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# PROCEEDINGS

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> Porto Seguro 2011

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## Preface

The productivity of each rotation of *Eucalyptus* plantation worldwide has increased by 10 to 20%, as a result of major advances in silviculture and genetics. But, can we continue increasing yields for the next rotation, and the one beyond that? Yes, but only if we develop fundamentally new ways to combine silviculture and genetics research, and applied them via adequate planned operations on the lookout for economical, social and environmental sustainability.

As in previous IUFRO *Eucalyptus* meetings (Bordeaux 1990, Hobart 1995, Salvador 1997, Valdivia 2001, Aveiro 2004 and Durban 2007), this was a great opportunity for scientists, foresters and plantation managers to lecture, discuss and foresee ways to improve our understanding and management of eucalypt forests.

Eucalypt plantations constitute approximately 15% of global plantations, and are being grown for a wide range of end-products for industrial and domestic uses. Currently, eucalypts are most often established replacing pasture or crops in tropical and subtropical areas, being a significant component of the carbon and water balances of these landscapes, with ecological, economical and social interfaces.

Eucalypt is the dominant and most productive planted forest in Brazil, covering around 3.5 million ha, of which 60% is certified according to international standards. Plantations are grown on short- and medium-rotations for the production of pulp, charcoal, fuelwood, reconstituted and solid wood. The Brazilian forestry sector produces approximately 4% of the gross domestic product and employs about 4.5 million people. At the early establishment of the forest plantations, on the second half of the sixties, the eucalypt yield was 10 m<sup>3</sup> ha<sup>-1</sup> yr<sup>-1</sup>, and many environmental problems were not adequately addressed. Now, four decades later, as result of investments in research and technology the average productivity is close to 40 m<sup>3</sup> ha<sup>-1</sup> yr<sup>-1</sup>, and eucalypts forests are included in a more systemic view, ranging from wood production to ecosystem biodiversity.

Eucalypt forestry fulfils multiple functions in landscapes within different ecosystems, and in all of them, some level of environmental stress is present, as a physical (water, temperature, nutrients) or biological (pests, diseases and competing vegetation) constraint, or both. Beyond that, climate changes can cause the decline in forest productivity due to the amplification of such stresses. The search, test and selection of appropriate genotypes and site management practices, improving the resource-use-efficiency of the eucalyptus forests, are imperative for sustain productivity and maintain environmental services of these forests for the generations to come.

With all that in mind, the central goal of this Conference was to highlight how silviculture and genetic knowledge can be used in a predictive way to guarantee the yield of the plantations, and health of the ecosystems in the long run, with extra challenges due to the future climate and economic uncertainties.

The scientific program comprised formal sessions for each major theme related with eucalypt production: forest establishment and regeneration, genetics, breeding, biotechnology, ecophysiology, hydrology, environmental stress, mixed plantation and agroforestry, and wood technology. The synthesis and application of such concepts and researches into practical and predictive tools were, as said, an important aspiration of this conference. Each theme was introduced by a keynote speaker presenting an overview of the subject area of that session, follow by oral presentations and posters.

An one-day field trip to visit high productive *Eucalyptus* forests was taken place at the middle of the conference, and for those able to stay longer, a post-meeting Brazilian *Eucalyptus* Field Trip was accomplished to expose the participants to direct contact with practices and experiences of local foresters and researchers, as well as our traditional landscapes and culture.

We felt very honored to host this conference of IUFRO in Brazil.

Cordially,

Prof. José Leonardo de Moraes Gonçalves – Chair The "Luiz de Queiroz" College of Agriculture (ESALQ) University of São Paulo, Brazil

Prof. José Luiz Stape – Co-chair

North Carolina State University, USA

#### Dr. Dario Grattapaglia – Co-chair

Brazilian Enterprise for Agricultural Research (EMBRAPA), Brazil

## **International Year of Forests 2011**

In 2006, the United Nations General Assembly declared 2011 the International Year of Forests and invited Governments, the United Nations system, relevant non-governmental organizations, the private sector and other actors to make concerted efforts to raise awareness at all levels to strengthen the sustainable management, conservation and sustainable development of all types of forests for the benefit of current and future generations.

The General Assembly also requested the secretariat of the United Nations Forum on Forests of the Department of Economic and Social Affairs of the Secretariat to serve as the focal point for the implementation of the Year, in collaboration with Governments, the Collaborative Partnership on



Forests and international, regional and subregional organizations and processes as well as relevant major groups. It also encouraged voluntary partnerships among Member States, international organizations and major groups to facilitate and promote activities related to the Year at the local and national levels, including by creating national committees or designating focal points in their respective countries.

The International Year of Forests 2011 (Forests 2011) offers a unique opportunity to raise public awareness of the challenges facing many of the world's forests and the people who depend on them. Great success stories and valuable lessons on how to promote sustainable forest management already exist. The Year provides a means of bringing those voices together and building momentum towards greater public participation in forest activities around the world.

Forests 2011 is a unique opportunity to highlight the key role of forests in our lives. By showcasing success stories and solutions, the year will galvanize greater public participation in forest-related activities. "Forests for People" is the main theme of the Year. It highlights the dynamic relationship between forests and the people who depend on them.

The success of Forests 2011 will be a combination of actions on the local, national and regional level. All organizations, from governments to schools, are encouraged to hold activities in celebration of the Year.

Quick Facts

• Over 1.6 billion people's livelihoods depend on forests.

• 80 percent of the world's forests are publicly owned, but ownership and management of forests by communities, individuals and private companies is on the rise.

- 30 percent of forests are used for production of wood and non-wood products.
- Forests are home to 300 million people around the world.

• The annual value of wood removed from forests is estimated to be more than \$100 billion.

### PORTO SEGURO, BAHIA, BRAZIL

Brazil is continental in size, the fourth largest national territory in the world. In fact, its land area is greater than Europe and larger than the continent of United States (excluding Alaska and Hawaii). A visit to Brazil means the unexpected discovery of a place of warm tropical sun and 5,000 miles of white-sand beaches, coconut groves and mango trees, music and dance, baroque colonial towns and villages, and impressive cities of 21st century opulence and sophistication.

Brazil's allure is not only in the climate, the landscapes and the architecture, it lies in the people themselves, whose sense of cordial hospitality and friendship create the perfect environment for your travel. As a whole the climate is excellent all year round. Whatever choice you make, personal attention and warm hospitality will be yours everyday of your stay.

Bahia is one of the 26 states of Brazil, and is located in the northeastern part of the country on the Atlantic coast. It is the fourth most populous Brazilian state after São Paulo, Minas Gerais and Rio de Janeiro, and the fifth-largest in size. It is also one of the most important states in terms of history and culture in Brazil. Bahia's capital is the city of Salvador, located at the junction of the Atlantic Ocean and the Bay of All Saints.

The name "bahia" is an archaic spelling of the Portuguese word baía, meaning "bay", and comes from All Saints' Bay, first seen by European sailors in 1501. Bahia's nature wonders and also its culture and people, fruits of the miscegenation of the Indigenous, the European and the African, who connected here, generating a magical, involving and mysterious energy.

The Portuguese Pedro Álvares Cabral landed at what is now Porto Seguro, on the southern coast of Bahia in 1500, and claimed the territory for Portugal. Considered the starting point of the Brazilian history and culture, the Discovery Coast was declared World Natural Heritage by UNESCO, in 1999. Surrounded by several natural attractions - such as beaches, bays, inlets, cliffs, coral reefs, mangroves and navigable rivers - the region has favorable conditions for adventure tourism and ecotourism.

The region's nature wonders are well known by visitors, especially those who enjoy extreme sports that for now seem to have a closer contact with such attractions. During ultra-light flights it is possible to admire all the beauty of the region, from a different angle. The Discovery Coast has ideal spots for diving, windsurf, surf, kite surf, trekking, horse rides, among others. It is worth remembering that the fun doesn't end here.

The region has a vibrant nightlife. Contrary to what happens in most beach towns, where the day activities are more attractive than the night ones, along the Discovery Coast night leisure is also a must. It is difficult to decide whether to enjoy the day or the night. Generally, visitors end up enjoying both, showing that sleeping is not the priority.

The fun never ends. For more than 500 years this region has been visited by various explorers seeking for the most unusual products. Nowadays, the explorers are better known as "tourists", that look for high doses of adrenaline and lots of history, in a scenery composed by sun, sea and tranquility.

## **KEYNOTE SPEAKERS**

#### Acelino Alfenas

Forest Engineer (1974), M.Sc. in Plant Pathology (1978) at the Federal University of Viçosa (UFV) and Ph.D. in Forest Pathology at the University of Toronto, Canada (1983), Full Professor at the Department of Plant Pathology of the Federal University of Viçosa, Viçosa –MG, Brazil, Research Fellow (1A) of the National Council for Scientific and Development (CNPq). His research involves "Etiology, epidemiology and control of diseases" especially eucalyptus-pathosystems. In recent years, he has worked mainly on genetic basis of disease resistance, genetic variability of pathogens in host tissue, in water, in soil and in potting media to minimize risks of disease in forest plantations.

#### Antonio Carlos da Gama-Rodrigues

Ph.D. Modeling the Dynamics of Phosphorus in Soil at the University of Florida. Fellow of CNPq productivity, full Professor at the Universidade Estadual do Norte Fluminense Darcy Ribeiro. Works in Agronomy, Soil Science, with emphasis on "balance and nutrient cycling and modeling of nutrient dynamics in agroforestry and forestry". Assistant Editor of the Journal of Soil Science, Editor-guest (Guest Editor) of Agroforestry Systems and Applied and Environmental Soil Science. Member of the Brazilian Society of Soil Science and the Brazilian Society of Agroforestry.

#### Alexander (Zander) Myburg

He is an Associate Professor in the Department of Genetics at the University of Pretoria. His research program in the Forestry and Agricultural Biotechnology Institute (FABI) focuses on the genomics and molecular genetics of wood formation in Eucalyptus trees. In particular, his research has focused on the transcriptional regulation of cellulose biosynthesis. By analyzing the promoters of primary and secondary cell wall associated cellulose synthase (CesA) genes of Eucalyptus with orthologous promoters in Arabidopsis and Populus his group has identified conserved cis-regulatory elements that may underlie the tissue-specific expression of the CesA genes (Creux et al. 2008). This work has been expanded to include other upstream components of the transcriptional network controlling cellulose biosynthesis (Creux, Hussey et al. unpublished), genomics analysis of diurnal variation in gene expression during wood formation (Solomon et al. 2010) and whole-transcriptome sequencing of a range of xylogenic and non-xylogenic tissues using Illumina mRNA-Seq analysis (Mizrachi et al. submitted). Zander is currently serving as President of the South African Genetics Society (SAGS) and he coordinates the International Eucalyptus Genome Network (EUCAGEN). He is also the lead investigator of the US Department of Energy (DOE) - Joint Genome Institute (JGI) project to sequence the Eucalyptus grandis genome.

