic correlations between wood quality traits of *Pinus pinaster* Ait. Ann For Sci 65 (7):703.

Gilmour, A., Gogel, B., Cullis, B., Welham, S., and Thompson, R. 1998. ASREML Users Manual. *In* VSN International. New south Wales Agriculture, Orange.

Hannrup, B., Cahalan, C., Chantre, G., Grabner, M., Karlsson, B., Le Bayon, I., Jones, G.L., Muller, U., Pereira, H., Rodrigues, J.C., Rosner, S., Rozenberg, P., Wilhelmsson, L., and Wimmer, R. 2004. Genetic parameters of growth and wood quality traits in *Picea abies*. Scand J of For Res 19 (1): 14-29.

Harris, J.M. 1989. Spiral grain and wave phenomena in wood formation, Berlin, Heidelberg, and New York, 230 pp.

Ilic, J. 1995. Advantages of Pre-freezing for reducing shrinkage-related degrade in Eucalypts - General considerations and review of the literature. Wood Sci Technol 29 (4): 277-285.

Johnson, G.R., and Gartner, B.L. 2006. Genetic variation in basic density and modulus of elasticity of coastal Douglas fir. Tree Gen Geno 3 (1): 25-33.

Kennedy, R.W. 1995. Coniferous wood quality in the future - Concerns and strategies. Wood Sci Technol 29 (5): 321-338.

Kumar, S. 2004. Genetic parameter estimates for wood stiffness, strength, internal checking, and resin bleeding for Radiata pine. Can J Forest Res 34 (12): 2601-2610.

Kumar, S., Dungey, H.S., and Matheson, A.C. 2006. Genetic parameters and strategies for genetic improvement of stiffness in Radiata pine. Silvae Genet 55 (2): 77-84.

Kumar, S., Jayawickrama, K.J.S., Lee, J., and Lausberg, M. 2002. Direct and indirect measures of stiffness and strength show high heritability in a wind-pollinated Radiata pine progeny test in New Zealand. Silvae Genet 51 (5-6): 256-261.

Liu, J.Y., and Ross, R.J. 2005. Relationship between radial compressive modulus of elasticity and shear modulus of wood. Wood Fiber Sci 37 (2): 201-206.

Machado, J.S., and Cruz, H.P. 2005. Within stem variation of Maritime Pine timber mechanical properties. Holz Als Roh-Und Werkstoff 63 (2): 154-159.

Ohbayashi, H., Booker, R.E., Ball, R.D., and Ridoutt, B.G. 2001. Radial modulus of rupture in Radiata pine measured by individual rings. J Wood Sci 47 (3): 233-236.

Perez, D.D., Guillemain, A., Alazard, P., Plomion, C., Rozenberg, P., Rodrigues, J.C., Alvess, A., and Chantre, G. 2007. Improvement of *Pinus pinaster* Ait elite trees selection by combining near infrared spectroscopy and genetic tools. Holzforschung 61 (6): 611-622.

Perstorper, M., Pellicane, P.J., Kliger, I.R., and Johansson, G. 1995. Quality of timber products from Norway spruce. 1. Optimization, Key Variables and Experimental-Study. Wood Sci Technol 29 (3): 157-170.

Rodrigues, J., Alves, A., Pereira, H., Perez, D.D.S., Chantre, G., and Schwanninger, M. 2006. NIR PLSR results obtained by calibration with noisy, low-precision reference values: Are the results acceptable? Holzforschung 60 (4): 402-408.

Santos, J.A., and Pinho, A.C.M. 2004. New advances for the application of *Eucalyptus* as a structural wood. Silva Lusitana 12(1): 43-50.

Sharp, A.Z. 2003. The mechanical properties of wood. *In* Wood quality and its biological basis. *Edited by* J. R. Barnett, and G. Jeronimidis. Blackwell Publishing, Oxford. pp. 187-210.

Steffenrem, A., Saranpaa, P., Lundqvist, S.O., and Skroppa, T. 2007. Variation in wood properties among five full-sib families of Norway spruce (*Picea abies*). Ann For Sci 64 (8): 799-806.

Timell, T.E. 1986. Compression Wood in Gymnosperms. Springer-Verlag, 2150 pp.

West, P.W. 2006. Growing Plantations Forests. Berlin, 304 pp.

Xavier, J., Avril, S., Pierron, F., and Morais, J. 2007. Novel experimental approach for longitudinal-radial stiffness characterization of clear wood by a single test. Holzforschung 61 (5): 573-581.

Xavier, J.C., Garrido, N.M., Oliveira, M., Morais, J.L., Camanho, P., and Pierron, F. 2004. A comparison between the iosipescu and off-axis shear test methods for the characterization of *Pinus Pinaster* Ait. Composites Part a - Applied Science and Manufacturing 35 (7-8): 827-840.

Zamudio, F., Rozenberg, P., Baettig, R., Vergara, A., Yanez, M., and Gantz, C. 2005. Genetic variation of wood density components in a Radiata pine progeny test located in the south of Chile. Ann For Sci 62 (2): 105-114.

Zobel, B.J., and Jett, J.B. 1995. Genetics of Wood Production. Springer-Verlag ed, Berlin, Heidelberg, and New York, 377 pp.

Zobel, B.J., and Sprague, J.R. 1998. Juvenil Wood in Forest Trees. Springer-Verlag ed, Berlin, Heidelberg, and New York, 300 pp.

Zobel, B.J., and Talbert, J. 1984. Applied Forest Tree Improvement. John Wiley & Sons, New York, 511 pp.

Zobel, B.J., and van Buijtenen, J.P. 1989. Wood Variation - Its causes and control. Springer-Verlag ed, Berlin, 363 pp.

CHAPTER 6

Does selecting for improved growth affect wood quality of *Pinus pinaster* in Portugal?

Published as: Gaspar, M.J.; Louzada, J.L.; Rodrigues, J.; Aguiar, A. and Almeida, M.H. (in press). Does selecting for improved growth affect wood quality of *Pinus pinaster* in Portugal? Forest Ecology and Management.
Doi:10.1016/j.foreco.2009.03.046

6.1. ABSTRACT

The selection criteria for the first generations in the Portuguese *Pinus pinaster* improvement program have been the growth rate and form traits. In this work we study the consequences of this selection on wood quality traits. This study assesses genetic and phenotypic correlation between growth, wood density components, lignin content and mechanical traits (radial modulus of elasticity and radial modulus of rupture) of 46 half sib families from a progeny trial located in Leiria, Portugal, originated from seed collected in a clonal seed orchard. A total of 552 seventeen-years-old trees (about half of full rotation age) were sampled at 2m height. Height measured at 12 years old presented a higher genetic control (h^2=0.34) relatively to DBH, measured at 12 and 17 years old respectively (h^2=0.17 and h^2=0.15). The results of this study also showed that DBH growth is more dependent on latewood components than earlywood components and that higher growth in Mediterranean regions can be due to an increase of the period of latewood formation. Further, we can conclude genetic selection based on growth will not result in a decrease of wood density, will not affect the occurrence of spiral grain, and is possible to obtain an increase in the radial modulus of elasticity. The present study also showed that it is possible to select for increased growth with lower lignin proportion. Results also suggest that selection for growth at 12 years will probably not affect negatively the wood properties at 17 years.

6.2. INTRODUCTION

Maritime pine (*Pinus pinaster* Ait.) is a highly valuable coniferous species (*Pinaceae*), broadly distributed in the western Mediterranean Basin, in Southern Europe and Africa, and the Atlantic coast of Portugal, Spain and France (Alía and Martín 2003). It is one of the most important forest species in France, Portugal and Spain, used mainly for carpentry, construction, chipboard, pulp and paper production, floor boards and palettes. In Portugal, according to the National Forest Inventory (2005/2006), 23% of the national forest area is occupied by *P. pinaster* (DGRF 2007), representing 11% of the total of forest product exports in 2000

(Correia et al. 2004). Due to the economic importance of *P. pinaster*, a national tree improvement plan for this species has been in operation since the early 1980's (Roulund et al. 1988). Presently, the maritime pine breeding programme is in its second generation of selection (the progeny trials reported in this study are part of this programme), and the selection criteria for the first generations have been the growth rate and form traits. Such selection criteria might have an indirect effect on wood properties (Zobell and Jett 1995), therefore, it's essential to know the nature and magnitude of the relationship between growth traits, wood density and other quality traits. The relationship between growth rate and wood density has been studied intensively, but the results obtained are contradictory. Zobel and van Buijtenen (1989) reviewed 55 hard pine studies up until 1986; 35 showed no relationship, 11 a significant reduction in density with increased growth and 4 reported that when trees grew faster, a higher density was observed. Wu et al (2008) reviewed several studies on *Pinus radiata* and found that in a total of 64 genetic correlations between density and DBH (or ring width) the values ranged between -1.08 and 0.6 with a mean value of -0.51.

More attention has been given to breeding for wood density and quality in recent years, because of shorter rotations and the recognition that increasing rotation markedly raises the effective growing cost (Wu et al 2008). In a previous work by Gaspar et al (2008a) on the heritability of wood density components in *P. pinaster*, it was concluded that ring density (RD) was the trait under strongest genetic control (h^2= 0.63), and heritability values of earlywood components exceeded those of latewood components. Heritabilities increased with ring number from pith for almost all wood-density components, and there were high age-age genetic correlations for wood density traits (r_g> 0.98).

Sound knowledge of genetic correlations is important for both defining breeding goals and selecting right genotypes (Kumar et al. 2008). If improving one desired characteristic results in a reduced value for a second desirable property, a very careful consideration must be made as to the most effective breeding plan (Zobel and van Buijtenen 1989). Consequently it is important to avoid problems derived from adverse correlations.

The aim of this study was to estimate genetic and phenotypic correlations between growth and wood quality traits and to evaluate the possible consequences of selection for growth at early ages on wood quality of maritime pine populations in Mediterranean conditions. These findings are of great importance for the continued development of maritime pine breeding programmes, providing a guideline to future generation's selection.

6.3. MATERIAL AND METHODS

6.3.1. Location

The field trial location was at Pinhal de Leiria, established in 1987 and situated in central coastal Portugal (lat 39°50', long 8°55', alt 30m). The site is characterized by a Mediterranean climate, with a mean air temperature between 12.5°C and 15°C, relative humidity between 80 and 85% and yearly rainfall values of 700mm to 800mm, with a 4 month period of summer drought. The Spodic Podzols (PZ sd) soils are derived from sands dunes of maritime origin. Seedlings were nursery raised for 9 months in plastic bags (250cm^3 volume). Planting (2m×2m spacing) took place in March 1987 after harrowing and ploughing the site; no fertilizer was applied.

6.3.2. Genetic material

The progeny test used in this study was established in 1987. The trial includes 46 open-pollinated families, derived from seed collected in the Escaroupim clonal seed orchard II. The ortets were obtained from plus-trees selected in the Mata Nacional de Leiria by senior forester D. H. Perry in 1963-1964. The selection criteria used were based on volume, stem form, spiral grain and branch habits (Perry and Hopkins 1967). This seed orchard includes 49 genotypes; it was established by grafting in 1975-1980 and belongs to the first generation of the breeding program. Only 46 families were considered in order to avoid poor seed production. The 46 families were replicated in 8 blocks with 2x4 trees plots. In 2004, wood samples were collected from a subset of 12 trees for every family in 3 blocks, giving a total number of 552 trees (the four trees per plot were selected by random). A single wood disc with a thickness of 10 cm was collected at a predetermined sampling height of 2m. The disk was sawn into a 2mm-thick radial strip segment from the pith to the bark, and then conditioned at 12% moisture content. Radial samples were X-rayed perpendicular to the transverse section and their images scanned by microdensitometric analysis (Hughes and Sardinha 1975; Polge 1978). The time of exposure to radiation was 300s, at an intensity of 18mA and an accelerating tension of 12kV, with a 2.5m distance between X-ray source and film. The data comprising the radial density profiles were recorded every 100µm with a slit height (tangential direction) of 455µm.

The first and last annual rings of each sample were excluded since they were usually incomplete. Growth-ring boundaries were identified on the radial profiles by locating the sharp density variations through visual observation of the macroscopic anatomical features. For each ring, average ring density (RD), minimum density (MND), maximum density (MXD), earlywood density (EWD), latewood density (LWD), earlywood width (EWW), latewood width (LWW), ring width (RW) and latewood percentage (LWP) were determined. The earlywood (EW) latewood (LW) boundary in each growth ring was assigned a fixed density of 500 Kgm^{-3} as the limit between EW/LW. We used one fixed value of density as has been used for several conifer species (Barbour et al. 1994; Fujimoto et al. 2006), even though some researchers have been using the average of minimum and maximum ring density to define the transition point for EW/LW (Nicholls et al. 1980; Vargas-Hernandez and Adams 1991; Zamudio et al. 2005). The advantages of this criterion for the EW/LW boundary based on a fixed density value were well explained by Jozsa et al. (1987) and Rudman (1968). This value of 500Kgm^{-3} was a result of an adjustment to the value proposed by Louzada (2000) for *P. pinaster,* where it compares different criteria and indicates that this one is the most correct for this species with 18 years old. Although this criterion does not allow identifying the beginning of the latewood, it allows indentifying the portions of the ring with a density higher than a threshold, which we call LW. Intra-ring density variation was quantified by the heterogeneity index (HI) proposed by Ferrand (1982), defined by the standard deviation of all density values across the annual ring. Because rings close to the pith have less volume than those near the bark, they contribute less to the whole disc volume. To compensate for this effect, wood density components were weighed for each ring density component by its respective cross-sectional area. These area-weighed cumulated traits are henceforth referred to simple as cumulated traits or values (Hylen 1999).