#### Ana Gabriela Monnerat Carvalho Bassa

She has a Forest Engineer degree from, Federal University of Viçosa (1999), Masters in Forestry Science, University of São Paulo (2007) and a post Graduation in Pulp and Paper Technology, Federal University of Viçosa (2003). She worked as a research scientist for VCP, being responsible for the company's wood property studies for the breeding program and also for the wood supply at the mills (2000-2010). While in the research area she worked also with pulp and paper mill waste treatment and implemented a tree farmer program in one of the company's site. In 2010 she became a Research and Product Development Manager for ArborGen, where her job is focused in developing biotechnology. Throughout her career she published many scientific papers in conferences and reputed journals.







#### Andre Giacini de Freitas

He is a forester by training, has extensive background in forest certification. Prior to his current role as FSC Executive Director, Mr. de Freitas served as FSC Head of Operations and FSC Head of Policy and Standards. Previously, he managed Imaflora (a Brazilian NGO and SmartWood affiliate), developed the social and environmental policy for Rabobank in Brazil, and worked as regional forest coordinator for forest workers unions in Latin America.

#### **Brad Potts**

Professor of Forest Genetics in the School of Plant Science at the University of Tasmania. He is a program manager in the Cooperative Research Center for Forestry where he also leads their Biodiversity research project. He specializes on the genetics of eucalypts, and is well known for his collaborative work with both molecular and quantitative geneticists. He has undertaken extensive research on reproductive biology, hybridization and breeding, focusing on the temperate plantation species Eucalyptus globulus. He has published 199 refereed journal articles and 13 book chapters on eucalypts. In 2006 he was awarded a Doctorate of Science for this work and in 2009 was awarded the Royal Society of New South Wales' 2008 Clarke Medal for distinguished work in a natural science done in Australia and its Territories.

#### Carlos Frederico Wilcken

Undergraduation in Agronomy from Sao Paulo State University - UNESP (1986), master degree (1991) and Doctorate degree (1997) in Sciences - Entomology from ESALQ / University of São Paulo - USP. Carlos Wilcken was Coordinator of Graduation Program in Plant Protection (2002-2004 and 2007-2010) and is Scientific coordinator of Forest Protection Program (PROTEF) in IPEF (Forest Studies and Researches Institute). He has experience in Agronomy, acting on the following subjects: Forest Protection, Plant protection, Eucalyptus, insect forest pest management and biological control of forest pests.

#### Cesar Santana

Forest Engineer (2002) at the Federal University of Viçosa (UFV), Masters in Forest Management at Federal University of Paraná (UFPR) in 2008. He is currently doctoral student in Forest Management at UFPR. He is coordinator of planning and research forest at Klabin S/A – Paraná and coordinated the forest planning of Boise Cascade of Brazil in Rio Grande do Sul between 2007 and 2009, where he also worked in the silviculture and management activities. He was Forest planning expert from Stora Enso Arapoti Agricultural Projects in 2006/2007 and planning engineer and measuring forest from International Paper of Brazil (Arapoti unit) between 2004/2006. Began career as a trainee from International Paper in February 2003, where he developed activities in all units.

10) and is Scientific coordinator of Forest Protection dies and Researches Institute). He has experience in bjects: Forest Protection, Plant protection, Eucalypand biological control of forest pests.







## IUFRO 2011, Brazil | Improvement and Culture of Eucalypts xiii

#### Daniel Saloni

Ph. D Wood Science from Department of Wood and Paper Science, Assistant professor in the Department of Forest Biomaterials at North Carolina State University. Master's degree in Integrated Manufacturing System Engineering at North Carolina State University. He also obtained a bachelor's degree in Industrial Engineering and a Master's degree in Project Management at Andres Bello Catholic University. Currently a chair of Milling and Machining group of the Forest Products Society. Specialized in the area of Manufacturing and process improvement for the forest products industry. He has published various papers and participated in several conferences on biomass supply chain, techno-economical analysis on energy densification (briquettes and pellets). His areas of interest include life cycle analysis, supply chain management for biomass and bioenergy, and process improvement.

#### David Bush

He is a research fellow in the Department of Forest and Ecosystem Science at the University of Melbourne, Australia. He obtained a Bachelor of Science degree (2000) and PhD (2004) from the Australian National University before spending the next six years at the University of Melbourne. His research focuses on how changes in forest structure and species composition influence plant interactions, growth dynamics, and water, carbon and nutrient cycles.

#### David Ian Forrester

Principiante He is a Forest Scientist who has worked with Australia's Commonwealth Scientific Industrial Research Organisation (CSIRO) since 2001. He is the Leader of the Australian Tree Seed Centre which is a repository of documented wild and genetically improved seed of Australian tree taxa. His main research interests are eucalypt domestication and breeding and forest genetic resource exchange.

#### Don White

Leading forest ecophysiologist based in Western Australia. He is the leader of a Forest Systems group (CSIRO) and Forests and Water program (CRC for Forestry). His long term research has focussed on understanding forest functions in response to environmental changes, especially those impacting on water availability, and how to manage the ecosystem for sustained production of wood and non-wood values from planted forests. His work with his team also extends to managing drought and salinity prone landscapes using woody perennials. Don's collective contributions have advanced our understanding of key processes determining water use efficiency in eucalypts plantations, and he has developed judicious ways for managing drought risk and productivity, in partnership with the industry. He is a strong advocate for focussing research from single leaf to landscape level processes and delivering decision making tools for mangers at appropriate spatial scales.









#### Eckehard G. Brockerhoff

He is a forest entomologist and ecologist with Scion (the New Zealand Forest Research Institute). He studied in Germany and Canada and received his Ph.D. from the University of Toronto. His research interests span from biological invasions to forest biodiversity conservation, particularly in the context of plantation forestry. For more than 10 years he has been leading several research projects on biodiversity and conservation matters relevant to plantation forests in New Zealand and elsewhere. This included studies on successions of vascular plants, insects and birds, relationships with forestry activities, as well as the occurrence and management of threatened and endangered species. Along with several colleagues from around the world he has conducted a review of the effects of plantation forestry on biodiversity, under the umbrella of IUFRO, which resulted in numerous publications. He has also been intrigued by the role of forest biodiversity in ecosystem functioning and the provision of ecosystem goods and services. Eckehard serves on the editorial boards of Biodiversity and Conservation and several other journals, he is the coordinator of IUFRO's Forest Entomology Research Group, and a past president of the Entomological Society of New Zealand.



#### **Edival Zauza**

Forest Engineer, Federal University of Viçosa (1997), Masters in Plant Pathology, Federal University of Viçosa (2000) and Ph.D. in Plant Pathology, Federal University of Viçosa (2007). Has experience in the area of Forestry, acting on the following topics: disease resistance, forest protection and eucalyptus breeding. In the private sector acted as Coordinator of Forest Technology in the Stora Enso Group (Rio Grande do Sul), where he developed management activities of projects and experiments in forest protection, plant breeding, biotechnology, soil and nutrition. Currently, is part of the team o researchers at Suzano Pulp and Paper, with works in the area of eucalyptus breeding and forest protection.



#### Fernando Dalla Tea

Forest and Agronomist Engineer (1983), MSc.School of Forest Resources and Conservation, Universidad de Florida.Research on southern pines productivity as affected by nutrition and weed competition. Currently he is work in Forest Argentina SA with silvicultural operations.

#### Gabriel Dehon S. P. Rezende

Worked as a research scientist for Aracruz Celulose S. A., being responsible for the company's eucalypt breeding program (1996-2003). In 2004 he was moved to the Forest Technology Manager position in the same company. Up to 2008 he managed a qualified group of scientists working in about 50 forest research projects related to traditional breeding, biotechnology, plant propagation, soil and nutrition, forest protection and ecophysiology. In 2008 he accepted a professional challenge in Portugal, by assuming the Forest Research Director position in RAIZ Institute, belonging to PortucelSoporcel group. In this position (current) his mission is to manage forest research projects in the key areas previously mentioned, and also to contribute to forest business prospecting projects in different countries of the southern hemisphere. Throughout his career published scientific papers in reputed journals and took part in different qualifying programs and conferences for foresters and managers.





#### Jaime Rodriguez

Forest Engineer, Universidad Austral de Chile, and has a Diploma in Business Management from Universidad Adolfo Ibáñez. At the beginning of his carrer he worked for several research projects at Universidad Austral de Chile and is the co-author of various articles. Later he worked for Mininco Timber company dedicated to plantations, Forestal y Agrícola Monte Águila S.A and presently he leads forest operations in Masisa S.A. timber company, working mainly in the management of forest plantations and to a less extent in native forests. His main areas of expertise are strategic planning, growth and yield modeling, forest inventory, Project evaluation, intensive silviculture of forest plantations and forest research and development.



#### Jean-Pierre Bouillet

He is a Cirad scientist, currently visiting professor at the University of Sao-Paulo (USP-Esalq) in Brazil, in charge of research on the silviculture of mixed-species Eucalyptus plantations. He obtained his Diploma (1984) and PhD (1993) in Forest Sciences from Engref, Nancy (France). In 1985-1986 he worked in CTFT (French Guyana) on silviculture and breeding of forest plantation species. From 1986 to 1995 he worked in DRFP-FOFIFA (Madagascar) when he was in charge of research programs on the silviculture of commercial pine plantations (100,000 ha) and eucalyptus coppices managed by smallholders. Thereafter he was during 6 years the director of UR2PI (Congo) devoted to the productivity of eucalypt commercial plantations, and was the head of the silviculture and environment research program. From 2002 to 2008 he worked as senior scientist in Ciradforêt (France) and was the head of research unit on the functioning and the management of tree-based tropical ecosystems. He has published over 35 peer-reviewed journal articles and more than 200 scientific documents.



#### José Tarcísio Lima

He has a degree in forest engineering from Universidade Federal de Viçosa (1979), m.s. in Forest Science from Universidade Federal de Viçosa (1983), specialization in Wood Industrial Machinery by Industrial Research Institute (Japan, 1987) and PhD in Forest Science-University of Wales Bangor (1999). Currently is Associate Professor III da Universidade Federal de Lavras, where he was the coordination of the graduate program in science and technology of wood. Has experience in the area of forest resources and forest engineering, with emphasis on Physico-mechanical properties of wood and Wood Processing, mainly with wood of Eucalyptus (density, tension, growth of reaction wood, sawmill and drying). Coordinate or participate in various research projects. Coordinated the Brazilian part of an international cooperation agreement-funded Européia (Alfa program). Currently, coordinates cooperation agreement with the CIRAD Montpelier, funded by Vallourec & Mannesmann.



#### Mario Tomazello Filho

He has a degree in Agronomy from the University of São Paulo (1972), MSc. in Agronomy (Phyto) from the University of São Paulo (1975), PhD in Agronomy (Phyto) from the University of São Paulo (1980) and Professor (2006) by the Department of Forest Sciences of ESALQ/USP. He is currently Professor of the Department of forest science of the Scola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo. Is a visiting Professor at the University of Cuyo-Argentina and the Universidad Nacional Agraria La Molina-Peru. Has experience in the area of forest resources and forest engineering, with emphasis on Anatomy and identification of forest products, mainly in the subjects of Anatomy and identification of Woods, dendrochronology, Dendrology, x-ray densitometry applied in the study of wood and tree ring growth. Was coordinator of the graduate program in Forest Sciences (1990-91) and in Wood Science and technology (196-97, 1998-99). Coordinator of the graduate program in forest resources ESALQ/USP.



#### Mark Adams

He received his B.Sc. Honors and PhD From the University of Melbourne. Mark is currently Professor and Dean of the Faculty of Agriculture at the University of Sydney. He has held Professorial appointments at the University of Western Australia, the University of Melbourne, and most recently at UNSW. Mark publishes widely with a focus on sustainability and biogeochemistry of natural and managed ecosystems. His published work includes more than 150 peer-reviewed journal articles and book chapters, a major text on nutrition of eucalypts, and many commissioned reports for governments and industries. He led the first state-based assessment of forest carbon stocks, conducted for the then State Electricity Commission of Victoria and published in the Australian Journal of Botany in 1992. He was also responsible for the carbon component of the assessment of Ecologically Sustainable Forest Management for the WA State Government as part of expert panels between 1998 and 2003.



#### Michael Wingfield

He was born in South Africa where he obtained his early education (B.Sc. Hons, Natal; M.Sc. Stellenbosch). In 1983, he completed PhD. in Plant Pathology at the University of Minnesota, specialising in forest pathology and forest entomology. He returned to South Africa to establish the first formal, forest pathology programme in the country, required to serve a rapidly expanding plantation industry. In 1988 he became a full professor and member of Senate at the University of the Free State and together with industrial partners, established the Tree Protection Co-operative Programme (TPCP). This is now one of the best-recognized tree protection groups in the world. In 1998, he joined the University of Pretoria as Professor and member of Senate, specifically tasked with establishing the Forestry and Agricultural Biotechnology Institute (FABI). This Institute now houses the TPCP and other internationally, recognized plant biotechnology programmes (http:// fabinet.up.ac.za) and was honored in 2004 by being selected by the Department of Science and Technology to house one of the first six Centers of Excellence (CoE in Tree Health Biotechnology; CTHB) in South Africa. He is an alumnus of the Harvard Business School Advanced Management Programme, AMP175.



#### Michael Ryan

My primary research interest is understanding what controls ecosystem metabolism-the exchange of carbon and water between forests and the atmosphere. I'm interested in understanding how changes in climate, land use, forest age, and disturbance will affect forest productivity and the role of forests in the global carbon cycle. Within this area, I've focused on understanding what controls changes in productivity and carbon cycling with stand development, the role of plant respiration in controlling productivity, ecosystem respiration, carbon and nitrogen interactions, and decomposition of soil carbon.



#### Nuno M.G. Borralho

He is currently Forestry Consultant in Tree Breeding and Genetics. He has been involved in several Research and Operational breeding projects in eucalypts, pines and teak, in Europe, South America and Australia. Key areas include economic and organizational aspects of breeding, measurement strategies and statistical procedures for estimation of breeding values and genetic parameters. Recently he has been given in a number of short courses in forest genetics and breeding in Europe and South America. Research Associate of Centro de Estudos Florestais (CEF), a Research Unit from the Technical University of Lisbon, and coordinator of Tree Genetics at the Instituto de Investigação Científico Tropical (IICP), in Portugal.



xvi

## IUFRO 2011, Brazil | Improvement and Culture of Eucalypts xvii

#### Peter Dye

He obtained his tertiary qualifications at the University of the Witwatersrand, Johannesburg, South Africa. His MSc was based on a study of plant species distributions on sodic soils in Zimbabwe, while his PhD was concerned with modeling rangeland grass production at Matopos Research Station near Bulawayo, Zimbabwe. He subsequently moved to South Africa when he joined the South African Forestry Research Institute, and researched forest hydrology in the eastern forest plantations of Mpumalanga province. This focus broadened to a variety of land use hydrology studies when the Institute was taken over by the South African Council for Scientific and Industrial Research (CSIR). Currently, Peter is a researcher in the Ecological Engineering and Phytotechnologies Programme within the School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa. His work involves investigation of the effectiveness of trees in the containment of contaminated mine seepage water in the vicinity of gold tailings dams situated in the Witwatersrand goldfields.

#### Philip Smethurst

He first joined CSIRO in 1984 in Mount Gambier, South Australia, with the Division of Forestry. He focused on organic matter and nitrogen dynamics in pine plantations. Between 1989 and 1992 he completed a Doctorate in soil and water science at the University of Florida, USA, during which his research focused on the mathematical modeling of phosphorus and potassium uptake by competing roots of grasses and pine seedlings. In 1992 Dr Smethurst rejoined CSIRO, in Hobart, Tasmania, Australia, to work on soil and nutrient management of eucalypt plantations. In 2004, while in the same position, he started working with phosphorus fertilizer management in intensive dairy systems, and the water issues associated with the placement of forest plantations in mixed rural landscapes that mainly contain pastures, forest plantations, and native forests. These types of projects remain his main focus.

#### Robert Hubbard

He primary research interests focus on plant water relations and the role plants play in regulating hydrologic processes. I am particularly interested in how changes in stand age and species composition affect stream flow at watershed scales. Other research and interests focus on the controls and mechanisms of stomatal behavior, environmental and physiological limits to plant water use, understanding the role of hydraulic constraints in determining tree and forest productivity, and understanding how changes in nutrition affects stand level transpiration.







#### **Rod Griffin**

He is an Honorary Research Associate at the School of Plant Science, University of Tasmania. He is also Managing Director of Australian based consulting company Griffin Tree Improvement Pty. Ltd. and a partner in the Chilean company Seed Production Technologies Ltd. For 26 years he worked for the CSIRO researching reproductive biology and breeding systems of both pines and eucalypts. He then moved to UK to manage tree improvement and supporting research for the worldwide portfolio of companies owned by Shell International Forestry Ltd. In 2002 he returned to Australia to take up the positions of Director of the Hobart based CRC for Sustainable Production Forestryand CEO of its successor CRC for Forestry, which he held for 5 years. This wide experience of research management and applied forest science formed the platform for his current consulting businesses which provide tree improvement support for companies in South America and Asia growing both Eucalypt and Acacia plantations.

#### Sadanandan Nambiar

Dr. Sci. – Highest Distinction, University of Ghent, Belgium, M. Sc. – University of Madras, India, B.Sc. – University of Madras, India. In CSIRO he was Chief Research Scientist in 1989 which I held until retirement.





#### Silvio F. B. Ferraz

He completed a degree in forest engineering from University of São Paulo in 1998. Ph.D in forest resources, University of São Paulo in 2004. Did post-doctoral work at the University of São Paulo in 2005. He was professor of Ecology Department of Biosciences Institute-UNESP Rio Claro from 2006-2008. Since 2009 he is a professor of the Department of Forest Sciences/ESALQ/USP. His research focuses on watershed management, GIS, land-scape ecology and forest hydrological modeling with emphasis on biodiversity and water conservation planning.

#### Teotônio Francisco de Assis

He is a Forest Engineer and MSc in Genetics and Plant Breeding from the Federal University of Viçosa. During 11 years he was in charge of a Eucalyptus breeding programs at Acesita Energetica, a steel industry, when he developed novel Eucalyptus hybrid combinations for high wood density. After a passage at Bioplanta, where he started working on mini-cutting technologies, he joined Riocell, where he consolidated his innovative developments of hybrid combinations and propagation technologies. He pioneered the micro-cutting propagation system an intensive cloning method that revolutionized commercial scale Eucalyptus clonal forestry worldwide. At Klabin and Aracruz he optimized indoor Eucalyptus orchards, early flower induction methods, super-intensive controlled pollination techniques and clonal propagation systems for loblolly pine. Since 2005, he is an independent consultant for companies in Brazil, Chile and Uruguay working with Eucalyptus, Pinus, and Acacia mearnsii. Since 2006 he teaches in the International Course of Forest Tree Breeding and Biotechnology held by the University of Concepcion and NC State University. Besides being the "father" of the micro-cutting technology, he is also recognized as the "architect" of the introgression of E. globulus in tropical eucalypts, a trend that is currently reshaping industrial forests in Brazil with a new wave of clones displaying radically improved wood quality for pulp, paper and energy.

#### **Trevor Booth**

He led the Natural Ecosystems Theme in CSIRO's Climate Adaptation Flagship (CAF) for three years until March 2011, when he returned to his own forestry research. His early work at CSIRO was concerned with developing and applying new bioclimatic analysis methods, particularly to assist the introduction of eucalypt species outside Australia. He led the Australian Tree Resources Program, including the Australian Tree Seed Centre, for four years in the early 1990s. He wrote his first paper on climate change in 1987 and contributed to the early work (AR2) of the Intergovernmental Panel on Climate Change (IPCC). While leading the Natural Ecosystems theme Booth maintained an interest in forestry research contributing to a major 2009 IUFRO report on 'Adaptation of Forests and People to Climate Change – A Global Assessment Report' as well as a chapter on commercial forestry for a 2010 book on 'Adapting Agriculture to Climate Change' in Australia.

#### Yann Nouvellon

He is a CIRAD ecophysiologist, based at USP (University of Sao Paulo) since 2006 (department of atmospheric sciences; and department of forest sciences). His research has focused on soil-plant-atmosphere interactions, carbon and water cycles in tropical plantation ecosystems (Eucalyptus plantations in Congo, and monospecific/mixed-species Eucalyptus grandis/Acacia mangium plantations in Brazil since 2006): he is currently leading or co-leading projects that aim at measuring and modeling water vapor and CO2 exchanges between plantations and the atmosphere (eddy-covariance), and understanding of key processes controlling gross primary production, carbon allocations, wood production, resource-use and resource-use efficiencies over the lengths of rotations. Measurements obtained at different scales (from the leaf to the canopy) are used to developed processbased forest models that are applied at scales relevant to the managers using satellite data (MODIS), soil maps, and meteorological data.