The same strips were used to evaluate the radial modulus of elasticity (MOE_{Rad}) and the radial modulus of rupture (MOR_{Rad}), through three-point bending tests (Brancheriau et al. 2002). Before testing the specimen dimensions (thickness and width) were measured with a digital calliper (0.01mm resolution). Bending tests were carried out in an electro-mechanical testing machine (Instron 5848 MicroTester®). The experiments were carried out at room temperature under displacement control (0.5mm/min) and with a span of 40 mm.

The other traits assessed were spiral grain (SPG) determined for all trees as explained in Gaspar et al. (2008b) In each disc, the grain angle was measured at the most recent formed latewood of the last ring, using a grain slope detector (Harris 1989) and the pith as a reference (Hannrup et al. 2002).

The proportion of lignin content (LG) was estimated by near infrared spectroscopy (NIR) on extractive-free wood meal samples according to Rodrigues et al. (2006) and Perez et al. (2007). Being an indirect method, NIR needs calibration. The accuracy of NIR spectroscopy is influenced by the spectral noise and the reference data noise (Geladi 2002). However statements such as "NIR predictions can never be better than the primary reference method" were refuted (DiFoggio 1995; Rodrigues et al. 2006).

Height (HT) was assessed with a telescopic rod at age 12, and breast height diameter (DBH) was measured over bark using standard procedures, at age 12 and 17, in all trees of the trial.

6.3.3. Data analysis

The mixed linear model associated with the data for a given trait was:

$$Y_{ijk}=\mu + B_j + F_i + B\times F_{ij} + \varepsilon_{ijk} \quad [6.1]$$

where Y represents the phenotypic individual observation; μ is the overall mean; B_j: the effect of the j^th^ block (fixed); F_i: the effect of the i^th^ family (random); $B\times F_{ij}$: the effect of the interaction between the i^th^ family and the j^th^ block (random) and ε- the residual error. All terms in the model 6.1, except B, is assumed to be normally and independently distributed with mean zero and corresponding variances (σ^2). Variance components for family (σ_f^2), family-block interaction (σ_{f*b}^2) and residual errors (σ_ε^2), with the respective associated standard errors, were estimated by restricted maximum likelihood, using the average information REML algorithm implemented in the ASREML programme (Gilmour et al. 1998).

Narrow sense heritability (*h²*) was calculated for each trait as:

$$h_i^2=\frac{\sigma_a^2}{\sigma_P^2} \quad [6.2]$$

where σ^2_a represents the additive genetic variance and σ^2_P the total phenotypic variance. Total phenotypic variance was estimated as:

$$\sigma^2_P=\sigma^2_f+\sigma^2_{f\times b}+\sigma^2_\varepsilon \quad [6.3]$$

and estimated additive variances as:

$$\sigma_a^2=\frac{1}{2\theta}\times\sigma_f^2 \quad [6.4]$$

where the coancestry coefficient (Θ =0.13) was obtained (in the absence of selfing) from correlated paternity r'_p estimated by molecular markers see (Gaspar et al. In Press) as:

$$\Theta = 0.250\times r'_p + 0.125\times(1- r'_p) \quad [6.5]$$

Standard errors for heritability were estimated by ASREML using a Taylor series approximation (Gilmour et al. 1998).

Genetic and phenotypic correlations between all wood traits were calculated using a multivariate extension of model [6.1]. When the family effect was found to be null for any of the traits analysed in the univariate analysis, this effect was not included in the multivariate analysis. Genetic correlations between traits (*X* and *Y*) were evaluated as follows:

$$r_a = \frac{Cov_a(x,y)}{\sqrt{\sigma^2_{a_x} . \sigma^2_{a_y}}} \qquad [6.6]$$

where $\mathrm{cov}_g\,(x,y)$ is the additive genetic covariance, and $\sigma^2_{a_x}$and $\sigma^2_{a_y}$ are the additive variance components for traits *X* and *Y*, respectively.

The phenotypic correlation between traits (*X* and *Y*), was estimated as:

$$r_P = \frac{Cov_P(x,y)}{\sqrt{\sigma^2_{P_x} . \sigma^2_{P_y}}} \qquad [6.7]$$

where $\mathrm{cov}_P\,(x,y)$ is the phenotypic covariance between traits *X* and *Y*, estimated as $\mathrm{cov}_P\,(x,y) = \mathrm{cov}_f\,(x,y) + \mathrm{cov}_{fb}(x,y) + \mathrm{cov}_\varepsilon\,(x,y)$, which is the sum of the family, interaction, and residual covariance components. $\sigma^2_{P_x}$ and $\sigma^2_{P_y}$ are the phenotypic variances for traits X and Y, respectively. Standard errors for genetic and phenotypic correlations were estimated by ASREML a Taylor series approximation (Gilmour et al. 1998).

Selecting for one trait x will result in a correlated response of other traits, and the correlated response of a trait y can be estimated by using the equation of indirect response (Falconer and Mackay 1996):

$$Cr_{y/x} = i.\ h_x . h_y . r_{g(xy)} . \sigma_{Py} \qquad [6.8]$$

where h_x and h_y are the square root of appropriate narrow sense heritabilities for traits X and Y respectively; $r_{g(xy)}$ is the additive genetic correlation between traits X and Y and $\sigma^2_{P_y}$ is the phenotypic standard deviations of trait y.

6.4. RESULTS AND DISCUSSION

Summary statistics for growth traits and respective heritability estimates are given in Table 6.1.

Results showed a lower heritability for DBH ($h^2_{i\,(12)}$=0.17 and $h^2_{i\,(17)}$=0.15) compared to HT (h^2_i= 0.34) because the phenotypic variance is relatively higher than the additive variance.

Results for DBH are in agreement with those observed for the same species by Kusnadar et al. (1998) and Zas et al. (2004), but lower than those found by Costa and Durel (1996). Our estimated heritabilities for HT correspond well with those found in previous studies of maritime pine (0.27-0.45) (Cotterill et al. 1987; Danjon 1995; Kremer and Lascoux 1988; Pot et al. 2002), but lower values (0.11-0.17) for this trait were observed by (Kusnadar et al. 1998; Zas et al. 2004).

Table 6.1 Descriptive statistics table for diameter measured at 17 and 12 years old and total height measured at 12 years old, respective narrow heritability(h^2) estimates and phenotypic and additive variances (V_P and V_a, respectively). Standard errors given in brackets.

	DBH_{12} (cm)	HT_{12} (m)	DBH_{17} (cm)
Mean	10.28	6.9	13.26
SD	2.66	1.16	3.20
CV (%)	29	18	27
Max	18.0	10.0	22.0
Min	5.0	9.0	5.1
Va	1.15	0.41	1.50
Vp	6.68	1.19	9.80
h^2	0.17 (0.06)	0.34 (0.10)	0.15 (0.05)

Although a higher genetic control of HT relatively to DBH was reported by Zas et al. (2004), other authors have suggested the opposite (Costa and Durel 1996; Kusnadar et al. 1998); i.e. higher heritability values for DBH. According to Costa and Durel (1996) diameter is more affected by competition between trees and microenvironment effects than height. In fact, larger additive and phenotypic variances observed for DBH were observed in this work. Contributing to this higher competition between trees may the fact that this trial was installed with a narrower spacing (2x2) than usual for this species. Relatively small changes in DBH heritability with age (0.17 versus 0.15), were also observed by Costa and Durel (1996); Danjon (1994) and Kusnadar et al. (1998) although most of these authors mentioned that heritability increases with age.

Phenotypic (r_p) and additive genetic (r_g) correlations between DBH measured at 17 years old and the other wood traits are presented in Table 6.2. In contrast to *Picea* species, where negative genetic correlation between wood density and growth traits is usually reported (Hannrup et al. 2004; Yanchuk and Kiss 1993; Zhang et al. 1996), studies on several *Pinus* species have revealed often contrasting results (Fries and Ericsson 2006; Wu et al. 2008; Zobel and Jett 1995). Even in *P. pinaster*, correlation values between wood density and growth rate are contradictory. While Keller (1973) and Louzada (2003) obtained positive correlations between density and growth (0.43 and 0.11 respectively), Pot et al. (2002) and Chaperon et al. (1989) found negative genetic correlations in the same species (-0.48 and -0.89). For *P. pinaster* in France Bouffier et al. (2008), reported that these two traits were weakly negatively correlated both phenotypically and genetically. In the present study, phenotypic correlations between DBH_{17} and RD were weakly positive (0.24) but genetic correlation was close to zero (0.04), indicating that selection based on growth will not result in a decrease of wood density.

Table 6.2 Phenotypic (r_p) and additive genetic (r_g) correlations between diameter measured at 17 years old and the other wood traits (Standard errors given in brackets).

	r_p	r_g
RD	0.24 (0.04)	0.04 (0.32)
MND	0.00 (0.04)	-0.05 (0.22)
MXD	0.24 (0.04)	0.04 (0.25)
EWD	0.07 (0.05)	-0.04 (0.22)
LWD	0.20 (0.05)	-0.09 (0.28)
LWP	0.33 (0.04)	0.24 (0.22)
EWW	0.00 (0.05)	-0.17 (0.26)
LWW	0.42 (0.04)	0.13 (0.25)
SPG	0.24 (0.04)	0.15 (0.23)
MOR_{rad}	0.07 (0.05)	-0.02 (0.25)
MOE_{rad}	0.49 (0.03)	0.30 (0.24)
LG	-0.19 (0.05)	-0.41 (0.23)

DBH17=diameter measured at 17 years old; RD = Average Ring Density, MND = Minimum Density, MXD = Maximum Density, EWD = Earlywood Density, LWD = Latewood Density, LWP = Latewood Percentage, EWW= Earlywood Width, LWW= Latewood Width, SPG=Spiral Grain; Rrad =Radial Modulus of Rupture ; MOErad= Radial Modulus of Elasticity; LG= Lignin Content

In a comprehensive review on the reasons for these contradictory results, Louzada (2003) suggested that the relationship between wood density and growth rate is site specific. From our data (Table 6.2) it can be observed that DBH was more dependent on latewood components (MXD, LWD) than earlywood components (MND, EWD). Increase in DBH_{17} was due to an increase in LWP (0.42) and not an enhancement in EWW (0.0). Further, it is clear that larger trees tended to present higher values of RD, LWD, LWW and LWP, explaining why faster growing trees do not have necessarily lower densities. Results suggest that one possible reason for higher or lower wood density in fast growing trees depends upon growth being due to an increase of LWW or EWW, respectively. In a study on *Abies balsamea* (Koga and Zhang 2004) reported a negative phenotypic correlation between RD and RW (-0.48), a strong positive correlation between RW and EW (0.96), but no correlation between RW and LW (0.05), i.e., larger radial growth was caused by an increase in EW. In *Picea* *abies* Steffenrem (2008) refers that RW variation can be explained by earlywood zone width, while the width of the latewood barely varies. The existence of more LW or EW can be associated with the growing conditions of trees. Higher growth rates observed in higher latitudes can be a consequence of the early onset of cambial initiation, resulting in a higher proportion of EW. On the other hand, higher growth in Mediterranean regions can be due to an increase of the period of latewood formation, since the main tree growth constraining factors in this region are the high temperatures and water stress during the summer. When growth conditions are favourable in summer (water availability), trees present higher RW due to an enlargement of LWW and, consequently, higher RD. Conversely, during drought summers trees will present less LWW, RW and RD. A significant association of *P. pinaster* growth with water supply was observed in a study performed in central Spain (Bogino and Bravo 2008). Also for three pine species (*P. sylvestris*, *P. nigra* and *P. uncinata*) a positive effect of summer rainfall on growth has also been observed in Spain and Portugal (Andreu et al. 2007).

Also Campelo et al. (2007) in a study performed in *P. pinea* in Portugal, verified that climate explained between 59% and 76% of the tree ring variance, that latewood width was more sensitive to climate variations than earlywood and that earlywood development was mostly pre-determined at the beginning of the growing season. Moreover, results from a previous work (Gaspar et al. 2008a), performed on the same samples used in this study, showed that heritability estimates for earlywood components ($h^2_{i\,(\mathrm{MND})}$=0.54, $h^2_{i\,(\mathrm{EWD})}$=0.60) were superior to those obtained for latewood components ($h^2_{i\,(\mathrm{MXD})}$=0.34; $h^2_{i\,(\mathrm{LWD})}$=0.26), confirming that for *P. pinaster* earlywood characteristics are subject to stronger genetic control and that latewood components are more subject to environmental factors. Earlywood formation is made partially at the expense of stored carbohydrates rather that products of current photosynthesis (Hill et al. 1995), while latewood formation depends mainly on current photosynthesis and, hence latewood traits are more closely related to current climate conditions that earlywood (Lebourgeois 2000; Zhang 1997).