## CONTENTS

INVITED SPEAKERS	01
A re-appraisal of eucalyptus plantations from the point of view of its quality-cost competitiveness and how to move them to the next level – Nuno M.G. Borralho	03
Eucalypt plantations and climate change – Trevor H. Booth	04
Site and stand management: theory and practice in Australian plantation forests – Philip Smethurst	07
An intensive eucalypt silviculture to cope with environmental stresses in Argentina – Fernando Dalla Tea, Federico Larocca	11
Eucalyptus production in temperate regions – Jaime Rodríguez Schäfer	15
Drought responses of Eucalypts in plantations – from physiology to management – Donald A. White, Anthony P. O'Grady, Michael Battaglia, Jody L. Bruce, Elizabeth A. Pinkard	17
Silviculture and management of <i>Eucalyptus</i> plantations in the frost occurrence areas of southern brazil – James Stahl, Cesar J. O. Santana, Djalma M. Chaves	20
Plantation eucalypt species: recent trends in testing and planting – David Bush	23
Advances in eucalypt genetics: from genes to ecosystems – Brad Potts, João Costa e Silva, Corey Hudson, Rebecca Jones, Matthew Hamilton, Greg Dutkowski, Julianne O'Reilly-Wapstra, Jules Freeman, Dorothy Steane, Joseph Bailey, Jennifer Schweitzer, Thomas Whitham, René Vaillancourt	26
Twenty years of <i>Eucalyptus</i> molecular breeding: from discrete marker-trait associations to whole-genome prediction of complex traits – D. Grattapaglia, M.D.V. Resende	33
Environmental stress and growth of eucalypts – Mark A. Adams	37
Measuring, modelling and managing water-use by <i>Eucalyptus</i> plantations: a South African perspective – Peter Dye	40
Factors controlling carbon and water balances on fast growing Eucalyptus plantations – Y. Nouvellon, J.L. Stape, G. Le Maire, D. Epron, J.L.M. Gonçalves, J.M. Bonnefond, O. Campoe, R. Loos, J.P. Bouillet, J.P. Laclau	43
Unsolved problems in whole-tree physiology: how to make progress for clonal Eucalyptus forests – Michael G Ryan	47
Well established as well as new technologies provide options to reduce increasing insect pest and pathogen damage to eucalypt plantations – Michael J. Wingfield, Jolanda Roux, Bernard Slippers, Brett Hurley, Jeff Garnas, Brenda Wingfield	49
Minimizing disease risks in eucalyptus plantations – Acelino Couto Alfenas, Lúcio Mauro da Silva Guimarães, Rodrigo Neves Graça, Rafael Ferreira Alfenas, Talyta Galafassi Zaperlon, Carla Cristina Gonçalves Rosado, Edival Ângelo Valverde Zauza, Reginaldo Gonçalves María, Marcolo Magalhãos Coutinho	51
Gonçalves Mafia, Marcelo Magalhães Coutinho Breeding Eucalyptus for insect resistance – Carlos F. Wilcken	51

Strategy of <i>Eucalyptus</i> breeding for disease resistance – Edival A. V. Zauza, Acelino Couto Alfenas, Lúcio Mauro da Silva Guimarães, João Flávio da Silva	55
Clonal Forestry – is it always the best deployment option? – Rod Griffin	58
Hybridization and cloning eucalyptus – Teotônio Francisco de Assis	59
State of the art of eucalypts breeding in the world – Gabriel Dehon S. P. Rezende	62
Tree growth and stand production in mixed-species plantations of <i>Eucalyptus</i> and <i>Acacia</i> mangium in Brazil and Congo – J.P. Bouillet, J.L.M. Gonçalves, J.L. Gava, F.P. Leite, R. Hakamada, L. Mareschal, A. Mabiala, F. Tardy, J. Levillain, M. Voigtlaender, Y. Nouvellon, J.P. Laclau	64
Quantifying the effects of species interactions in mixed species plantations – David I. Forrester, Jürgen Bauhus	68
Soil Organic Carbon Dynamics in Eucalyptus Silvopasture in Brazil: A Review – A.C. Gama- Rodrigues, E.F. Gama-Rodrigues	71
Coverting Eucalyptus biomass into ethanol – Daniel Saloni	74
Effect of climate and soil on the quality of Eucalyptus wood – Mário Tomazello Filho .	75
Technologies and uses of eucalyptus as raw-material for solid Products – José Tarcísio Lima, Paulo Fernando Trugilho, José Reinaldo Moreira da Silva	76
The genome of Eucalyptus grandis: A resource for genetic improvement of eucalypts – Alexander A. Myburg, Dario Grattapaglia, Gerald A. Tuskan	79
<b>Transgenic Technologies for <i>Eucalyptus</i></b> – Ana Gabriela M.C. Bassa, Maud Hinchee, William Rottmann, Brian Kwan, Michael Cunningham	82
Biosafety, environmental and health risk assessments of genetically modified eucalypts – Giancarlo Pasquali, Rochele Patrícia Kirch, Leandro Vieira Astarita, Eliane Romanatto Santarém, Karen A. de Freitas, Juliana Gerhardt, Eduardo Cassel, Aline M. Lucas, Betina Blochtein, Annelise Rosa, Mariana Zaniol, Daniela Loose, Sídia Witter, Rogério Margis, Joseane B. de Carvalho, Rafael Roesler, Maria Noêmia Martins de Lima	83
Forestry for rural development and poverty alleviation in the tropics : can we do better? – Sadanandan Nambiar	85
Role of plantation forests in biodiversity conservation and the provision of biodiversity- related ecosystem goods and services – Eckehard G. Brockerhoff, Hervé Jactel, John A. Parrotta	89
Trends in FSC forest certification – André G. Freitas	93
Forest plantations landscape management for water conservation – Silvio F. B. Ferraz, Walter de Paula Lima, Carolina Bozetti Rodrigues	96

## **ORAL SESSION**

1. FOREST ESTABLISHMENT AND REGENERATION	101
Harvest residue management in <i>Eucalyptus</i> plantations established on a tropical sandy soil largely influences soil CO <sub>2</sub> effluxes and tree growth without modifying C mineralization in the mineral soil – A. Versini, J.P. Laclau, J. Ranger, D. Epron, A. Kinana,	
L. Mareschal, Y. Nouvellon	103
Monitoring silvicultural quality using uniformity coefficients – Rodrigo Hakamada, Jose Luiz Stape, Cristiane Lemos	108
Thinning, pruning and fertiliser application interact to influence the growth, structure and resource-use of <i>Eucalyptus nitens</i> plantations – David I. Forrester, John J. Collopy, Christopher L. Beadle, Charles R. Warren, Thomas G. Baker	112
Restoration Treatments for <i>Eucalyptus</i> Plantations Infested by Introduced <i>Paspalum</i> to the Development of Native Forests in an Australian National Park (Ph.D. Thesis) – Benjamín Villa-Castillo	116
Comparing the accuracy of a process-based (3PG), a hybrid (Glob3PG) and an empirical (Globulus 3.0) model against long term permanent plot data from <i>Eucalyptus globulus</i> Labill. – Josep Crous, Susana Barreiro, José Tomé, Margarida Tomé	119
Evaluation of 3PG and Glob3PG models to predict growth of Eucalyptus globulus coppice stands – Susana Barreiro, Josep Crous, João Palma, José Tomé, Margarida Tomé	122
Harvesting impact on forest soil productivity. The "Extended Use Sampler", a new tool to estimate it – Federico Larocca, Roberto Raúl Filgueira, Fernando Dalla Tea	125
Developmental Homeostasis in the Leaves of Eucalyptus camaldulensis (Dehnhardt) – Sindhu Veerendra H. C., Sumita Sircar, Shilpa Gautam	129
2. ENVIRONMENTAL STRESS	133
Carbon allocation and leaf gas exchange of <i>Eucalyptus</i> hybrid clones under different climates – Rodolfo Araujo Loos, Jupiter Israel Muro Abad, Ricardo Miguel Penchel Filho, Tatiana da Silva Lopes	135
Environmental drivers of eucalypt productivity – J.T. Brawner, D.J. Lee Mark Hunt, Auro Almeida	139
Drought tolerant <i>Eucalyptus globulus</i> provenances have thick bark, high basic density and grow poorly on wet sites – Gregory W. Dutkowski, Brad M. Potts	143
Substitution of K+ by Na+ in <i>Eucalyptus grandis</i> plantations: consequences on the biological cycle of nutrients – J.P. Laclau, C.R. Sette Jr, J.C.R. Almeida, J.L.M. Gonçalves, J.S.R. Delgado, J.P. Bouillet, Y. Nouvellon, P.R.F. Battie-Laclau, R.M. Moreira, J. Ranger	149
Phenology of eucalyptus clones and hybrids growth under contrasting water environments – Rafael A. Rubilar, Manuel A. Acevedo, Matías P. Pincheira, León Bravo, Veronica Emhart, Oscar Mardones, J. Luis Stape, Thomas Fox	153
Mapping of the Brazil eucalyptus potential productivity – Clayton Alcarde Alvares, José Luiz Stape, José Leonardo de Moraes Gonçalves	156

Drought response at age 9 years of 5 <i>Eucalyptus</i> species planted at 3 different densities at Highflats plantation, South Africa – Jacob Crous, Louisa Burger, Giovanni Sale	160
Clonal variability for water use efficiency and carbon isotope discrimination (D <sup>13</sup> C) in selected clones of a few Eucalyptus species – B. Mohan Raju, V. Kumar, T.N. Narasimhamurthy, M.S. Sheshshaayee, S.D. Verryn, Nuveshen Naidoo, H. Bindumadhava, Mohan Varghese, K.T. Prasanna, T.G. Prasad, M. Udayakumar	163
Estimating potential productivity and identifying the factors affecting growth and growth efficiency in forest plantations in eucalyptus stands in Venezuela – Omar Carrero, José Stape, Lee Allen, María Arrevillaga, Mario Ladeira	167
Growth response of eucalyptus grandis in colombia to fertilization with various rates and frequencies of nitrogen – Thomas Fox, Rafael Rubilar	171
3. GENETICS	173
Genotype x environment interactions for growth and wood traits for eucalyptus hybrids – Gilles Chaix, M. Denis, J. Polidori, P. Vigneron, G. Makouanzi, Abdoulaye Diombokho, Sophie Nourissier, Jean Marc Bouvet	175
Plantation productivity of sixty-five taxa across eight ecosystems in north-eastern Australia – David J. Lee, Jeremy T. Brawner, Bruce W. Hogg, Roger Mederc David O. Osborne, John R. Huth	180
Recurrent Selection of F2 Genotypes based on GCA and Breeding Values for Improvement of yield in <i>Eucalyptus tereticornis</i> Smith Progeny trial – H.C. Sindhu Veerendra, Sumita Sircar	185
Relationship between non-additive and additive genetic variances from full-sib <i>Eucalyptus globulus</i> progeny trials: implications for deployment and breeding strategies – Edgardo L. Velilla, A. Rod Griffin, Verónica I. Emhart	188
Combining high-throughput sequencing and <i>in silico</i> prediction to identify miRNA in <i>E. globulus</i> xylem tissues formed upon gravitropic stimulation – Victor Carocha, Nuno D. Mendes, Andreia J. Amaral, Clara Graça, Joana Amado, Jorge Oliveira, Hélène San-Clemente, Hua Wang, Teresa Quilhó, José Carlos Rodrigues, Fernanda Bessa, Lucinda Neves, Clara Araújo, Jacqueline Grima-Pettenati, Ana Teresa Freitas, Jorge A. Paiva	190
Identification, phylogeny and tissue-specific expression of some members of multigene families involved the lignin biosynthesis in <i>Eucalyptus</i> – Victor Carocha, Marçal Soler, Mohammed Saidi, Hong Yu, Eduardo Camargo, Clara Graça, Hélène San-Clemente, Nathalie Ladouce, Pedro Fevereiro, Lucinda Neves, Clara Araújo, Hua Wang, Jorge Paiva, Jacqueline Grima-Pettenati	194
4. ECOPHYSIOLOGY AND HYDROLOGY	197
Estimating potential productivity of cold-tolerant <i>Eucalyptusbenthamii</i> in the Southestern US – Jose S. Alvarez, Jose L. Stape, Thomas Fox, Rafael Rubilar, Timothy Albaugh	199
Aboveground biomass in Eucalyptus grandis plantations fertilized with different sewage sludges – Alexandre de Vicente Ferraz, Paulo Henrique Müller da Silva, Fábio Poggiani	202

Ecophysiological processes and wood anatomy related to growth and drought resistance in genotypes of <i>Eucalyptus</i> grandis – N. Tesón, S. Monteoliva, J.A. Licata, M.E. Fernández	205
Soil water retention characteristics explain growth variability within Eucalyptus plantations: a modelling analysis – Claire Marsden, Yann Nouvellon, Jose-Luiz Stape, José-Leonardo de Moraes Gonçalves, Jean-Paul Laclau, Guerric le Maire	209
Quantifying water use and biomass production by forest and pasture at plot- to catchment-scales – Auro Almeida, Anders Siggins, Craig Baillie, Philip Smethurst	213
Predicting long term growing stock and carbon sequestration in Portuguese eucalyptus stands under different wood demand and climate scenarios – Margarida Tomé, Susana Barreiro, Josep Crous, Luís Fontes, João Palma, Sónia Palma, José Tomé	217
Using the remote sense technique to monitor Eucalyptus forest plantation in Brazil – Cristiane Lemos, Rodrigo Hakamada, Thiago Freitas, Jose Luiz Stape, Kevin Hall, Amanda Vergani, Israel Lima, Stephen Kinane	219
6. BREEDING	223
Cooperative eucalypt breeding program of nucleus population – PCPN/IPEF – Brazil – Edson Seizo Mori, Paulo Henrique Muller da Silva, Mario Luiz Teixeira de Moraes	225
The role of seed orchard factors in genetic gain and plantation yield of <i>Eucalyptus nitens</i> in South Africa – Tammy Swain, Steve Verryn	229
Evaluation of fertility and progeny performance in seedling and clonal seed orchards of Eucalyptus camaldulensis in southern India – M. Varghese, R. Kamalakannan, R. Mathew, P.G. Suraj, T. Arutselvan	232
Simultaneous Selection for Productivity, Stability and Adaptability in progenies of <i>Eucalyptus grandis</i> – Aline Cristina Miranda, Paulo Henrique Muller da Silva, Edson Seizo Mori, Mario Luiz Teixeira de Moraes, Alexandre Magno Sebbenn	236
Breeding E.tereticornis x E.grandis and E.tereticornis x E.alba dihybrids: From Seed Production to Macropropagation – B. Nagarajan, V.K.W. Bachpai, K.S. Venkatramanan, M. Ganesan	239
Evaluation of mini-cuttings as a propagation system for <i>Eucalyptus</i> hybrids – R.D. Naidu, N.B. Jones	243
Influence of stockplants management on cutting production and rooting efficiency of cold-tolerant <i>Eucalyptus grandis x nitens</i> hybrids in KwaZulu-Natal – M. Ziganira, I. Bertling, M.E.O. Mokotedi, F. Blakeway, B. Pollard, I. Thompson	246
Provenance Variation of <i>Eucalyptus urophylla</i> forPhysical and Chemical Wood Properties in Genetic Trials in FiveDifferent Countries – G.R. Hodge, W.S. Dvorak, J.A. Espinoza, W. Woodbridge	250
NIR spectral heritability: a promising tool for wood breeders – P.R.G. Hein, M. Denis, P.F. Trugilho, J.T. Lima, A.M. Rosado, G. Chaix	251

7. MIXED PLANTATIONS AND AGROFORESTRY	255
Belowground carbon allocation in mixed <i>Eucalyptus</i> – <i>Acacia</i> plantations: a comparison between Brazil and Congo – Daniel Epron, Yann Nouvellon, Louis Mareschal, Rildo Moreira e Moreira, Lydie-Stella Koutika, Blandine Geneste, Juan Sinforiano Delgado Rojas, Jean-Paul Laclau, José Leonardo de Moraes Gonçalves, Jean-Pierre Bouillet	257
Dynamics of soil N mineralization in mixed-species plantations of <i>Eucalyptus grandis</i> and <i>Acacia mangium</i> in southern Brazil – Maureen Voigtlaender, Jean-Paul Laclau, José Leonardo de Moraes Gonçalves, Marisa de Cássia Piccolo, José Luiz Gava, Fernando Palha Leite, Rodrigo Eiji Hakamada, Jean-Pierre Bouillet	260
8. WOOD TECHNOLOGY	265
Improving the pulp productivity of a eucalypt suited to challenging environments - Corymbia citriodora – J.T. Brawner, Roger Meder, D.J. Lee	267
Using NIR to assess of Kraft pulp yield in breeding trials: In-field sampling considerations – R. Meder, G.M. Downes, N. Ebdon, J.T. Brawner	271
Variation of wood stiffness and genetic control within eucalyptus hybrid stems from clonal plantations in Brazil – P.R.G. Hein, G. Chaix, A.M. Rosado, P.F. Trugilho, J.T. Lima	275
Wood Quality studies of ITC Bhadrachalam Clones of Eucalyptus – H.D. Kulkarni	278
Preliminary first global NIRS models to predict chemical properties of <i>Eucalyptus</i> woods – Gilles Chaix	294
9. BIOTECHNOLOGY	295
Genomic selection in eucalyptus breeding: assessing the gain per unit time in recurrent selection scheme for clone production – Marie Denis, Jean-Marc Bouvet	297
Towards elite <i>Eucalyptus dunnii</i> using conventional tree breeding applications and molecular technologies in South Africa – Andrea K. Louw, Elize C.L. Ade, Arnulf Kanzler	301
Plant regeneration from somatic embryogenesis in Eucalyptus globulus: current status and future perspectives – Glória Pinto, Clara Araujo, Conceição Santos, Lucinda Neves	303
Plant growth regulator choice and properties dictate the rooting ability and root physiology of <i>Eucalyptus</i> spp. clones in vitro – M. Nakhooda, M.P. Watt, D. Mycock	306
Treeforjoules, a Plant KBBE project to improve eucalypt and poplar wood properties for bioenergy – J. Grima-Pettenati, J.C. Leplé, J.M. Gion, L. Harvengt, M. Fladung, J. Puls, U. Schmitt, D. Meyer, B. Kamm, C. Araujo, J. Pinto Paiva, J. Rodrigues, G. Lopez, F.R. Canton, G. Gallardo, I. Allona, H. Sixto-Blanco	309
Cold-tolerance and initial growth of 38 Eucalyptus species in the Southeastern of the United States – Jose Luiz Stape, Thomas Fox, Tim Albaugh, Jose Alvarez, Rafael Rubilar	311

## **POSTER SESSION**

1. FOREST ESTABLISHMENT AND REGENERATION	313
Tree and stand growth for clonal E. urophylla x grandis over a 16 years following a range	
of fertilizer treatments in southern China – Shaoxiong Chen, Jiefeng Liu	315
Growth patterns in widely spaced clonal <i>Eucalyptus urophylla</i> × grandis plantations on different site classes up to age 16 years – Shaoxiong Chen	316
Growth and aboveground biomass of an <i>Eucalyptus regnans</i> stand, managed for high value timber production, located in the coast of Arauco, Biobío, Chile – Magdalena Lisboa, Jorge Cancino, Ruth Maturana, Fernando Muñoz	317
<b>Nutrient Export and Cycling at Harvest of <i>Eucalyptus dunnii</i> Maiden</b> – Alejandro González, Jorge Hernández, Amabelia Del Pino, Gimena Arrarte	320
Analysis of the economical viability of <i>Eucalyptus</i> spp. Coppice compared to replanting using genetically improved seedlings on different productivities sites in Minas Gerais State, Brazil – Antonio Carlos Ferraz Filho, José Roberto Soares Scolforo	323
Effect of container type on root architecture of Eucalyptusglobulus Cuttings – A. Filipa Rodrigues, L. Oliveira Neves, M. Sousa Louro, A. Kidelman Dantas, J. Majada Guijo, M. Clara Araujo	327
Genetic Diversity among different provenances of <i>Eucalyptus</i> spp with phenotypical markers base in greenhouses – Leonardo S. Souza, Ricardo F.C. Moreira, Tuany P.P. Costa, Teresa A.S. de Freitas, Andrea V.R. Mendonça, Mariana D.S. Fonseca, Thamara M. Lima, Jesus M.D. Mendez	329
Background on the growth and yield of <i>Eucalyptus globulus</i> coppice in chile, an opportunity for efficient forest management – Juan Carlos Pinilla S., María Paz Molina B., Mauricio Navarrete Torres	332
Planting of cuttings eucalyptus with biodegradable containers – José Junior, Fabrício Sebok, Marcello Pizzi, José Gonçalves	335
Production of cuttings in biodegradable containers – José Junior, Marcello Pizzi, Fabrício Sebok, José Gonçalves	340
Impact of mechanized logging on physical properties of forest soils – C.A.S. Morales, J.A. Sampietro, E.S. Lopes, J.M. Reichert, D. Oliveira, C.K. Rodrigues, B.P. Morales	344
Initial growth of Eucalyptus saligna smith in function of tillage and soil characteristics – Cedinara Arruda Santana Morales, Jean Alberto Sampietro, José Miguel Reichert, Bruno Pimentel Morales, Eloi Paulus, Rodrigo Luiz Ludwig	349
<i>Eucalyptus</i> seedlings <i>urophylla</i> in different concentrations of hydrogel and irrigation time – Adalberto Brito de Novaes, Glauce Taís de O. Sousa, Helane França Silva, Gileno	343
Brito de Azevedo	353