The relationship between wood density and growth also differs with location to some extent some extent (Zhang et al. 1996). To this author it seems that in a species where exists a negative relationship between wood density and growth this relation tends to be weaker in trees growing in a more favourable environment. For Downes et al. (2002) and Wimmer and Downes (2003) it is evident that the relationship between annual growth rate and density will depend largely on the relative size of the increments produced at different times of the year. These authors stated that when large rings are accompanied with the increased proportion of latewood, a positive relationship between density and growth is also possible. It is important to point out that in order to assess the real influence of growth rate on wood density, analyses must be carried out among homogeneous age classes. According to Zobel and van Buijtenen (1989) it is not acceptable to relate wood properties to ring width with rings of different ages. Nevertheless, this has been and still is frequently done, resulting in false and controversial ideas on the effect of growth rate, and may be another factor contributing for multiplicity of results obtained. It is of great importance to define difference between juvenile and mature wood when studying the effect of growth rate on basic density (Saranpää 2003). Wide growth rings and low density are associated with juvenile wood, and narrow growth rings with high density are characteristic for mature wood. Therefore, a negative correlation between ring width and density will be evident if juvenile and mature wood are both included in the analysis (Saranpää 2003).

Formation of spiral grain is under considerable genetic control, while its expression may be at least partly dependent on factors affecting the growth conditions of trees (Harris 1989). Other works have revealed high level of spiral grain to be correlated with a fast growth rate (Eklund et al. 2003). These authors demonstrated a positive relationship between growth, ethylene evolution and a high left-handed spiral grain. Results from this work indicate the existence of a positive but low phenotypic and genetic correlation between growth and spiral grain (0.24 and 0.154 respectively), indicating that grain inclination tend to be maintained in fast growing trees. Similar findings were reported for *P. abies* by Costa e Silva et al. (2000) and Hallingback et al. (2008). Lower but inconsistent correlations were obtained for *Pinus caribaea* (Harding et al. 1991), *Pinus sylvestris* (Hannrup et al. 2003) and *P. abies* (Hannrup et al. 2003; Hansen and Roulund 1997). As observed in the present work, phenotypic correlations between these traits were higher than genetic ones, meaning that the relation between spiral grain and growth traits is more influenced by environmental conditions than genetic conditions. Thus, selecting for fast growing trees will not affect the occurrence of spiral grain since environmental conditions can exert a more pronounced effect.

Correlations between growth and mechanical traits highlighted a modereate positive phenotypic correlation between diameter growth and MOE_{Rad} (0.49) while genetic correlation, although also positive, presented a more moderate value (0.30). For MOR_{Rad} a very weak positive phenotypic correlation was obtained (0.07) while the genetic correlation presented a weak but negative value. Several works on *P. radiata* (Baltunis et al. 2007; Kumar 2004; Kumar et al. 2002), *Pseudotsuga menziesii* (Johnson and Gartner 2006) and *P. abies* (Steffenrem 2008) gave negative correlations between growth and modulus of elasticity, but it should be highlighted that the mechanical traits were evaluated in the longitunal direction in these studies. Results from a previous work (Gaspar et al. Submitted-b) performed on the same samples used in the present study, indicated that MOE_{Rad} was more dependent on latewood components, either the density (MXD and LWD) or growth components (LWP and LWW). The higher positive genetic correlation observed in this work was between MOE_{Rad} and LWW, indicating that selecting for and enlargement of the latewood percentage would increase wood stiffness. So, it is possible that LWW, which also has a high association with growth rate, is responsible for the positive correlation between growth and radial MOE.

Phenotypic and genetic correlations between the DBH and lignin were negative (-0.19 and -0.41, respectively). The moderate to high value obtained for genetic correlation indicates that it is possible to select for increased growth with a lower lignin proportion, which is beneficial to pulp production. This relation can also be explained by the fact that lignin proportion was more dependent on latewood portion presented at the ring (LWP and LWW), suggesting that these traits may be controlled, at least partially by the same set of genes, and that increasing the existence of more latewood would decrease the lignin proportion (Gaspar et al. Submitted-a). Thus, trees with higher DBH and higher proportion of latewood are expected to have lower LG. Results observed in this work are opposite those observed for other conifers including *P. pinaster* where positive genetic correlations were observed between growth traits and lignin proportion (Costa e Silva et al. 1998; Pot et al. 2002; Sykes et al. 2006). These results are understandable since the relation between growth and wood density in these works was negative, and wood density is usually negatively correlated with lignin proportion.

Table 6.3 presents phenotypic (r_p) and additive genetic (r_g) correlations between wood quality traits evaluated at 17 years old, and DBH and HT measured at 12 years old, while in Table 6.4 are presented the expected correlated response for each trait considering a selection for growth traits with an intensity of 10%.

Table 6.3 Phenotypic (r_p) and additive genetic (r_g) correlations between different wood traits and DBH (diameter) and HT (total height) measured at 12 years old.

	DBH $_{12}$		HT $_{12}$	
	r_p	r_g	r_p	r_g
RD	0.25 (0.04)	0.11 (0.21)	0.31 (0.04)	0.06 (0.19)
MND	-0.13 (0.05)	0.09 (0.21)	0.11 (0.05)	-0.05 (0.19)
MXD	0.25 (0.04)	0.03 (0.24)	0.29 (0.04)	-0.01 (0.22)
EWD	0.08 (0.04)	0.05 (0.21)	0.13 (0.05)	-0.03 (0.19)
LWD	0.21 (0.04)	-0.08 (0.27)	0.25 (0.04)	-0.08 (0.24)
LWP	0.32 (0.04)	0.29 (0.21)	0.36 (0.04)	0.25 (0.20)
EWW	-0.05 (0.05)	-0.32 (0.24)	-0.13 (0.04)	-0.13 (0.32)
LWW	0.40 (0.04)	0.14 (0.24)	0.37 (0.04)	0.23 (0.21)
SPG	0.20 (0.04)	0.22 (0.21)	0.12 (0.05)	0.17 (0.20)
R_{rad}	0.03 (0.05)	-0.14 (0.24)	0.04 (0.05)	-0.11 (0.22)
MOE_{rad}	0.44 (0.04)	0.16 (0.25)	0.41 (0.04)	0.29 (0.22)
LN	-0.23 (0.04)	-0.31 (0.24)	-0.28 (0.04)	-0.42 (0.23)

Note: See Table 6.2 for trait abbreviations

Early evaluation of genotypes for adaptative and volume traits are an important component of tree improvement strategy when long rotations are used in forestry. However, attention should be given to the effect of this selection process on wood quality. Our results indicate that since genetic and phenotypic correlations between wood quality traits and growth traits (DBH and HT) at age 17 were of the same order and direction as correlations with the same traits at 12 years old, we can conclude that selection for growth at 12 years will not negatively affect the wood properties at 17 years. In some situations like for RD and EWW, the correlations were higher with DBH_{12} (0.11 and -0.32) than with DBH_{17} (0.04 and -0.17), indicating that early selection can be suitable for these traits. The correlated responses presented in table 6.4 between these traits corroborate this point; however we have to stress out the high errors associated with the correlation estimates. Concerning the genetic correlations with HT_{12}, all were lower than the ones observed with DBH_{12}, excepting for the correlations with LWW and MOE_{Rad}, but the expected correlated responses are not very different.

Table 6.4 Expected correlated response for each trait considering selection for growth traits with an intensity of 10%, genetic gain give in trait units (GG) and in percent (CG%).

	HT_{12}		DBH_{12}		DBH_{17}	
	GG	CG%	GG	CG%	GG	CG%
RD (Kg m^{-3})	0.53	11	0.64	35	0.58	22
MND (Kg m^{-3})	0.27	-13	0.43	41	0.23	-26
MXD (Kg m^{-3})	0.70	-1	0.74	5	0.69	-2
EWD (Kg m^{-3})	0.36	-7	0.46	19	0.34	-13
LWD (Kg m^{-3})	0.57	-7	0.54	-12	0.53	-14
LWP (%)	38.20	1	38.39	1	38.38	1
EWW (mm)	2.59	-3	2.34	-13	2.52	-6
LWW (mm)	1.69	9	1.69	10	1.82	18
SPG	4.27	1	4.34	3	4.33	3
MOR_{rad} (MPa)	13.61	-1	13.53	-1	13.54	-1
MOE_{rad} (GPa)	1.32	16	1.30	14	1.48	30
LG (%)	27.86	-1	27.79	-1	27.60	-2

Note: See Table 6.2 for abbreviations

6.5. CONCLUSIONS

Based on the results of this study we can conclude that for the *P. pinaster* Portuguese breeding programme a genetic selection based on growth can be performed without a consequent decrease of wood density, it will not affect the occurrence of spiral grain, and is possible to obtain an increase in the radial modulus of elasticity. The present study also showed that it is possible to select for increased growth with lower lignin proportion. Further, our results suggests that for Maritime pine in Mediterranean conditions, DBH growth is more dependent on latewood components that earlywood components, and that an increase in this trait is due to an increase in LWP and not due to an enhancement in EWW. Results also suggest that selection for growth at 12 years will not negatively affect the wood properties at 17 years.

Acknowledgements

M.J. Gaspar was funded by the Portuguese Foundation for Science and Technology (Doctoral grant SFRH/BD/27412/2006).

6.6. REFERENCES

Alía, R., and Martín, S. 2003. Euforgen. In: Technical Guidelines for Genetic Conservation and Use for Maritime pine (*Pinus pinaster*). International Plant Genetic Resources Institute.Rome, p. 6.

Andreu, L., Gutierrez, E., Macias, M., Ribas, M., Bosch, O., and Camarero, J.J. 2007. Climate increases regional tree-growth variability in Iberian pine forests. Global Change Biol 13 (4): 804-815.

Baltunis, B.S., Wu, H.X., and Powell, M.B. 2007. Inheritance of density, microfibril angle, and modulus of elasticity in juvenile wood of *Pinus radiata* at two locations in Australia. Can J Forest Res 37 (11): 2164-2174.

Barbour, R.J., Fayle, D.C.F., Chauret, G., Cook, J., Karsh, M.B., and Ran, S.K. 1994. Breast-height relative density and radial growth in mature Jack Pine (*Pinus banksiana*) for 38 years after thinning. Can J Forest Res 24 (12): 2439-2447.

Bogino, S.M., and Bravo, F. 2008. Growth response of *Pinus pinaster* Ait. to climatic variables in central Spanish forests. Ann For Sci 65 (5): 506.

Bouffier, L., Charlot, C., Raffin, A., Rozenberg, P., and Kremer, A. 2008. Can wood density be efficiently selected at early stage in maritime pine (*Pinus pinaster* Ait.)? Ann For Sci 65: 106.

Brancheriau, L., Bailleres, H., and Guitard, D. 2002. Comparison between modulus of elasticity values calculated using 3 and 4 point bending tests on wooden samples. Wood Sci Technol 36 (5): 367-383.

Campelo, F., Nabais, C., Freitas, H., and Gutierrez, E. 2007. Climatic significance of tree-ring width and intra-annual density fluctuations in *Pinus pinea* from a dry Mediterranean area in Portugal. Ann For Sci 64 (2): 229-238.

Chaperon, H., Raoux, H., Siohan, A., and Alazard, P. 1989. Variabilité génetique des proprietés technologiques du bois de pin maritime. Annales Afocel, Annales de Recherches sylvicoles 174 (7): 327-345.

Correia, I., Almeida, M.H., and Aguiar, A. 2004. Variabilidade do Crescimento e da Forma de Proveniências de *Pinus pinaster* Aiton aos 8 anos, na Mata Nacional do Escaroupim. Silva Lusitana 12 (2): 151-182.

Costa e Silva, J., Borralho, N.M.G., and Wellendorf, H. 2000. Genetic parameter estimates for diameter growth, pilodyn penetration and spiral grain in *Picea abies* (L.) KARST. Silvae Genet 49 (1): 29-36.

Costa e Silva, J., Wellendorf, H., and Pereira, H. 1998. Clonal variation in wood quality and growth in young sitka spruce (*Picea sitchensis* (Bong.) Carr.): estimation of quantitative genetic parameters and index selection for improved pulpwood. Silvae Genet 47 (1): 20-33.

Costa, P., and Durel, C.E. 1996. Time trends in genetic control over height and diameter in Maritime pine. Can J Forest Res 26 (7): 1209-1217.

Cotterill, P.P., Dean, C.A., and Vanwyk, G. 1987. Additive and dominance genetic effects in *Pinus pinaster*, *Pinus radiata* and *Pinus elliottii* and some implications for breeding strategy. Silvae Genet 36 (5-6): 221-232.

Danjon, F. 1994. Stand Features and Height Growth in a 36-Year-Old Maritime pine (*Pinus pinaster* Ait) Provenance Test. Silvae Genet 43 (1): 52-62.

Danjon, F. 1995. Observed selection effects on height growth, diameter and stem form in Maritime pine. Silvae Genet 44 (1): 10-19.

DGRF. 2007. Inventário Florestal Nacional 2005/2006. Direcção Geral das Florestas.

DiFoggio, R. 1995. Examination of some misconceptions about near-Infrared analysis. Applied Spectroscopy (1): 67-75.

Downes, G.M., Wimmer, R., and Evans, R. 2002. Understanding wood formation: gains to commercial forestry through tree-ring research. Dendrochronologia 20 (1-2): 37-51.

Eklund, L., Sall, H., and Linder, S. 2003. Enhanced growth and ethylene increases spiral grain formation in *Picea abies* and *Abies balsamea* trees. Trees-Struct Funct 17 (1): 81-86.

Falconer, D.S., and Mackay, T.F.C. 1996. Introduction to quantitative genetics. Longman Scientific & Technical ed, New York, 464 pp.

Ferrand, J.C. 1982. Réflexions sur la Densité du Bois. 2ère Partie: Calcule de la densité et de son hétérogénéité. Holzforschung 36 (3): 153-157.

Fries, A., and Ericsson, T. 2006. Estimating genetic parameters for wood density of Scots pine (*Pinus sylvestris* L.). Silvae Genet 55 (2): 84-92.

Fujimoto, T., Akutsu, H., Kita, K., Uchiyama, K., Kuromaru, M., and Oda, K. 2006. Age trends of genetic parameters of spiral grain in hybrid larch F-1 and implications for efficiency of early selection. J Wood Sci 52 (2): 101-106.

Gaspar, M.J., Alves, A., Louzada, J.L., Santos, A., Simões, R., Fernandes, C., Almeida, M.H., and Rodrigues, J.C., submitted-a. Phenotypic and genetic correlations between lignin and wood density components in *Pinus pinaster* AIT. Ann For Sci.

Gaspar, M.J., Lousada, J.L., Morais, J., Fernandes, C., Rodrigues, J.C., and Almeida, M.H. Submitted-b. Genetic variations of wood mechanical traits in 46 half-sib families of *Pinus pinaster* Ait. Silvae Genet.

Gaspar, M.J., de-Lucas, A., Alia, R., Paiva, J.A.P., Hidalgo, E., Louzada, J.L., Almeida, M.H., and González-Martínez, S.C. In Press. Use of molecular markers for estimating breeding parameters: a case study in Maritime pine progeny trial. Tree Gen Genom. DOI 10.1007/s11295-009-0213-1

Gaspar, M.J., Louzada, J.L., Silva, M.E., Aguiar, A., and Almeida, M.H. 2008a. Age trends in genetic parameters of wood density components in 46 half-sibling families of *Pinus pinaster*. Can J Forest Res 38 (6): 1470-1477.

Gaspar, M.J., Louzada, J.L., Aguiar, A., and Almeida, M.H. 2008b. Genetic correlations between wood quality traits of *Pinus pinaster* Ait. Ann For Sci 65 (7):703.

Geladi, P. 2002. Some recent trends in the calibration literature. Chemometrics and Intelligent Laboratory Systems 60: 211-224.

Gilmour, A., Gogel, B., Cullis, B., Welham, S., and Thompson, R. 1998. ASREML Users Manual. VSN International. New south Wales Agriculture, Orange.

Hallingback, H.R., Jansson, G., and Hannrup, B. 2008. Genetic parameters for grain angle in 28-year-old Norway spruce progeny trials and their parent seed orchard. Ann For Sci 65 (3): 301.

Hannrup, B., Cahalan, C., Chantre, G., Grabner, M., Karlsson, B., Le Bayon, I., Jones, G.L., Muller, U., Pereira, H., Rodrigues, J.C., Rosner, S., Rozenberg, P., Wilhelmsson, L., and Wimmer, R. 2004. Genetic parameters of growth and wood quality traits in *Picea abies*. Scan J Forest Res 19 (1): 14-29.

Hannrup, B., Grabner, M., Karlsson, B., Müller, U., Rosner, S., Wilhelmsson, L., and Wimmer, R. 2002. Genetic parameters for spiral-grain angle in two 19-year-old clonal Norway spruce trials. Ann For Sci 59: 551-556.

Hannrup, B., Säll, H., and Jansson, G. 2003. Genetic parameters for spiral grain in Scots pine and Norway spruce. Silvae Genet 52: 215-220.

Hansen, J.K., and Roulund, H. 1997. Genetic parameters for spiral grain, stem form, pilodyn and growth in 13 years old clones of Sitka spruce (*Picea sitchensis* (Bong.) Carr.). Silvae Genet 46 (2-3): 107-113.

Harding, K.J., Kanowski, P.J., and Woolaston, R.R. 1991. Preliminary genetic parameter estimates for some wood quality traits of *Pinus caribaea* Var Hondurensis in Queensland, Australia. Silvae Genet 40 (3-4): 152-156.

Harris, J.M. 1989. Spiral grain and wave phenomena in wood formation, Berlin, Heidelberg, and New York, 230 pp.

Hill, S.A., Waterhouse, J.S., Field, E.M., Switsur, V.R., and Aprees, T. 1995. Rapid recycling of triose phosphates in Oak stem tissue. Plant Cell Environ 18 (8): 931-936.

Hughes, J.F., and Sardinha, R.M.A. 1975. The application of optical densitometry in the study of wood structure and properties. Journal of Microscopy 104.

Hylen, G. 1999. Age trends in genetic parameters of wood density in young Norway spruce. Can J For Res 29: 135-143.

Johnson, G.R., and Gartner, B.L. 2006. Genetic variation in basic density and modulus of elasticity of coastal Douglas-fir. Tree Genet Geno 3 (1): 25-33.

Jozsa, L., Richards, J., and Johnson, S. 1987. Calibration of Forintek's direct reading x-ray densitometer. Report No 36a. Forintek Canada Corp., Vancover, p.16.

Keller, R. 1973. Caractéristiques du bois de Pin Maritime- Variabilité et Transmission Héréditaire. Annales Science Forestires 30: 31-62.

Koga, S., and Zhang, S.Y. 2004. Inter-tree and intra-tree variations in ring width and wood density components in balsam fir (*Abies balsamea*). Wood Sci Technol 38 (2): 149-162.

Kremer, A., and Lascoux, D.M. 1988. Genetic architecture of height growth in maritime pine (*Pinus pinaster* Ait.). Silvae Genet 37 (1): 1-8.

Kumar, S. 2004. Genetic parameter estimates for wood stiffness, strength, internal checking, and resin bleeding for Radiata pine. Can J Forest Res 34 (12): 2601-2610.

Kumar, S., Burdon, R.D., Stovold, G.T., and Gea, L.D. 2008. Implications of selection history on genetic architecture of growth, form, and wood-quality traits in *Pinus radiata*. Can J Forest Res 38 (9): 2372-2381.

Kumar, S., Jayawickrama, K.J.S., Lee, J., and Lausberg, M. 2002. Direct and indirect measures of stiffness and strength show high heritability in a wind-pollinated Radiata pine progeny test in New Zealand. Silvae Genet 51 (5-6): 256-261.

Kusnadar, D., Galwey, N.W., Hertzler, G.L., and Butcher, T.B. 1998. Age trends in variances and heritabilities for diameter and height in Maritime pine (*Pinus pinaster* Ait.) in Western Australia. Silvae Genet 47 (2-3): 136-141.

Lebourgeois, F. 2000. Climatic signals in earlywood, latewood and total ring width of Corsican pine from western France. Ann For Sci 57: 155-164.

Louzada, J.L.P.C. 2000. Variação fenotípica e genética em características estruturais na madeira de *Pinus pinaster* Ait. *In* Departamento Florestal. Universidade de Trás-os-Montes e Alto Douro, Vila Real, 293 pp.

Nicholls, J.W.P., Morris, J.D., and Pederick, L.A. 1980. Heritability estimates of density characteristics in juvenile *Pinus radiata* wood. Silvae Genet 29 (2): 54-61.

Perez, D.D., Guillemain, A., Alazard, P., Plomion, C., Rozenberg, P., Rodrigues, J.C., Alvess, A., and Chantre, G. 2007. Improvement of *Pinus pinaster* Ait elite trees selection by combining near infrared spectroscopy and genetic tools. Holzforschung 61 (6): 611-622.

Perry, D., and Hopkins, E. 1967. Importation of breeding material of *Pinus pinaster* Ait. from Portugal. For. Dep. W. A. Bulletin 75.

Polge, H. 1978. Fifteen years of wood radiation densitometry. Wood Sci Technol 12: 187-196.

Pot, D., Chantre, G., Rozenberg, P., Rodrigues, J.C., Jones, G.L., Pereira, H., Hannrup, B., Cahalan, C., and Plomion, C. 2002. Genetic control of pulp and timber properties in Maritime pine (*Pinus pinaster* Ait.). Ann For Sci 59 (5-6): 563-575.

Rodrigues, J., Alves, A., Pereira, H., Perez, D.D.S., Chantre, G., and Schwanninger, M. 2006. NIR PLSR results obtained by calibration with noisy, low-precision reference values: are the results acceptable? Holzforschung 60(4): 402-408.

Roulund, H., Alpuim, M., Varela, M.C., and Aguiar, A. 1988. A tree improvement plan for *Pinus pinaster* in Portugal. EFN.

Rudman, P. 1968. Growth ring analysis. Journal of the Institute of Wood Science 4: 58-63.

Saranpää, P. 2003. Wood density and growth. *In* Wood Quality and its Biological Basis. *Edited by* J. R. Barnett, and G. Jeronimidis. Blackwell publishing, Victoria. pp. 87-117.

Steffenrem, A. 2008. Genetic variation in structural wood quality traits in Norway spruce and implications for tree breeding. *In* Department of Ecology and Natural Resource Management. Norwegian University of Life Sciences, 51pp.

Sykes, R., Li, B.L., Isik, F., Kadla, J., and Chang, H.M. 2006. Genetic variation and genotype by environment interactions of juvenile wood chemical properties in *Pinus taeda* L.. Ann For Sci 63 (8): 897-904.

Vargas-Hernandez, J., and Adams, W.T. 1991. Genetic-variation of wood density components in young coastal Douglas-fir - Implications for tree breeding. Can J Forest Res 21 (12): 1801-1807.

Wimmer, R., and Downes, G.M. 2003. Temporal variation of the ring width-wood density relationship in Norway spruce grown under two levels of anthropogenic disturbance. Iawa Journal 24 (1): 53-61.

Wu, H.X., Ivkovic, M., Gapare, W.J., Matheson, A.C., Baltunis, B.S., Powell, M.B., and Mcrae, T.A. 2008. Breeding for Wood Quality and profit in *Pinus radiata*: a review of genetic parameter estimates and implications for breeding and deployment. N Z J of For Scie 38 (1).

Yanchuk, A., and Kiss, G. 1993. Genetic variation in growth and wood specific gravity and its utility in the improvement of interior spruce in British Columbia. Silvae Genet 42 (2-3): 141-148.

Zamudio, F., Rozenberg, P., Baettig, R., Vergara, A., Yanez, M., and Gantz, C. 2005. Genetic variation of wood density components in a Radiata pine progeny test located in the south of Chile. Ann For Sci 62 (2): 105-114.

Zas, R., Merlo, E., and Fernandez-López, J. 2004. Genetic parameter estimates for maritime pine in the Atlantic coast of North-West Spain Forest Genetics 11 (1): 45-53.

Zhang, S.Y. 1997. Variations and correlations of various ring width and ring density features in European oak: implications in dendroclimatology. Wood Sci Technol 31 (1): 63-72.

Zhang, S.Y., Simpson, D., and Morgenstern, E.K. 1996. Variation in the relationship of wood density with growth in 40 Black spruce (*Picea mariana)* families grown in New Brunswick. Wood Fiber Sci 28 (1): 91-99.

Zobel, B.J., and Jett, J.B. 1995. Genetics of Wood Production. Springer-Verlag ed, Berlin, Heidelberg, and New York, 377 pp.

Zobel, B.J., and van Buijtenen, J.P. 1989. Wood Variation - Its causes and control. Springer-Verlag ed, Berlin, 363 pp.

CHAPTER 7

Use of molecular markers for estimating breeding parameters: a case study in maritime pine progeny trial.

In press. Gaspar, M.J.; de-Lucas, A.; Alia, R.; Paiva, J.A.P.; Hidalgo, E.; Louzada, J.L.; Almeida, M.H. and González-Martínez, S.C. Use of molecular markers for estimating breeding parameters: a case study in maritime pine progeny trial. Tree Genetic and Genomes. DOI 10.1007/s11295-009-0213-1

7.1. ABSTRACT

The management of a genetic improvement program is based on the knowledge of the genetic parameters and their relationships to determine the genetic gains. Knowledge of the coefficient of coancestry (θ) is a requirement for efficient progeny testing scheme and for estimating additive variance components for any quantitative trait. When using open-pollinated families, most authors assume that the seedlings are related as half-sibs, but this is not always true. Our aim was to estimate a mean value of the coancestry coefficient of the families present in a maritime pine *Pinus pinaster* Ait. (maritime or cluster pine) progeny trial originating from seed collected in a clonal seed orchard, and to study how deviations from the standard assumption of θ=0.125 affect heritability estimations. Five highly polymorphic microsatellite markers were scored in 125 offspring from a sub-sample of five families from the progeny trial. The mean value of the coancestry coefficient of the families present in this progeny trial was 0.130. Differences between the unadjusted and adjusted heritability estimates were more pronounced in wood density (0.629 and 0.586, respectively) than in diameter (0.166 and 0.154, respectively). We conclude that in the trial, the associated error in heritability estimates due to the inclusion of full-sibs, when assuming a standard coefficient of relationship amongst open-pollinated sibs of 0.250, was low and that this result is robust with respect to the number of families sampled, given unbiased estimates of average relationship among offspring within sib-families.

7.2. INTRODUCTION

Pinus pinaster Ait. (maritime or cluster pine) is an important commercial species in south-western Europe. In Portugal, *P. pinaster* is one of the most important native species, covering 1 Mha, it is the only source of long fibre for pulp and paper industry and the main one for solid sawn timber industries. A tree improvement program is being developed for this species since the early 1980s based on a selection of plus trees, the establishment of first-generation seed orchards and open-pollinated progeny tests (Aguiar et al. 2003; Perry et al. 1970), with the aim of increasing volume per hectare and quality of stem form. Progeny tests allow the estimation of genetic parameters and provide information about the ability of a species to respond to selection and thus inform the deployment strategy for that species (Zobel and Talbert 1984). To estimate genetic variance components it is necessary to establish the relationship structure among the individuals tested (Falconer and Mackay 1996; Lynch and Walsh 1997). We focus on the traditional population genetic definition of coefficient of relatedness or relationship (r) for diploid individuals, which is twice the coefficient of coancestry (θ) (Lynch and Walsh 1997; Wright 1976).