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2. ENVIRONMENTAL STRESS	357
Carbon isotope discrimination and differential drought tolerance in eucalypt clones – Flancer Novais Nunes, Ivo Ribeiro da Silva, Nairam Félix de Barros, Roberto Ferreira de Novais	359
Cold acclimation for plant cryopreservation: some physiological and biochemical responses of <i>Eucalyptus grandis</i> to different nonfreezing low temperatures and exposure periods – C.H. Ting, K. Padayachee, D.J. Mycock	364
Tools for early selection of drought tolerant eucalyptus – Hachemi Merouani, Maria Helena Almeida, José Alexandre Araújo, Mariana Matos Moreira, Filipe Costa e Silva, Helena Martins, Gabriel Dehon Rezende, João Santos Pereira	368
Evaluation of climate effects on Eucalyptus grandis Hill Ex Maiden provenances tests – Rosana Clara Victoria Higa, Antonio Rioyei Higa, Joel M Corrêa das Rosas, Marcos Silveira Wrege, Josiléa Accordi Zanata	369
Effects of saline stress over mineral nutrition on <i>Eucalyptus</i> seedlings – Andrea V.R. Mendonça, José G. de A. Carneiro, Teresa A.S. de Freitas, Leonardo S. Souza, Josival S. Souza, Jesus M.D. Mendez, Ricardo F.C. Moreira	374
Low temperatures risk analysis for eucalyptus introduction in south east us – Omar Carrero, Lee Allen, José Stape, José Alvarez	377
Standardization and optimization of core sampling procedure for carbon isotope analysis in eucalyptus and variation in carbon isotope ratios across species and growth conditions – B. Mohan Raju, Nuveshen Naidoo; M.S. Sheshshaayee, S.D. Verryn, R. Kamalkannan, H. Bindumadhava, Mohan Varghese, Cyndi Snedden, T.G. Prasad, M. Udayakumar	381
Inter-clonal variability in fine root distribution in very deep soil layers: a poorly explored criterion for tree breeding in regions prone to water stress – R.C. Pinheiro, J.C. Deus-Junior, I.A. Silva, R.M. Moreira, E.A. Silva, O. Campoe, Y. Nouvellon, J.L. Stape, J.L.M. Gonçalves, J.P. Bouillet, J.C. Pereira, J.P. Laclau	385
3. GENETICS	389
The influence of tree improvement and cultural practices on dry timber production of <i>Eucalyptus</i> pulpwood plantations over a rotation in temperate South Africa – Jacob Crous, Louisa Burger, Giovanni Sale, Wesley Naidoo	391
Performance of association genetics and genomic selection of wood physical traits in large populations of Canadian mature spruces – Jean Beaulieu, Trevor Doerksen, John MacKay, Jean Bousquet	395
Genetic variation for growth in a <i>Eucalyptus pellita</i> breeding population in Colombia – V. Nieto, N.M.G. Borralho, A.G. Gasca	397
Genetic diversity within and between subpopulations of Handroanthus heptaphyllus (vell.) mattos by microsatellite markers – Neide Tomita Mori, Mario Luiz Teixeira de Moraes, Caroline Midori Morita, Edson Seizo Mori	401
Estimates of genetic parameters for <i>Eucalyptus globulus</i> in Colombia – Víctor Nieto, Nuno Borralho, Martha Salas, Liliana Perafán, Jairo Silva, Mauricio Moreno	404

Did you ask for something small? The microRNAs power in a Eucalyptus tension world!         (the microEGo project) - Paiva, Jorge A. P.         40:
4. ECOPHYSIOLOGY AND HYDROLOGY
The effect of propagation methods on some growth and physiological characteristics of seed- and vegetatively propagated Eucalyptus varieties – Oscar Mokotedi, Paula Watt and Norman Pammenter
Interaction between rainfall and potassium availabilities on the cycles of C, H <sub>2</sub> O and nutrients in <i>Eucalyptus grandis</i> plantations. Preliminary results on tree growth. – J.C. Deus-Junior, R.C. Pinheiro, I.A. Silva, L. Mietton, P.R.F. Battie-Laclau, J.S.R. Delgado, R.M. Moreira, E.A. Silva, J.L.M. Gonçalves, J.P. Bouillet, Y. Nouvellon, J. Ranger, J.C. Pereira, J.P. Laclau
Improving the soil water content in the 3-PG model to estimate growth in Eucalyptusplantation - Cristiane Lemos, Jose Luiz Stape, Omar Carrero, Rodrigo Hakamada410
Project Nenus: a 3PG version that run in diary bases – Mariano Silva, Aristides Ribeiro 420
Photosynthetic parameters and Eucalyptus clones growth under different climatic conditions in Brazil – Aline Regina Vergani, Arthur Vrechi, Sérgio Ricardo Silva, Marina Shinkai Gentil, Antônio Natal Gonçalves, José Luiz Stape
Relating growth and physiological responses of an Eucalyptus clonal plantation to soilwater availability in northeastern Brazil – Arthur Vrechi, Helton Maycon Lourenço, AlineRegina Vergani, Sérgio Ricardo Silva, Marina Shinkai Gentil, José Luiz Stape424
Distribution of throughfall in an Eucalyptus dunnii plantation, Rosário do Sul, RS, Brazil       423         - Catarine Consensa       423
Gains with supplementary fertilization and weed control on the Eucalyptus yield –Ricardo Martins, Atus Lemos, Marcos Santana, Marcelo Rezende, Edival AngeloValverde Zauza430
Assessing the relationship between stem production and leaf area index (LAI) at canopy closure for <i>Eucalyptus</i> clones in Brazil – Eduardo Mattos, Estela Foltran, Otávio Campoe, José Luiz Stape, Jean Paul Laclau, Yann Nouvellon
Seasonal specific stream flow in Eucalyptus catchments in tropical region – Isis da SilvaFranco, Maureen Voigtlaender, Silvio Frosini de Barros Ferraz, Walter de Paula Lima,Carolina Bozetti Rodrigues430
Growth dominance and the effect of dominant trees to stand level productivity of anEucalyptus grandis plantation in Brazil – Otávio Campoe, José Luiz Stape, Jean PaulLaclau, Yann Nouvellon439
5. DISEASES AND PESTS
Assay on distribution, preferences and performances of <i>Phoracantha recurva</i> and <i>Phoracantha semipunctata</i> (Coleoptera: Cerambycidae) in various <i>Eucalyptus</i> species in Tunisia – Samir Dhahri, Mohamed Lahbib BenJamaa, Faouzia Charfi-cheikhrouha

and Francois Lieutier .....

447

Approaches to predicting current and future distributions of Puccinia psidii in SouthAmerica under climate change scenarios – N.B. Klopfenstein, J.W. Hanna, R.N. Graça,A.L. Ross-Davis, P.G. Cannon, A.C. Alfenas, M.S. Kim450
Comparing field screening of Eucalyptus for rust resistance with artificial infection in controlled conditions – Rodrigo Hakamada, Cristiane Lemos, José Teixeira, Simone Takahashi, Carla Garcia, Flávio Augusto, Adriano Almeida
Variability of the fungus Puccinia psidii Winter in commercial plantations of Eucalyptus         sp. – Karine Kettener, Julio César Santos Otto, Juliana Erika Teixeira, Edson Luiz Furtado,         Celso L Marino       458
6. BREEDING 459
Early growth performance and wood properties of <i>Eucalyptus</i> inter specific hybrids atCoastal and Inland sites in Southern India – Kamalakannan R., Suraj P.G., Arutselvan T.,Mohan Varghese461
A study on superior eucalypt species and provenances introduction for China's Central Sub-tropical area (Hunan Province) – Lin Mujiu
Genetic variability in Eucalyptus clones for rust Puccinia psidii resistance – CristianoBueno de Moraes, Cristiane de Pieri, Alexandre Martins Fragoso, João Grande, GláuciaUesugi, Maria Rita Silva Gilli Martins, André Carignato, Débora Palheta Rodrigues,Léo Zimback, Rildo Moreira Moreira, Edson Seizo Mori465
Genetic variability in eucalypt clones for frost tolerance – Cristiano Bueno de Moraes,Talitha Casella M. de Freitas, Gustavo B. Pieroni Léo Zimback, Edson Seizo Mori468
Comparison of methods for controlled pollination in hybrids Eucalyptus globulus Labili P. Molina, R. Sanhueza, J. Elizaul471
Effect of environmental conditions of mother plants in rooting of Eucalyptus globulusLabill. mini cuttings – M. Sousa Louro, L. Oliveira Neves, I. Mourão, L. Miguel Brito, F.Fôlgoa Baptista, J. Majada Guijo, Clara Araujo474
Development of species-specific markers to five Eucalyptus species – Maria C.P. Fuchs,Aletea Madacki, Karine Kettener, Tania M. Bortolotto, Shinitiro Oda, Celso L. Marino476
Establishment of barbatimão (Stryphnodendron adstringens mart) progeny trial – Enzo         Antonio Lecciolle Paganini, Edson Seizo Mori, Adriano Ballarin de Lima, Eliete Aparecida         Branco, Léo Zimback       480
Eucalyptus benthamii: considerations on the genetic improvement program carriedout by Embrapa and its partners in Brazil – Paulo Eduardo Telles dos Santos, EstefanoPaludzyszyn Filho, Luís Carlos Valtrin, Élson Luiz Tussolini
In vitro and ex vitro adventitious rooting of Eucalyptus benthamii – Gilvano EblingBrondani, Evandro Vagner Tambarussi, Francisco José Benedini Baccarin, José LuizStape, Antonio Natal Gonçalves, Marcilio de Almeida
Seed Source Variation in Growth Performance of Eucalyptus species Provenance cumProgeny Trials in South India – R. Seenivasan, P. Chezhian, G. Venkatesan, V. Prasath487
Genetic variation in growth, cold tolerance and coppicing in Eucalyptus dunnii trials inHunan – Li Bohai, Luo Jianzhong, Roger Arnold, Li Zhihui490