Open-pollinated families have often been used for forest tree progeny trials, due to easy operative management and simplicity of calculations (Borralho 1994), especially in first generation breeding programs or in ecological studies where seeds are collected in natural populations. Genetic relatedness among members of a wind-pollinated family is usually assumed to be mathematically equivalent to the covariance among half-sibs, which is equal to 0.25 (Falconer and Mackay 1996). This involves assuming that families are true half-sib families, i.e., the female trees from which the progenies were collected are unrelated, crosses are based on a high effective number of unrelated males and there is no self-fertilization (Borralho 1994). However, these assumptions are not usually met due to unaccounted relatedness between parents, nonequal contributions of pollen, or the occurrence of selfed progeny (Borralho 1994; Hansen and Kjaer 2006). This failure to meet assumptions also implies that the family variance component is inflated by non-additive effects when families include some proportion of full-sibs. Random mating and panmitic equilibrium assumptions are often unrealistic for natural populations and, in many cases, for seed orchards too (El-Kassaby and Ritland 1986). For example, several studies in conifers have demonstrated that not all the clones within a seed orchard (SO) make an equal contribution to the next generation (Goto et al. 2002; Hansen and Kjaer 2006; Moriguchi et al. 2005; Plomion et al. 2001). In these conditions, the use of r=0.25 would result in a biased estimation of additive genetic variance (Squillace 1974). According to Askew and El-Kassaby (1994), any testing program that depends on wind–pollinated progenies for estimation of genetic parameters have to cautiously evaluate the factors that determine the relationships among the progeny.

The use of molecular marker technologies for parental analysis in breeding programs can provide a solution to these uncertainties about the coancestry coefficient, because they provide a mean to infer the relationship structure among the individuals (Blouin 2003; Lynch and Ritland 1999). Some of the preferred genetic markers for obtaining precise estimates of relatedness are microsatellites markers (Gerber et al. 2000; Moriguchi et al. 2005), either from the nuclear or the chloroplast genomes, because they usually display many alleles per locus (Lynch and Ritland 1999) and are co-dominant (Hardy 2003). In pines, chloroplast microsatellites are haploid and paternally inherited. Because they don't recombine, multiple chloroplast fragments can be combined in haplotypes providing a paternal marker ideal for pollen flow studies, as they allow direct identification of paternal gametes (Plomion et al. 2001; Robledo-Arnuncio et al. 2004)

The aim of this work is to estimate a mean value of the coancestry coefficient (θ) of the families present in a progeny trial originated from seed collected in a clonal seed orchard (CSO), and to study in what way deviations from the standard assumption of Θ=0.125 (i.e., r=0.25) in open-pollinated progeny tests of *P. pinaster* would affect heritability estimation. It is also an objective of this study to show how differences in coancestry coefficient across the families evaluated in a progeny test would affect quantitative genetics estimations.

7.3. MATERIAL AND METHODS

7.3.1. Plant material and common-garden experiment

The progeny test used in this study belongs to a series replicated at three sites and established in 1987 (Aguiar et al. 2003). The trials included 46 open-pollinated families, originated from seed collected in the Escaroupim clonal seed orchard II (Aguiar 1993). This CSO includes 49 genotypes and was established by grafting in 1975-80, but only 46 families were considered in the progeny test due to poor seed production in the rest. 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 about the plus phenotypes selected and the scoring system employed are described in (Perry and Hopkins 1967). The 46 families were randomly replicated in 8 blocks with 8 trees per plot. In 2004 (age 17), wood samples were collected from a subset of 12 trees from every family in 3 blocks, giving a total number of 552 trees for evaluation of wood quality traits.

A subsample of 125 offspring from five families (25 offspring per family), representative of the same seed lot used in the establishment of the progeny trial, were genotyped in our study. These five families showed extreme and contrasting values of the inter-individual variance for different quantitative traits (Gaspar et al. 2008a). This way, i.e., by selecting families from the tails of the distribution for molecular analysis, we were able to test whether quantitative genetics estimates are affected by standard assumptions on sib relationship, with a limited genotyping effort.

7.3.2. DNA isolation and molecular markers

Total genomic DNA was isolated from needles following the Doyle and Doyle protocol (Doyle and Doyle 1990), with some modifications. Offspring were genotyped for five microsatellite markers: two chloroplast microsatellite loci (Pt87268 and Pt1254) and three nuclear microsatellites (Itph4516, Ctg275 and Ctg4363). These markers were chosen from previous studies (see de-Lucas et al. 2008) because of their high level of polymorphism and unambiguous scoring. The amplification conditions for the different molecular markers are described in Robledo-Arnuncio et al. (2004) (cpSSRs), González-Martínez et al. (2002) (Itph4516) and Chagné et al. (2004) (Ctg275 and Ctg4363).

Microsatellite fragments were scored in an ABI-PRISM 310 genetic analyzer (Applied Biosystems, Foster City, CA, USA) using GeneScan ROX-500 as internal ladder and standard running parameters.

7.3.3. Data Analyses

7.3.3.1. Mating system parameters

Single- and multi- locus estimates of outcrossing rates (t_s and t_m, respectively) per family were computed using a moment method based only on nuclear microsatellites using MLTR (see Ritland 2002, for details). Confidence intervals were obtained by bootstrapping (1000 bootstraps). The difference between multilocus and single-locus estimates of outcrossing (t_m-t_s) was used to estimate biparental inbreeding (i.e., inbreeding due to mating among relatives).

The percentage of full-sibs in each progeny was estimated by computing correlated paternity within families (r_p=2×F_{ij}) following Hardy et al. (2004) and using SPAGeDi 1.2 (Hardy and Vekemans 2002). This method uses molecular markers to score progeny arrays from mothers with known genotypes. In a first stage, pollen gametes are inferred by discounting the mother genotype from the offspring diploid genotypes (see Hardy et al. 2004 for details on how to deal with the classical 'double heterozygote' issue). Then, in a second stage, co-ancestry among inferred pollen gametes within families is computed using Nason's relative kinship estimator (see Loiselle et al. 1995). Correlated paternity (r_p) was computed separately for chloroplast and nuclear markers and averaged, as in de-Lucas et al. (2008).

7.3.3.2. Quantitative genetic parameters

The traits assessed were ring density (RD) determined using X-ray densitometry procedures, as described in Gaspar et al. (2008) and diameter at 1.30m (DBH).

Traits were analysed using the following model:

$$Y_{ijk}=\mu + B_j + F_i + B\times F_{ij} + \varepsilon_{ijk} \qquad [7.1]$$

where Y represents the phenotypic individual observation; μ is the overall mean; B_j is the effect of the jth block (fixed); F_i is the effect of the ith family (random); $B\times F_{ij}$ is the effect of the interaction between the ith family and the jth block (random); and ε is the residual error. Variance components for family (σ_f^2), family-block interaction (σ_{f*b}^2) and residual errors (σ_ε^2), with the respective associated standard errors, were estimated by restricted maximum likelihood, using the average information REML algorithm implemented in the ASREML program (Gilmour et al. 1998). Narrow sense heritability ($\mathrm{h_i^2}$) was calculated for each trait as:

$$\mathrm{h_i^2} = \frac{\sigma_a^2}{\sigma_P^2} \qquad [7.2]$$

where σ^2_a represents the additive genetic variance and σ^2_P the total phenotypic variance. Total phenotypic variance was estimated as:

$$\sigma_P^2 = \sigma_f^2 + \sigma_{f*b}^2 + \sigma_\varepsilon^2 \qquad [7.3]$$

and estimated additive variances as:

$$\sigma_a^2 = \frac{1}{2\theta} \times \sigma_f^2 \qquad [7.4]$$

where the coancestry coefficient (θ) was obtained from estimates of outcrossing rates and correlated paternity (obtained by molecular markers) as:

$$\theta = \frac{0.2500 \times fs + 0.3335 \times sfs + 0.1250 \times hs + 0.2040 \times shs}{fs + sfs + hs + shs} \qquad [7.5]$$

where *fs* is the number of full-sibs, *sfs* the number of self full-sibs, *hs* the number of half-sibs and *shs* the number of self half-sibs (Squillace 1974). The number of full-sibs is estimated here directly from correlated paternity ($n \times r_p$, where n is the number of offspring considered). Given that outcrossing rates are not different from 100% (see Results), selfing can be considered negligible. Therefore, the number of half-sibs can be estimated as 1- r_p while the number of self full-sibs and self half-sibs would be zero.

Coancestry coefficients estimated in this way are similar to those computed directly for each family using nuclear markers (0.0773-0.1746 using Nason's approach, depending on the family), but they are more precise as this approach also incorporates the information obtained from chloroplast haplotypes (as in de-Lucas et al. 2008). Standard errors for heritability were estimated by ASREML using a Taylor series approximation (Gilmour et al. 1998).

7.3.3.3. Simulation study

The main objective of the simulation study was to analyse the impact on heritability estimates of different coancestry coefficients (estimated with molecular markers) of the families included in a progeny trial. We simulated 46 families with different structure (measured by the coancestry coefficient). Several scenarios were then simulated for populations of 46 families presenting different degrees of correlated paternity (between 5% and 60% of full sibs, corresponding to coefficients of relationship of 0.26-0.40) and coefficients of variation (1, 10 and 25%). Although the coefficient of relationship of open-pollinated families has a lower bound in 0.25, when estimated with molecular makers it can have lower values due to statistical error. Therefore, the range of the coefficients of variation for the coefficient of relationship included in the simulations does not take into account this lower bound. The value of correlated paternity of each simulated family was drawn from a normal distribution, considering the same average (simulated) value of correlated paternity and standard deviations according to assumed among-family coefficient of variation. For each scenario, we computed narrow-sense heritability (h^2), the mean coefficient of relationship of the 46 families, and the coefficient of variation for the coefficient of relationship.

7.4. RESULTS

Average (over families) out-crossing rates were high, 1.063±0.063, CI (95%): 0.992-1.200 and 0.911±0.039, CI (95%): 0.866-1.031 for multilocus and single locus estimates, respectively. The high values obtained, in particular for the multilocus estimates of outcrossing (t_m=1.063±0.063), indicate absence (or at best a very low proportion) of selfed offspring. The apparent difference between single and multilocus outcrossing rates (t_m-t_s=0.152±0.067) is related to biparental inbreeding (i.e., mating among relatives; (Ritland 2002).

Estimates of correlated paternity within families varied between -0.0019 and 0.0738 with an average of 0.0418±0.0273, CI (95%): 0.0174-0.0662 (Figure 7.1).

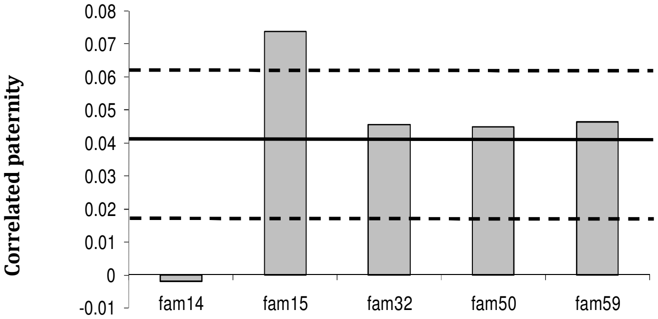


Figure 7.1 Marker-based estimates of correlated paternity for each of five maritime pine families (as estimated by Hardy et al.'s 2004 approach); the average (continuous line) and 95% confidence intervals (dashed lines) are also indicated.

We did not find any trend between correlated paternity and the level of inter-individual variance shown for different quantitative traits (see Gaspar et al. 2008) by the families included in this study. Correlated paternity estimates were not significantly different, as judged by overlapping confidence intervals at 95% probability, to those obtained using other estimation methods such as the TwoGener approach [average of 0.0546±0.0297, CI (95%): 0.0290-0.0822; see description of the TwoGener method in (Austerlitz and Smouse 2001; Smouse et al. 2001)].

The effective number of males mating with a given mother tree was 24 (N_{ep}=1/r_p= 23.9), which is about half the census number (49 genets). Given that similar numbers of effective males are typically found in large natural populations of the species (see de-Lucas et al. 2008), our results suggest either more even male contribution to sib families than normally expected in pine seed orchards or pollen contamination from a source outside the CSO. The mean value of the coancestry coefficient obtained considering 4% of full-sibs was 0.130, very close to the expected for half-sib progenies (0.125), with a coefficient of variation (based on estimates from molecular markers) of 2.5%.

Adjusted heritabilities and standard errors (used here as a rough approximation for inferring confidence intervals) calculated for different coancestry coefficients and associated coefficients of variation are shown for the two traits under study, diameter (DBH) and wood ring density (RD), in Figure 7.2 (it should be noted that the heritability scale is different in the two figures).

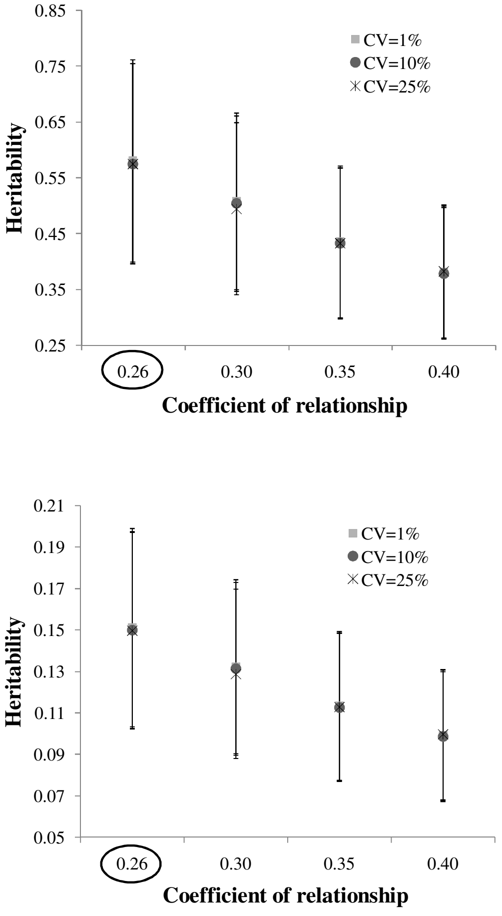


Figure 7.2 Adjusted heritability and standard errors for different averages and among-family coefficients of variation (CV) of the relationship coefficient (r), for two quantitative traits: wood density (top) and diameter (bottom). The circle indicates the observed values for the coefficient of relationship in our study case.

Differences between the unadjusted and adjusted heritability values were more pronounced in wood density -the trait with higher heritability- (0.629 and 0.586, respectively) than in diameter (0.166 and 0.154, respectively), but they did not imply significant bias (bias <5%) in any of the two. The effect of the coefficient of variation was not noteworthy in any trait, indicating that it does not affect heritability estimates at all under the conditions analysed in this experiment.

7.5. DISCUSSION

Conifers are wind-pollinated and known to be predominantly outcrossers. Recent studies on the pine mating system indicate that, for most species, outcrossing rates are higher than 0.9 (de-Lucas et al. 2008; Fernandes et al. 2008; Robledo-Arnuncio et al. 2004; Wasieliwska et al. 2005). The multilocus outcrossing rate (t_m) of *Pinus pinaster* in this study, based on progenies from a SO, was 1.063±0.063 (with confidence intervals at 95% overlapping one), matching well those found in natural populations of the species. González-Martínez et al. (2003) found a value of outcrossing of approximately 0.96, based on natural regeneration (i.e., after seed germination and seedling establishment) in natural conditions. Similarly, de-Lucas et al. (2008) obtained an average (over 61 families from three populations) outcrossing rate of 0.977 based on germinated seeds (i.e., without including early natural selection). In addition, Fernandes et al. (2008), in a study performed in a *P. pinaster* CSO, concluded that the probability that a seed embryo sampled from a mother tree was derived from an outcrossing event was 90.1±2.3%. The absence of selfing found in our study was not surprising in spite of the self-fertilization rates of around 5% found in most seed orchards examined to date (Moriguchi et al. 2007). In fact, many of the seeds and seedlings produced by inbreeding have a lower viability than outcrossed individuals, so that the inbreds often remain undetected because of large-scale early mortality (Linhart 2000). In *Pinus* species, selection against inbreds is likely to be common at the seed stage (Leding 1998). In *P. pinaster*, most lethal or sub lethal alleles are probably eliminated during seed formation and germination as well as during the first growing season, so that the proportion of selfed offspring is expected to be very low in mature populations (González-Martínez et al. 2003). Durel et al. (1996), however, observed that *P. pinaster* survival rates after a first growing season in the nursery were the same, independently of the level of inbreeding.

Nevertheless, in cases where outcrossing is high, mating may still involve only a limited number of males or the contribution of a few males may be responsible for the majority of mating, leading to high values of correlated paternity (r_p) and, consequently, high coancestry coefficients among offspring of the same family. Several studies in different conifer species performed in seed orchards reported that contribution as pollen donor differs significantly among clones (El-Kassaby et al. 1984; Goto et al. 2002; Hansen and Kjaer 2006; Kumar et al. 2007; Moriguchi et al. 2005; Plomion et al. 2001). Possible explanations for this fact may include flowering asynchrony, and differences in male flowering intensity and pollen competitive ability. In fact, Varela (1989), in a study of reproductive behavior performed in the same CSO as this study, observed flowering phenology asymmetry, which could have promoted substantial differences in the contribution of pollen donors. Fernandes et al. (2008), in a study performed in a CSO that has some families in common with our study, observed a male and female unbalanced contribution to the progeny. In our study, a relatively low level of correlated mating (4-5%) was obtained, which is similar to the values obtained in natural populations of *P. pinaster* (de-Lucas et al. 2008; González-Martínez et al. 2002). This result was not expected, given the low number of males available for mating in the CSO studied (only 49 genotypes) in comparison to the large natural populations studied by de-Lucas et al. (2008) and the typically uneven male contributions in pine seed orchards. However, according to Askew and El-Kassaby (1994), the intrusion of foreign pollen usually increases the effective size of the paternal population. The same authors note that wind-pollinated seed orchards dominated by effectively large foreign pollen pools would produce the ‘idealized’ seed crops of virtually all half-sib relationships. Numerous studies have reported the occurrence of high pollen contamination in conifer seed orchards (Adams et al. 1997; Kaya et al. 2006; Moriguchi et al. 2007; Plomion et al. 2001; Slavov et al. 2005). Fernandes et al. (2008), in a *P. pinaster* CSO installed side by side with the CSO from which the trees of the present trial originated, found gene immigration rates from outside the CSO of 52.4%. The very possible existence of pollen contamination may explain why the r_p value is so low in our case study and so very close to the natural-population values.

Our results are similar to those expected from half-sib families. This situation is not always the case when the seeds are collected in natural populations, as mating can differ among the different populations. In populations that are a source of plus trees, pedigrees are usually unknown, and it is assumed that all plus trees are genetically unrelated (Kumar and Richardson 2005). This lack of relatedness may not always be true, causing the occurrence of biparental inbreeding within the families in the following stages of the breeding program, as it may be the case in the present progeny trial. Comparison of multi-locus and single-locus outcrossing rates in our families revealed some amount of biparental inbreeding, although it probably does not contribute much of the inbreeding coefficient. In contrast, Fernandes et al. (2008) obtained a minimum estimate of biparental inbreeding of 21.7%. These authors note that it is a very high value considering the care taken in the selection of plus trees; however, as they were collected from the same provenance, the existence of some family relationship among different first selections is still a reasonable hypothesis.

The coancestry coefficient was calculated based on outcrossing rates (t_m) and correlated paternity (r_p) estimates from molecular markers. We did not find any trend in r_p values when comparing families showing extreme and contrasting values of the inter-individual variance for different quantitative traits. The average value obtained (θ=0.130) was not very different from that expected for half-sib progenies (0.125). The mean value of the genetic covariance coefficient of the families present in this progeny trial was then 0.260. Differences between the unadjusted and adjusted heritability values were more pronounced in wood density (0.629 and 0.586, respectively) than in diameter (0.166 and 0.154, respectively), but were not significantly biased (<5%). Nevertheless, a relatively low number of full-sibs (~10%; i.e., a covariance coefficient of 0.28) would be enough to produce heritability overestimations of about a 10%.

Attention should be given to the fact that, as the number of full-sibs increase, the value of heritability does not depend only on the additive variance but also on the dominance variance, so that the value of heritability would be further inflated.

According to Borralho (1994), when dominance effects are large and selfing rates vary significantly among families, heritabilities can be substantially overestimated, especially for low heritability traits. On the other hand, bias does not appear to be important when dominance effects are small and heritabilities are moderate to high. This author also refers that the magnitude of the bias due to overdominance seems small compared with the potential bias from assigning a wrong genetic correlation among open-pollinated sibs.

The effect of the coefficient of variation (CV) of coancestry coefficients among families was not notable for either of the traits studied, indicating that even if larger variation among families was present, as far as the mean correlated paternity is correctly estimated (unbiased), increasing the number of families studied (for instance, from five to ten or more) would not, in our case, affect heritability estimates. The low importance of the CV in our results suggests that only under extreme conditions (high incidence of full-sib relationships) or extremely diverse values among families, the heritability estimates can be affected. Therefore, molecular markers, even scored only in a subsample of the families included in the test, can be a valuable tool in assessing mean coancestry coefficient for the estimate of quantitative genetic parameters in common garden experiments. We can conclude that in *P. pinaster* open-pollinated progeny tests (and probably in other species with a similar mating system), the associated error in heritability estimates due to the inclusion of full-sibs, when assuming a standard coefficient of relation amongst open pollinated sibs of ¼, is low and that this result is robust with respect to the number of families sampled with genetic markers, given unbiased estimates of average relationship among offspring within sib families.

Acknowledgements

Our most sincere thanks to Rowland Burdon for comments and edits that greatly improved the manuscript. M.J. Gaspar was funded by the Portuguese Foundation for Science and Technology (Doctoral grant SFRH/BD/27412/2006).

7.6. REFERENCES

Adams, W.T., Hipkins, V.D., Burczyk, J., and Randall, W.K. 1997. Pollen contamination trends in a maturing Douglas-fir seed orchard. Can J Forest Res 27 (1): 131-134.

Aguiar, A. 1993. Análise da variação do crescimento em altura de descendências maternais de pinheiro bravo. Silva Lusitana 1 (2): 267-274.

Aguiar, A., Almeida, M.H., and Borralho, N. 2003. Genetic control of growth, wood density and stem characteristics of *Pinus pinaster* in Portugal. Silva Lusitana 11 (2): 131-139.

Askew, G.R., and El-Kassaby, Y.A. 1994. Estimation of relationship coefficients among progeny derived from wind pollinated orchard seeds. Theor Appl Genet 88 (2): 267-272.

Austerlitz, F., and Smouse, P.E. 2001. Two-generation analysis of pollen flow across a landscape. II. Relation between Phi(ft), pollen dispersal and interfemale distance. Genetics 157 (2): 851-857.

Blouin, M. 2003. DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. Trends in Ecology and Evolution 18 (10): 503-511.

Borralho, N.M.G. 1994. Heterogeneous selfing rates and dominance effects in estimating heritabilities from open-pollinated progeny. Can J Forest Res 24 (5): 1079-1082.

Chagné, D., Chaumeil, P., Ramboer, A., Collada, C., Guevara, A., Cervera, M.T., Vendramin, G.G., Garcia, V., Frigerio, J.M.M., Echt, C., Richardson, T., and Plomion, C. 2004. Cross-species transferability and mapping of genomic and cDNA SSRs in pines. Theor Appl Genet 109 (6): 1204-1214.

de-Lucas, A.I., Robledo-Amuncio, J.J., Hidalgo, E., and González-Martínez, S.C. 2008. Matting system and pollen gene flow in Mediterraneam Maritime pine. Heredity 100: 390-399.

Doyle, J., and Doyle, J. 1990. Isolation of plant DNA from fresh tissue. Focus 12:13-15.

Durel, C.E., Bertin, P., and Kremer, A. 1996. Relationship between inbreeding depression and inbreeding coefficient in Maritime pine (*Pinus pinaster*). Theor Appl Genet 92 (3-4): 347-356.

El-Kassaby, Y.A., Fashler, A.M.K., and Sziklai, O. 1984. Reproductive phenology and its impact on genetically improved seed production in a Douglas-fir seed orchardd. Silvae Genet 33 (4-5): 120-125.

El-Kassaby, Y.A., and Ritland, K. 1986. The relation of outcrossing and contamination to reproductive phenology and supplemental mass pollination in a Douglas-fir seed orchard. Silvae Genet 35:240-244

Falconer, D.S., and Mackay, T.F.C. 1996. Introduction to quantitative genetics. Longman Scientific & Technical ed, New York. .

Fernandes, L., Rocheta M., Cordeiro, J., Pereira, S., Gerber, S., Oliveira, M., and Ribeiro, M.M. 2008. Genetic variation, mating patterns and gene flow in a *Pinus pinaster* Aiton clonal seed orchard. Annals Forest Science 65 (7): 706.

Gaspar, M.J., Louzada, J.L., Silva, M.E., Aguiar, A., and Almeida, M.H. 2008. Age trends in genetic parameters of wood density components in 46 half-sibling families of *Pinus pinaster*. Can J Forest Res 38 (6): 1470-1477.

Gerber, S., Mariette, S., Streiff, R., Bodenes, C., and Kremer, A. 2000. Comparison of microsatellites and amplified fragment length polymorphism markers for parentage analysis. Mol Ecol 9 (8): 1037-1048.

Gilmour, A., Gogel, B., Cullis, B., Welham, S., and Thompson, R. 1998. ASREML Users Manual. *In* VSN International. New south Wales Agriculture, Orange.

González-Martínez, S.C., Gerber, S., Cervera, M.T., Martinez-Zapater, J.M., Gil, L., and Alía, R. 2002. Seed gene flow and fine-scale structure in a Mediterranean pine (*Pinus pinaster* Ait.) using nuclear microsatellite markers. Theor Appl Genet 104: 1290–1297.

González-Martínez, S.C., Gerber, S., Cervera, M.T., Martinez-Zapater, J.M., Alía, R., and Gil, L. 2003. Selfing and sibship structure in a two-cohort stand of maritime pine (*Pinus pinaster* Ait.) using nuclear SSR markers. Ann for Sci 60 (2): 115-121.

Goto, S., Miyahara, F., and Ide, Y. 2002. Identification of the male parents of half-sib progeny from Japanese black pine (*Pinus thunbergii* Parl.) clonal seed orchard using RAPD markers. Breeding Science 52 (2): 71-77.