IUFRO 2011, Brazil | Improvement and Culture of Eucalypts xxix

7. MIXED PLANTATIONS AND AGROFORESTRY	491
Preliminary results on litter decomposition and soil microbial activity in <i>Eucalyptus grandis</i> and <i>Acacia mangium</i> plantations. Consistency with the Home Field Advantage theory? – Luciana Ruggiero Bachega, Daniel Bini, Marisa de Cássica Piccolo, Elke Jurandy Bran Nogueira Cardoso, José Leornardo Gonçalves, Jean Paul Laclau, Jean Pierre Bouillet	493
Leaf area index and eucalypt clone coppice growth in agroforestry systems in the cerrado region in Brazil – Geraldo G. Reis, Maria G.F. Reis, Hortênsia N.S. Lopes, Hélio G. Leite, Raul C.N. Melido, Felippe C. Souza, Ranieri R. Paula, Carlos H.R. Oliveira, Frederico F. Alves,	499
Amounts of structural and non structural carbon and nitrogen in <i>Eucalyptus</i> , grown in monoculture and in association with <i>Acacia</i> as nitrogen fixing species – Morgane Pluchon, Dominique Gérant, Daniel Epron	502
8. WOOD TECHNOLOGY	503
Near infrared spectroscopy (NIRS) as a tool to discriminate the source <i>Eucalyptus</i> species of solid wood samples – Jez W.B. Braga, Tereza C.M. Pastore, Dario Grattapaglia; Ana C. dos S. Azevedo, Ana L.M. da Costa; Layse E. dos S. Guimarães, Vera T.R. Coradin, Patrick L.A. Rousset, Elisabeth K. Takahashi, José L. Lima	505
Study of industrially produced chip dimensions using descriptive statistics – João Paulo Grande, Fábio Monteiro Leite de Souza, Cristiano Bueno de Moraes, Cláudio Angeli Sansígolo	508
Evaluation of wood surface roughness of eucalyptus species submitted to cutterhead rotation – Camila Santos Doubek Lopes, Adriana Maria Nolasco, Mário Tomazello Filho	512
Variation in pulp yield and paper properties of ITC Bhadrachalam clones of Eucalyptus – H.D. Kulkarni	515
Comparative study of the dendrometric parameters of <i>Eucalyptus urophylla</i> S.T.Blake and the hybrid <i>E. urophylla</i> x <i>E. grandis</i> in commercial (3x3 m spacing per tree) and silvopasture (9x2 m spacing per tree) stands – Fábio Monteiro Leite de Souza, João	
Paulo Grande, Felipe Manente Garcia, Cláudio Angeli Sansígolo	528
9. BIOTECHNOLOGY	533
Culture Establishment and Multiplication of superior recombinants emanating of mature <i>Eucaltyptus</i> hybrid <i>E. citrodora</i> Hook. × <i>E. torelliana</i> F.v. Muell – Ajay Thakur	535
R2R3 MYB transcription factors controlling of wood formation in Eucalyptus – Marcal Soler, Eduardo Leal Oliveira Camargo, Hong Yu, Hélène San Clemente, Bruno Savelli, Nathalie Ladouce, Jorge Paiva, Hua Wang, Jacqueline Grima-Pettenati	537
<b>Optimal time for the adventitious rooting of</b> <i>Eucalyptus benthamii</i> mini-cuttings – Gilvano Ebling Brondani, Francisco José Benedini Baccarin, Antonio Natal Gonçalves, Marcilio de Almeida, José Luiz Stape	539

10. SOCIAL AND ENVIRONMENTAL RESPONSABILITY	541
Sustainable Intensification of <i>Eucalyptus camaldulensis</i> in Southern India – R. Mathew, A.P. Upadhyay, M. Varghese, Navin Sharma	543
The Cultural objective and its Realization of <i>Eucalyptus</i> Plantation Management – Mo Xiaoyong Liu Liting	447

## **INVITED SPEAKERS**

## **INVITED SPEAKERS**

## A re-appraisal of eucalyptus plantations from the point of view of its quality-cost competitiveness and how to move them to the next level

Nuno M.G. Borralho<sup>1</sup>

### ABSTRACT

The enthusiastic spread of eucalyptus was initially driven by its fast growth and excellent form, but it was its potential as a prime source of pulpwood which brought eucalypts to stardom. It is now the most important plantation hardwood, both in planted area and in volume of wood harvested. From hundreds of potential species in the early days, six of them (camaldulensis, tereticornis, globulus, grandis, nitens and urophylla and its hybrids) are currently used in large scale plantation programs, with a few others being under investigation or in early development. Of those species, *E. globulus* is considered to have the best wood, especially in terms of pulp manufacturing costs, as well as in many aspects of paper mill runnability. This is due to its high pulp yield and density and excellent fiber physical and chemical properties. Unfortunately, the species adaptation is constrained in temperate climates by frost or drought, and in the tropics by excessive temperature and humidity. In this paper we will compare the economics of pulpwood production of the main plantation species being used, discuss the key reasons which have conditioned their selection in the past and the likely paths to ensure that future plantations are capable of improving its pulpwood quality and cost standards to E. globulus levels, whilst ensuring forests remain productive and healthy. In particular we will compare the advantages of continuing to select and breed pure species versus the development of interspecific E. globulus hybridization strategies, with the purpose of developing a "globulus-like" wood in "non-globulus" land. The emphasis will be on pulpwood production, as this is likely to remain the main driver of future eucalypt plantations and breeding programs. However, we will discuss briefly the implications of these trends for alternative uses such as solid timber and bio-energy.

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## Eucalypt plantations and climate change

Trevor H. Booth<sup>1</sup>

The introduction of eucalypts as plantation species around the world has been very successful and there are now about 20 million hectares of eucalypt plantations growing in more than 90 countries (GIT 2009).

For over 40 years CSIRO's Australian Tree Seed Centre has played an important role in collecting and distributing seed of the approximately 700 eucalypt species for trials. Results from species elimination trials in many countries helped to identify suitable plantation species and develop descriptions of where particular species could be grown. The development of climatic interpolation surfaces allowed estimates to be made of species climatic requirements (Booth 1996). Climatic data, along with information on soil requirements and potential uses, have provided an important part of books and computerised databases used to assist species selection (see, for example, CABI 2005).

As suitable species were identified for particular conditions the focus shifted from species selection to tree improvement (Eldridge *et al.* 1994). Enormous gains in productivity have been achieved through tree breeding and the potential for genetically modified eucalypts is now being explored.

In the last 20 years or so, while great strides have been made with the development of eucalypt plantations, there has been increasing concern about climate change (Booth 1991). It is now widely accepted that global temperatures will inevitably rise by at least 2°C and the more serious consequences of even greater increases are also being considered (Solomon *et al.* 2007).

Forestry has an important role to play in addressing climate change, as loss of natural forests are an important part of the problem, while increased tree planting has been proposed as part of the solution (Booth 1991). A key issue for plantations is how will climate change affect productivity and even tree survival? If plantation productivity is seriously affected, what options are available for alternative genotypes to plant? The main difficulties in addressing these issues are uncertainties around climate change scenarios and how trees will respond. The response issue is complex as tree growth will not only be affected by climatic and site interactions, but also atmospheric change. While changing temperature and rainfall may in some cases reduce productivity, increasing CO<sub>2</sub> levels will tend to

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affect photosynthesis and water use efficiency positively, offsetting some or all of the negative climatic changes (Booth *et al.* 2010).

A particularly interesting simulation study by Battaglia *et al.* (2009) has analysed likely impacts of climate change in 2030 and 2070 across Australia's plantation estate using the CABALA process-based simulation model. Uncertainty was dealt with by running the model for a range of different assumptions. The model was calibrated for Australia's main plantation species including *E. globulus* and *E. nitens.* In summary, the combinations were: 3 climate models x 3 time periods x 3 plant photosynthetic responses x 134 representative sites (species x site combinations) x 20 rotation length weather sequences. In all more than a million simulation runs were made, but these allowed uncertainties both in physiological responses and climate change scenarios to be incorporated. Maps of change and uncertainty were generated. While there was uncertainty about productivity implications in some areas, some regions clearly emerged as being likely to benefit from climate change, while others would clearly suffer. A similar analysis for major eucalypt plantations around the world would be useful.

One of the main areas of uncertainty in assessing impacts on eucalypt plantations around the world is how trees will respond to raised levels of atmospheric  $CO_2$ . Experiments growing eucalypts under increased  $CO_2$  levels are helping to reduce these uncertainties (Barton *et al.* 2010).

The entire genome was released for *E. grandis* in April 2011 (EUCAGEN 2011) and genomic studies are beginning to unlock the secrets of how particular genes influence the abilities of different eucalypts to cope with various climatic conditions. However, many key traits are likely to be influenced by multiple genes in complex ways.

As knowledge of the likely impacts and the characteristics of different genotypes improves appropriate climate change adaptations can be put in place (Seppälä *et al.* 2009). As eucalypts are generally grown in comparatively short rotations the adaptive capacity of plantations is reasonably high.

The success of eucalypt introductions around the world was in large part due to sharing of information. Responding to climate change will also be most effective if information is shared. More than 20 years ago a global climatological audit was proposed to assist both sustainable development and conservation (Booth 1991).

That approach could be updated to help us identify climate change impacts as soon as possible. We need to know where particular genotypes are grown and how they are responding to changing conditions. We should identify locations where particular genotypes are already being grown under relatively extreme conditions. By simple monitoring of these sites, often using data that are already collected, we could provide early warning of any climate change related problems that may arise in the core areas of the planted distributions of these genotypes and develop appropriate adaptations.

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# Site and stand management: theory and practice in Australian plantation forests

Philip Smethurst<sup>1</sup>

#### Background

Our understanding of processes that determine wood yield and quality from eucalypt plantations has advanced during the past few decades to the extent that a high level of site-species-product specificity is possible, but optimisation of wood production is not always achieved. This paper aims to summarise in an Australian context the theory of silvicultural optimisation, current practices, and the need for and potential for further innovation.

#### Theory

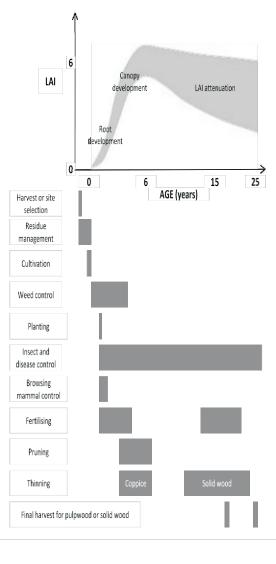
Most plantation operations affect the supply, capture or utilisation of light, water or nutrients, product amount or quality, and profitability. The main silvicultural operations are listed in Figure 1 in relation to the pattern of leaf area index (LAI) of a stand and its development phases. Assuming a manager aims to produce a large amount of good quality wood as quickly and profitably as possible, management should aim to appropriately select sites, conserve site resources (e.g. soil, organic matter and nutrients), and optimise inputs (e.g. genetics, nutrients, herbicides, energy, and costs generally). The timing of many operations is crucial for attaining the desired outcomes. Maximising tree growth rates might not be an appropriate goal if it compromises tree form, wood quality or profitability. Many silvicultural operations and effects are inter-related and non-additive.

#### **Current Practices**

The choice of site underpins resource availability (water and nutrient availability, micro-climate). Sites sought are ex-agricultural, because of their high nutrient capital, more than 1 m deep soil of good structure, few rocks, low slope, and suitable climate. For subsequent rotations, the harvesting system maximises nutrient availability if slash is retained evenly and compaction is avoided. Cultivation mainly affects nutrient and water availability or capture. Surface (0.3

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m) row- or spot-cultivation is common, along with mounding on nutrient-poor or imperfectly drained sites. Deep ripping (to 0.5-1.2 m depth) used to be practiced widely, but it is now rare because it provides few benefits. Seedlings are planted at a density of 1000-1500 per ha depending on rainfall zone; low-densities are restricted to dry sites. Weed control reduces competition for light, water, and



**Figure 1.** The of LAI development typically observed in Australian eucalypt plantations in relation to the main silvicultural operations.

nutrients; strip control is common. Browsing mammal control maximises survival and LAI (light capture), which is achieved mainly by poisoning and shooting. Fertilising increases nutrient availability and is achieved by spot, strip and broadcast applications of specific nutrients and rates during critical phases of the crop (during root and canopy development, and mid- to late-rotation). Phosphorus, nitrogen and potassium are the main nutrients applied. Pruning and thinning reduce LAI and therefore light capture, but they redirect carbon allocation into more highvalue products, and they are only used in solid wood regimes.