Hansen, O.K., and Kjaer, E.D. 2006. Paternity analysis with microsatellites in a Danish Abies nordmanniana clonal seed orchard reveals dysfunctions. Can J Forest Res 36 (4): 1054-1058.

Hardy, O.J. 2003. Estimation of pairwise relatedness between individuals and characterization of isolation-by-distance processes using dominant genetic markers. Mol Ecol 12 (6): 1577-1588.

Hardy, O.J., and Vekemans, X. 2002. SPAGEDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes 2 (4): 618-620.

Hardy, O.J., González-Martínez, S. C., Colas, B., Fréville, H., Mignot, A., Olivieri, I. 2004. Fine-scale genetic structure and gene dispersal in *Centaurea corymbosa* (Asteraceae) II. Correlated paternity within and among sibships. Genetics 168:1601-1614

Kaya, N., Isik, K., and Adams, W.T. 2006. Mating system and pollen contamination in a Pinus brutia seed orchard. New Forests 31 (3): 409-416.

Kumar, S., Gerber, S., Richardson, T.E., and Gea, L. 2007. Testing for unequal paternal contributions using nuclear and chloroplast SSR markers in polycross families of radiata pine. Tree Genetics & Genomes 3 (3): 207-214.

Kumar, S., and Richardson, T.E. 2005. Inferring relatedness and heritability using molecular markers in radiata pine. Molecular Breeding 15(1): 55-64.

Ledig, F.T. 1998. Genetic variation in Pinus. *In* Ecology and Biogeography of Pinus *Edited by* D. M. Richardson. Cambridge University Press. pp. 251-279.

Linhart, Y.B. 2000. Variation in woody plants; molecular markers, evolutionary processes and conservation biology. *In* Molecular biology of woody plants. *Edited by* S.M Jain, and S. C. Minocha. klower academic publishers, Dordrecht/Boston/London. pp. 341-373.

Loiselle, B.A., Sork, V.L., Nason, J., and Graham, C. 1995. S patial genetic-structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). Am J Bot 82: 1420-1425

Lynch, M., and Ritland, K. 1999. Estimation of pairwise relatedness with molecular markers. Genetics 152 (4): 1753-1766.

Lynch, M., and Walsh, B. 1997. Genetics and analysis of quantitative traits. Sinauer Associates,Inc., Massachusetts, 980 pp

Moriguchi, Y., Tani, N., Itoo, S., Kanehira, F., Tanaka, K., Yomogida, H., Taira, H., and Tsumura, Y. 2005. Gene flow and mating system in five Cryptomeria japonica D. Don seed orchards as revealed by analysis of microsatellite markers. Tree Genetics & Genomes 1 (4): 174-183.

Moriguchi, Y., Tsuchiya, S., Iwata, H., Itoo, S., Tani, N., Taira, H., and Tsumura, Y. 2007. Factors influencing male reproductive success in a *Cryptomeria japonica* seed orchard revealed by microsatellite marker analysis. Silvae Genet 56(5): 207-214.

Perry, D., and Hopkins, E. 1967. Importation of breeding material of *Pinus pinaster* Ait. from Portugal. For. Dep. W. A. Bulletin 75.

Plomion, C., LeProvost, G., Pot, D., Vendramin, G., Gerber, S., Decroocq, S., Brach, J., Raffin, A., and Pastuszka, P. 2001. Pollen contamination in a Maritime pine polycross seed orchard and certification of improved seeds using chloroplast microsatellites. Can J Forest Res 31 (10): 1816-1825.

Ritland, K. 2002. Extensions of models for the estimation of mating systems using n independent loci. Heredity 88: 221-228.

Robledo-Arnuncio, J.J., Smouse, P.E., Gil, L., and Alia, R. 2004. Pollen movement under alternative silvicultural practices in native populations of Scots pine (P*inus sylvestris* L.) in central Spain. Forest Ecol Manag 197 (1-3): 245-255.

Slavov, G.T., Howe, G.T., and Adams, W.T. 2005. Pollen contamination and mating patterns in a Douglas-fir seed orchard as measured by simple sequence repeat markers. Can J Forest Res 35 (7): 1592-1603.

Smouse, P.E., Dyer, R.J., Westfall, R.D., and Sork, V.L. 2001. Two-generation analysis of pollen flow across a landscape. I. Male gamete heterogeneity among females. Evolution 55 (2): 260-271.

Squillace, A.E. 1974. Average genetic correlations among offspring from ope-pollinted forest trees. Silvae Genet 23 (5): 149-156.

Varela, C. 1989. Comportamento reprodutivo de *Pinus pinaster* Ait. num pomar clonal de sementes. Universidade Técnica de Lisboa, Lisbon, 107pp.

Wasieliwska, M., LKlemm, M., and Burczyk, J. 2005. Genetic diversity and mating system of Scots pine plus trees. Dendrobiology 53: 57–62.

Wright, J.W. 1976. Introduction to Forest Genetics. Academic Press, New York, 463 pp.

Zobel, B.J., and Talbert, J. 1984. Applied Forest Tree Improvement. John Wiley & Sons, New York, 511 pp.

CHAPTER 8

General discussion, Conclusions and Perspectives

8.1. GENERAL DISCUSSION

The main objective of any tree improvement programme is to obtain populations of superior genetic value, leading to the establishment of more valuable plantations. It is then essential to clearly define the objectives, to indentify the characteristics that affect the final objective and to evaluate their genetic control. Based upon this information, deployment strategies can then be chosen. In a tree breeding programme that aims to produce material for wood processing industry, it is essential to consider wood properties as potential selection criteria.

The main objective of this work was to contribute to the Portuguese *Pinus pinaster* improvement programme with information regarding the genetic control of wood quality traits, and determine how these traits interact between themselves and with growth traits. It is also a goal of this work to develop an index selection to identify the families that better combine the different traits under study. Among the desirable wood-quality properties to breed for, wood density is the most frequently studied (Zobel and van Buijtenen 1989), since it is widely recognized as an important, easily measured trait that is that largely defines the appropriateness of wood for a variety of end products (Zobel and Jett 1995). However, the complex nature of this trait means that variations in wood density can be difficult to interpret, since wood density is not based upon a single property, but a complex of characteristics that make up density components. Overall ring density (RD) depends on earlywood (EW) and latewood (LW) densities, and the relative proportions of each (Vargas-Hernandez and Adams 1991).

In Chapter 2, the heritability of wood density components and ring-width characteristics as well as genetic correlations between cambial ages were studied. As expected for most wood quality traits, the results indicated that they were subject to genetic control [$0.26<h_i^2<0.63$]. RD had higher genetic control (h_i^2=0.63) and heritability values of earlywood components ($h_{i\,(\mathrm{MND})}^2$=0.54, $h_{i\,(\mathrm{EWD})}^2$=0.60) exceeded those of latewood components ($h_{i\,(\mathrm{MXD})}^2$=0.34; $h_{i\,(\mathrm{LWD})}^2$=0.26), confirming that for *P. pinaster*, EW characteristics are subject to a stronger genetic control, like other phonological traits, than LW components, which are more affected by the growth constrains acting in the end of the growing season. In Mediterranean regions usually springs provides optimal conditions for tree growth and it is during this period that genetic effects are stands out. In summer, there are constraining conditions to growth, due to high temperatures and water stress, which may increase phenotypic variance, leading to reduced heritability. Concerning the intra-ring growth components, the variation among families did not produce statistically significant differences ($p>0.05$) for ring width (RW) and heterogeneity index (HI), what conditions the possibility of obtaining genetic gains truth selection for this traits. Nevertheless, the moderate values obtained for earlywood width (EWW) and latewood width (LWW) provide a basis for the genetic manipulation of wood quantity and quality of this species. Further, the high heritability values obtained for EW components allows selection based on these components, that could result in an increase of wood density and a decrease in ring heterogeneity.

Heritability increased with ring number from pith for almost all wood-density components, and age-age genetic correlations for wood density components were generally high. The genetic correlations between rings 3 and 11 for most traits ($r_g>0.9$), open the possibility of performing early selection. Since tree breeding programs usually take many years to complete a breeding cycle; a trait in mature wood that is highly correlated with its value in juvenile material is advantageous, since the overall length of the programme will be reduced, making forest tree breeding more effective.

The high heritability values obtained for wood density components suggests that large gains can be obtained by selection for these traits, in the absence of adverse genetic correlations with other traits. Therefore, in Chapter 3 genetic and phenotypic correlations between the wood density components, traits and spiral grain were assessed. Data showed that genetic correlations for all wood density components were higher than corresponding phenotypic correlations, indicating that trait evaluation based on phenotypic correlations rather than genetic ones would result in an underestimate of potential gains from indirect selection. Furthermore, all wood density components were highly genetically correlated with ring density ($0.79<r_g<1$) and also closely associated among themselves, indicating that these traits are probably controlled, to a large extent, by the same set of genes, and that selection for one characteristic should result in a simultaneous response for associated traits. The strong genetic relationship between density and each one of its components highlights the fact that overall density is a complex trait resulting from different combinations of LW and EW, and the relative density of each component (Hylen 1997). RD presented a higher genetic correlation with earlywood density (EWD) than with latewood density (LWD), and a higher phenotypic correlation with LWD than with EWD, confirming results obtained in Chapter 2, namely that *P. Pinaster* earlywood characteristics are subject to stronger genetic control, and that latewood components are more strongly influenced by environmental factors. Therefore ring density (overall wood density) can be improved by increasing either EWD, LWP, or both of these traits.

Heigt genetic control was estimated for spiral grain (SPG) (h_i^2=0.47), indicating that potentially, tree breeders can modify SPG in future plantations. A moderate to high positive genetic correlation was observed between this trait and wood density characteristics (0.29-0.61). Also, SPG was more dependent on earlywood components than on latewood components. These higher correlations observed between earlywood and SPG, can be associated to the fact that spiral grain is a characteristic that may occur in association with the existence of reaction wood (compression wood in softwoods). Higher density values presented in compression wood, even by earlywood (Tsoumis, 1991), result in a shorter portion of the ring with lower density values than the threshold value between EW and LW, resulting in a smaller portion of EWW and a higher LWP.

In Portugal, Maritime pine is the only source of long fibre for both pulp and paper and the main source for solid sawn timber industries. Its chemical composition, as well as wood density, is of extreme importance as a source of long fibre. In Chapter 4 phenotypic and genetic correlations between lignin content (LG) and density components were studied. Results showed moderate genetic control of LG (h_i^2= 0.34), and very low or even null phenotypic correlations of lignin content with all other traits ($-0.16<r_p<0.15$), indicating that phenotypic correlation was unsuitable for mass selection of traits. Our findings concerning genetic correlations indicated that RD had negative but low genetic relationship with lignin (r_g=-0.16), suggesting that selection based on wood density will not result in an increase of LG.

The correlations between RD and LG have important industrial implications, since selection for increased density will increase pulp yield per ton and decrease production costs. Our results also showed that LG was more dependent on the proportion of latewood present in the ring, suggesting that these traits may be controlled at least partially by the same set of genes, and that increasing the existence of more latewood would decrease the lignin content. In Chapter 2, data showed that selection for higher density could be performed by increasing either r or EWD. Based on these results breeders can possibly adjust the course of selection depending on their objective. If the purpose is pulp production, improved density and reduced lignin content is desirable, and an increase in LWP appears to be the correct way to obtain higher wood density.

Genetic control of mechanical properties has not been well documented, compared with growth and other wood quality traits. In Chapter 5 the genetic variation of radial modulus of elasticity (MOE_{RAD}) and radial modulus of rupture (MOR_{RAD}) was analysed and correlation patterns between these traits and intra-ring wood characteristics, spiral grain and lignin content were also assessed. Both mechanical traits MOR_{Rad} and MOE_{Rad} are subject to only moderate levels of genetic control in Maritime pine (h_i^2=0.34 and h_i^2=0.31, respectively). Differences between families with highest and lowest modulus of rupture and modulus of elasticity, allied with the values of heritability estimates, indicated that selection for higher resistance and elasticity through breeding could yield some genetic gains. Results showed high phenotypic and genetic correlations between the two mechanical traits, indicating that these traits are at least for a large part controlled by the same set of genes and that indirect selection procedures could be carried out on these characters.

As observed for LG in Chapter 4, the results indicate that MOE_{Rad} was more genetically dependent on latewood components ($r_{g(MOE,MXD)}$=0.36; $r_{g(MOE,LWD)}$ =0.36 and $r_{g\ (MOE,\ LWW)}$ =0.48) than on earlywood components ($r_{g(MOE,\ MND)}$=0.11; $r_{g(MOE,\ EWD)}$ =0.16 and $r_{g(MOE,\ EWW)}$=-0.02), indicating that selection for greater proportion of latewood would increase wood stiffness. As observed in Chapter 3, RD is genetically highly positively correlated with LWW and negatively correlated with EEW. Thus, traits highly genetic correlated with RD like MOE_{Rad} are expected to present high positive correlations with latewood intra-ring traits, and negative correlations with earlywood. The high correlation between LWW and MOE_{Rad} can also explain the negative relation observed between MOE_{Rad} and LG, since LG presents a negative genetic correlation with RD. These results indicate that selecting trees with lower LG, for example for pulp production, might produce a decrease in radial modulus of elasticity of the wood, and that the final use of the material should be taken into account.

Genetic and phenotypic correlations between growth rate and wood quality traits have major implications for developing selection and breeding strategies. In the Portuguese *P. pinaster* improvement programme, selection criteria for the first generations have been the growth rate and form traits; however, it is important to be aware of the consequences of this selection on wood quality traits. We addressed this theme in Chapter 6 results showed that diameter at breast height (DBH) was more dependent on latewood ($r_{g(DBH,\ MXD)}$=0.24; $r_{g(DBH,\ LWD)}$=0.20 and $r_{g(DBH,\ LWW)}$=0.42) than earlywood components($r_{g(DBH,\ MND)}$=0.00; $r_{g(DBH,\ EWD)}$=0.07 and $r_{g(DBH,\ EWW)}$=0.00). In our opinion, the existence of more LW or EW could be associated with the growing conditions of trees. In fact, higher growth rates observed in higher latitudes can be a consequence of the early onset of cambial initiation, resulting in a higher proportion of EW. On the other hand, higher growth rates in Mediterranean regions may be due to an increase in the latewood formation period, since the main constraint regarding tree growth in this region is water stress during the summer. When growth conditions are favourable in summer (water availability), trees present higher RW due to an enlargement of LWW and, consequently, higher RD. Conversely, during summer drought conditions trees will present less LWW, RW and RD. Further, we can conclude that genetic selection for growth will not result in a decrease of wood density, nor affect the occurrence of spiral grain, and that it is possible to obtain an increase in the radial modulus of elasticity. The present study also showed that it is possible to select for increased growth with a lower proportion of lignin. Results also suggest that selection for growth at 12 years will probably not negatively affect the wood properties at 17 years.

A major difficulty estimating additive genetic parameters is the assumption that has to be made of the genetic relationship between the progeny in an open pollinated trial. Reliability of the estimates depends on knowledge of the relationship among the individuals recorded. In Chapter 7 the mean value of the coancestry coefficient of the families present in a progeny trial, with the same half-sib families present in the burned trial, was estimated and it was studied how deviations from the standard assumption of θ=0.125 affected heritability estimations. Five highly polymorphic microsatellite markers were scored in 125 offspring from a sub sample of five families from the progeny test. A mean coancestry coefficient value of 0.130 was derived. We concluded that the associated error in heritability estimates due to the inclusion of full-sibs in a *P. pinaster* open-pollinated trial, (assuming a standard coefficient of relation amongst open-pollinated sibs of 0.250) was low and that this result is robust relative to the number of families sampled, with unbiased estimates of average relationship among offspring within sib families. This determination allowed us to correct all subsequent genetic parameters estimates, and to make a more precise determination of expected gains and define better strategies for selection.

All tree improvement programmes aim to simultaneously improve more than one trait. Index selection presents tree breeders with the attractive option of combining information from several traits (Cotteril 1985). Knowledge of all genetic parameters enables the application of multi-trait index selection, which combines all information on phenotypic performance and genetic structure (Costa e Silva 1998). In order to explore the possibilities that the previous parameters estimates may have on *Pinus pinaster* improvement, expected genetic gains were estimated (White and Hodge 1989) for several family based multi-trait selection indices, for two possible final use scenarios, namely solid wood and other for pulp production (Table 8.1). The weighting coefficients indicated in the table represent the factors by which the “equal emphasis” weights were multiplied according to the iterative approach suggested by Cotterill and Dean (1990). We only chose to simulate the gains obtained from family selection, once the genetic trial has been burned. Thus, the only possibility was to select the best mothers, based on the index value of each family.

The ranking of the best 10 families considering the different scenarios are presented in table 8.2 (List of all families is presented in Appendix 1).

Table 8.1 Expected genetic responses in the target trait, from family selection based on multiple trait indices derived under different final use scenarios -1) solid wood, 2) pulp production- of alternative combinations of weighting coefficients. The expected genetic changes are expressed in the measurement units of the trait, as well as in percentage of the trait mean values (in parentheses).

Scenarios		Weighting coefficients					Expected genetic responses				
		DBH	RD	SPG	MOR$_{rad}$	MOE$_{rad}$	DBH (cm)	RD (gcm^{-3})	SPG	MOR$_{rad}$ (MPa)	MOE$_{rad}$ (GPa)
1)	a)	1	1	-1	1	1	0.07 (0.55)	0.05 (11.11)	1.32 (31.26)	1.22 (8.91)	0.12 (10.76)
	b)	3	3	-2	2	2	0.10 (0.84)	0.07 (14.02)	1.76 (41.72)	0.98 (7.13)	0.12 (10.42)
	c)	10	10	-5	5	5	0.12 (0.99)	0.07 (15.34)	1.97 (46.72)	0.80 (5.86)	0.11 (9.90)

Scenarios		DBH	RD	LG	DBH (cm)	RD (gcm^{-3})	LG (%)
2)	a)	1	1	-1	0.46 (3.88)	0.08 (18.97)	-0.80 (-2.84)
	b)	3	2	-3	0.57 (4.83)	0.07 (15.23)	-0.82 (-2.90)
	c)	10	7	-10	0.59 (4.72)	0.07 (15.23)	-0.82 (-2.90)

DBH- Breast high diameter; RD- Ring density; SPG- Spiral Grain; MORrad- Radial Modulus of Rupture; MOErad- Radial Modulus of Elasticity; LG- Lignin content proportion.

The expected gains presented clearly showed that it is possible to achieve simultaneous improvements in growth and wood quality traits through family selection for both solid wood and pulp production. An increase of 15% can be achieved in RD simultaneously with a 1% increase in DBH. No decline was observed for SPG, probably due to the positive genetic correlation observed between SPG and RD (Chapter 3). The physiological background for the variation of this trait is not well established, but it is likely that the pattern of variation is an adaptation to facilitate transport of water and photosynthetic products in xylem and phloem (Kubler 1991; Rosner et al. 2007) and increase stem strength against wind rupture (Skatter and Kucera 1997). According to Steffenrem (2008) zero grain angles at all radial positions is rare. Instead, selection should concentrate on eliminating trees with deviating spiral grain and excessive angles in juvenile wood from the breeding populations.

When final use is for pulp production it is possible to increase growth and RD with a simultaneous decrease in LG. Table 8.2 shows that several of the 10 best families are common for the two final use scenarios (signed at bold) and that it is possible to select a group of families that will give good results for solid wood end use or pulp production. In a small country with limited financial resources like Portugal, the optimization of resources with a multi-option use in a small breeding programme such as Portuguese *P. pinaster* programme is extremely important.

Table 8.2 Ranking of the best 10 families considering the different scenarios different final use scenarios - 1) solid wood, 2) pulp production- and the alternative combinations of weighting coefficients a), b and c).

1 a)	1 b)	1 c)	2 a)	2 b)	2 c)
32	**20**	**20**	**20**	**20**	**20**
15	**61**	**15**	**18**	**18**	**18**
20	**15**	32	**61**	53	19
61	**18**	**61**	19	19	53
13	32	**18**	**49**	**15**	**15**
68	**13**	**13**	53	**49**	**49**
18	**49**	**49**	**15**	**61**	**61**
49	68	68	**13**	**13**	**13**
22	53	53	78	78	78
59	19	7	7	7	7

8.2. CONCLUSION AND FUTURE PERSPECTIVES

The results obtained in this work clearly provide evidence that it is possible to perform genetic selection for improving wood quality in the Portuguese *Pinus pinaster* improvement program, once most of the studied traits presented moderate to high genetic control and favorable genetic and phenotypic correlations between them. The results obtained allow us to conclude that genetic selection based on growth can be performed without compromising wood density, will not affect the occurrence of spiral grain, and that is possible to obtain an increase in the radial modulus of elasticity. Also, the results showed that it is possible to select for increased growth with lower lignin proportion. Concerning the expected gains obtained through the application of multi-index selection indices, it is clear that is possible to achieve simultaneous improvements in growth and wood quality traits through family selection for the two final use scenarios, either solid wood as pulp production. However, it is necessary to further complement these indices by adding economic information on the several traits studied to obtain a more accurate estimation of the possible genetic gains.

Most scenarios for global climate change suggest increase aridity and increase frequency of extreme events in the near future. In this context, it is reinforced the interest in the study of morphological, physiological and biochemical responses of *Pinus pinaster* to this alterations, and to modulate our improvement population to face this changes. Also, little is known on the effect of climate changes on traits related with wood formation. Cambial activity is influenced by water balance variations during the growing season, which results in a variation of the morphology of the produced xylem cells. The ability of a given genotype to adjust its phenotype: i.e., phenotypic plasticity can minimized the impact of climate fluctuations. Changes in wood formation during cambial activity can be interpreted as a plastic response to climate variation. In this context some results obtained in this study are being used to study the genetic control of phenotypic plasticity, which is very useful tool to face the future climate changes.

In our opinion, knowledge on the genes responsible for controlling wood formation needs to be developed. As wood quality assessment is a long-time process, association between molecular markers and phenotypic expression should be established. Knowledge on these associations will be fundamental for early identification of superior genotypes. In this way we will be able to put together breeding populations in a more cost-effective and precise manner in the future.

Another aspect that should be highlighted, due to its importance and recent awareness, is the occurrence of the nematode (Bursaphelenchus xylophilus) in *P. pinaster*, the agent of the pine wilt disease, which is spreading to a vast area of our country and decimating several maritime pine stands. Tree improvement will play an important role on the control of this disease trough the investigation of the existence of possible resistant genotypes, and evaluating the genetic control of this resistance, so it should be a selection criterion to be included in the breeding programme.

Taking into account the results presented in this thesis, we believe that this work may be a valuable contribution to the Portuguese *P. pinaster* improvement programme, by adding information on the genetic control of wood quality and growth traits. Furthermore this work used as genetic material, wood disc samples from a progeny trial destroyed by a forest fire, gathering information that otherwise would be lost. This material allowed us to characterise for several wood quality properties, 46 families which belong to first generation of the improvement programme. These findings are of great importance for the continued development of maritime pine breeding programmes to guide future generation selection.

8.3. REFERECES

Cotterill, P.P., and Dean, C.A. 1990. Successful tree breeding with index selection. CSIRO Australia, Victoria, 80 pp.

Hylen, G. 1997. Genetic variation of wood density and its relationship with growth traits in young Norway spruce. Silvae Genet 46(1): 55-60.

Kubler, H. 1991. Function of Spiral Grain in Trees. Trees-Struct Funct 5(3): 125-135.

Rosner, S., Klein, A., Muller, U., and Karlsson, B. 2007. Hydraulic and mechanical properties of young Norway spruce clones related to growth and wood structure. Tree Physiol 27(8): 1165-1178.

Skatter, S., and Kucera, B. 1997. Spiral grain - An adaptation of trees to withstand stem breakage caused by wind-induced torsion. Holz Als Roh-Und Werkstoff 55(4): 207-213.

Steffenrem, A. 2008. Genetic variation in structural wood quality traits in Norway spruce and implications for tree breeding. In Department of Ecology and Natural Resource Management. Norwegian Univerity of Life Sciences.

Tsoumis, G. 1991. Science and Technology of Wood: Structure, Properties, Utilization. Chapman & Hall New York, 494 pp.

Vargas-Hernandez, J., and Adams, W.T. 1991. Genetic-variation of wood density components in young coastal Douglas-Fir - Implications for tree breeding. Can J Forest Res 21(12): 1801-1807.

White, T.L., and Hodge, G.R. 1989. Predicting breeding values with applications in forest tree improvement., Dordrecht, 367 pp.

Yeh, F.C. 1981. Analysis of gene diversity in some species of conifers. In Symposium of Isozymes of North American Forest Trees and Forest Insects, Berkeley, California

USDA Forest Service, Berkeley, Calif., USA. pp. 48-52.

Zobel, B.J., and Jett, J.B. 1995. Genetics of Wood Production. Springer-Verlag ed, Berlin, Heidelberg, and New York, 377 pp.

Zobel, B.J., and van Buijtenen, J.P. 1989. Wood Variation - Its causes and control. Springer-Verlag ed, Berlin, 363 pp.

APPENDIX 1

Apendix 1 - Ranking of the 46 families considering the different scenarios different final use scenarios - 1) solid wood, 2) pulp production- and the alternative combinations of weighting coefficients a), b and c).

1a)	1 b)	1 c)	2 a)	2 b)	2 c)
32	20	20	20	20	20
15	15	61	18	18	18
20	32	15	61	53	19
61	61	18	19	19	53
13	18	32	49	15	15
68	13	13	53	49	49
18	49	49	15	61	61
49	68	68	13	13	13
22	53	53	78	78	78
59	7	19	7	7	7
51	59	7	17	17	17
7	51	76	32	32	32
53	79	51	39	39	39
79	22	79	50	59	59
39	39	59	59	50	50
58	58	58	51	51	51
16	76	39	14	14	14
11	19	11	16	16	16
76	11	22	79	45	45
17	16	17	48	79	79
8	17	45	45	48	48
48	48	16	23	23	23
41	45	48	76	11	11
45	73	73	11	76	76
73	41	78	68	56	56
19	8	41	56	82	82
56	23	8	9	9	9
23	50	50	30	30	30
82	78	46	8	8	8
9	46	23	82	68	68
50	56	24	73	73	73
14	14	14	22	29	29
46	24	25	46	46	46
81	25	56	24	22	22
78	81	34	29	24	24
34	34	81	80	80	80
25	9	30	12	12	12
29	29	29	58	58	58
24	82	9	25	25	25
12	30	82	34	34	34
80	12	12	41	31	31
30	80	80	31	41	41
5	5	5	81	81	81
31	31	31	54	54	54
54	54	54	10	10	10
10	10	10	5	5	5