#### **Issues and Innovations**

Harvesting-Residue Management-Cultivation: Eucalyptus globulus and E. nitens residues in Australia are often removed or poorly distributed. Harvesting systems need to be employed that provide a high percentage recovery of the woody components while leaving foliage, twigs and bark evenly distributed near the stump. Harvesting should be seen as the first step in site preparation, because it influences residue management and cultivation options. Burning of retained residues is still used by some growers, but it should stop because of negative effects on site productivity and the carbon budget. Cultivation systems need to be developed that are effective with residue retention.

Weed control: Many Australian eucalypt plantations are quite weedy, which can reduce water, nutrient and light availability to trees, and limit the effectiveness of fertilisation. However, because herbaceous or shrub understoreys recycle nutrients, protect soil from erosion, enhance biodiversity, and don't compete for light with established trees, their retention can be beneficial.

Fertilisation: Cost and environmental pressures will probably require increased efficacy of fertilisation and a reduction in the use of herbicides. Careful fertiliser placement is needed when weeds are present, e.g. in the inter-row of strip weed control operations, but we need an improved knowledge of fertiliser efficacy in relation to the spatial-temporal development of tree and weed root systems and fertiliser placement. Nutritional diagnostics (soil and foliar analysis, visual symptoms, which are rarely well-calibrated) and fertiliser type, rate, timing and placement methods need continual refinement as climate, genotype and silvicultural systems change and fertiliser costs increase.

Water use: Regulation of plantations as water users is increasing, which affects site selection and management. We need methods of predicting water use (and stream flows or groundwater levels) for combinations of silviculture, genetics,

landscape and climate from plot- to catchment-scales. This requires linked wood production and hydrological modelling. The development of LAI in the plant model needs to integrate all site and stand variables, including genotype, planting density, pruning, and thinning.

Climate: Predictions of climate are becoming more refined spatially and temporally, which allows managers to better assess climate-related risks, e.g. droughts, frosts, and winds, which in-turn could affect silvicultural decisions like site selection, genotypes and stocking. These risks are least for short-rotation eucalypt plantations and increase with rotation length.

# An intensive eucalypt silviculture to cope with environmental stresses in Argentina

Fernando Dalla Tea<sup>1</sup>, Federico Larocca<sup>2</sup>

Culture of E. grandis plantations have evolved into more conservative tillage operations but more intensive silvicultural regimes in the Mesopotamia Argentina. Traditional mechanical site preparation and weed control have been changed into banded site prep and chemical weed control plus starter fertilization. These not only allow for large scale operations but also promote soil conservation. Minimum tillage operations have been adopted widely, in part as a result of the impressive change also imposed in the the agriculture sector.

With the increase in planted area, E. grandis not only was planted on good drained sandy soils but also on sites with more limited drainage. Mounding on these soils has proven to be a very effective technique according to different trials.

The strong eucalypt demand from the sawmill industry in Argentina is the main reason for the farmers to choose a sawmill over a pulpwood regime based on internal rate of return. This market factor influences directly on the planting density and the silvicultural regimes. This is different from neighboring countries where the pulp industry drives the market.

		-
	Mid 90's	Today
Genetics	South African seeds	SA seeds, local CSO and SSO, clones
Seedling production	Plastic bag containers	Containerized seedlings
Site preparation	Complete discing	Banded site preparation: subsoiling or bedding depending on soil drainage
Weed control	Cross discing as necessary	Chemical weed control: Banded residual and glyphosate in the interrow
Fertilization	No	Starter fertilizer
Planting	Manual	Mechanical or manual
Prunning	Basal pruning for visual purposes	Higher prunings from 6 to 9 m
Thinning	Νο	2-3 thinnings. Final density 200 to 300 trees/ha
Coppice	2 or more cycles	Replacement at the first rotation

 Table 1. Eucalypt forestry evolution in the Mesopotamia Argentina.

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Intensive silviculture (PT, pruning + thinning) was introduced in order to change production from small sawlog and pulpwood regimes into more diversified timber production. The PT regimes most applied don't affect productivity but increase residual tree diameters. Several trials are analyzed and only very intense pruning and/or thinning would reduce total productivity.

#### **Genetics and Site preparation**

Most plantations were generated with south African seed orchard seedlings whose improvement focused on stem form and saw timber quality. Less than 10% of the plantation base is clonal. In the open market different clones from INTA, CIEF and private companies breed for stem form, growth and reduced splitting can be purchased.

In FASA trials, company clones demonstrated increased growth compared with south African SO seedlings. The 10 best clones grow up to 20% more than south African seed orchard seedlings and have better form and higher density.

Older plantations are concentrated on sandy soils where less intensive site preparation is adequate for initial growth. New plantations moved into denser soils with some drainage restrictions. Mounding on these soils can increase survival and produce some 10% or more increment in growth.

Most of the plantation base has been coppiced for 2 or more rotations. These coppices are less productive and have lower log quality than the original forest. In the last 5 years, a program to replace all the harvested areas has been implemented in FASA and other private plantations.

Crop residue management appears to be the main challenge especially when clearcutting coppices. Burning is the most widely used method for post harvest residues disposal although uprises environmental issues, and certified companies are looking for a non burning policy. A V-shear + a D8 bulldozer is used for shearing and piling operations along with blading the stump. Forestry mulchers (i.e., FAE UMH 150) are now used in FASA for chopping and mulching the residues in a 2 m interrow and a ripper + disc operation completes the site prep.

Forest harvesting increases soil bulk density and its mechanical resistance to penetration (MRP). Bulk density increases up to 16% in loamy sandy soil with extreme conditions of surface disturbance (Wheel tracks), while the MRP can increase over 250% in extreme cases. Soil properties however, partially recover years after harvesting. A mechanization program developed in FASA aims at full tree processing besides returning branches and bark into the site.

Frost has become the most limiting factor for E. grandis in Entre Ríos. Different ways of dealing with it include species selection, intensive silviculture and soil cover management. The use of E. dunnii for frost sensitive sites has been discontinued because its sawnwood was not accepted by the sawmills. Hybridization of E. grandis with more frost tolerant species is one of the priorities of the genetic research programs.

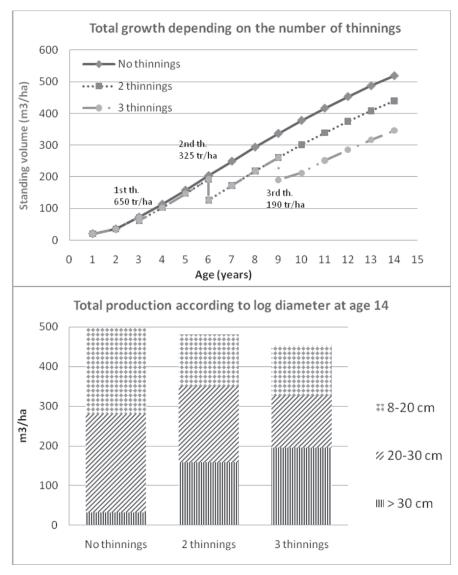


Figure 1. Effect of thinning on eucalypt growth and timber production.

Seasonal droughts common during summer can affect productivity dramatically, and annual increments can drop up to 30% in dry years.

#### Weed control and Fertilization

Weed competition is the practice most affecting initial growth. At least a 6month complete control period is necessary for E. grandis to have all the resources needed for exhibiting its potential. Small farmers are still using cross discing to control weeds although there are very old trials showing the advantages of using residual herbicides for early competition control. The most used herbicide is oxifluorfen on banded applications. For FSC certified companies, isoxaflutole is used instead.

Many trials demonstrated a significant response of grandis to starter fertilizers. Experience in Uruguay with similar soils and silviculture, have shown the same responses. A large and consistent P response is found during the first years after treatment. Nitrogen, however, only produces responses in sandy soils with extremely low organic matter.

#### Planting density and silvicultural regimes

A large and diversified sawmill sector besides the lack of large pulpmill industries are the reasons for choosing low planting densities whose objective is to increase the proportion of saw logs by the end of the rotation. Soils with high site index may be planted with higher stocking but in any case, initial density is recommended to be <1000 plants/ha and forest rotations are generally >12 yrs.

In the last years, few sawmills have been installing driers and manufacturing facilities to produce higher quality timber products. This market demands higher quality logs encouraging farmers to apply PT regimes. FASA installed 10 years ago trials and started operational PT silviculture, that vary according to sites. The most intensive treatments include 3 thinnings and 3+ prunnings, leaving 200 residual trees pruned up to 6+ meters.

The PT regimes appear to conserve more nutrients and carbon on the sites compared to short rotation pulpwood regimes. Furthermore, thinned stands have higher drought tolerance and less water consumption than unthinned plantations.

## Eucalyptus production in temperate regions

Jaime Rodríguez Schäfer

This paper analyses the structure and usage of the Eucalyptus sp. resource in Chile, wood supply, the silviculture and stand management practices, harvesting methods, growth rates as well as some new insights and results on biodiversity issues on plantation forestry.

Commercial plantations of Eucalyptus in Chile account for a total of 668,000 ha located between Maule and Los Lagos Regions, of which 72% and 28% are established correspondingly with E. globulus and E. nitens. According to INFOR, both species explain a gross wood supply of 14.5 to 16.2 million m3/year of internal demand from local pulpmills, OSB panel plants and woodchips for export to Japanese market as the main wood consumers.

Upstream, silviculture and establishment practices have showed minor changes during the last decade for pulpwood production.

Growth rates are strongly specie by site dependent: meanwhile E. globulus ranges from MAI 20 to 35 m3/ha/yr. between dry clay soil sites and high productive volcanic ashes soils, E. nitens does it between MAI 35 and 55 m3/ha/yr in the same sites. In both soil types, productivity measured as pulp-per-hectare-year basis is higher growing E. nitens than E. globulus.

Rotation length has increased as a result of higher wood density requirements from the pulp industry, ranging from 10 to 12 years old for E. globulus and 12 to 14 years old and even longer for E. nitens. Impact of wood properties on pulping economics are also discussed, where basic density seems to be the main driver given its positive correlation with age and with pulpmill output. Nevertheless, in a marginal analysis the optimal wood supply mix will strongly depend on price relationship of both, raw material and pulp prices. However, a latitudinal variation has been found with lower density values in the south and higher in the north range of the plantation distribution.

Despite some stand management regimes have been developed for solid wood products from E. nitens, the drying challenges have been a limiting factor for industrial development of this kind of products.

Harvesting methods are based preferably on full mechanised systems [feller

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#### 16 - Invited Speakers

bunchers, grapple skidders and whole tree road-side processing heads] with variable bucking patterns, and complementary, road side chipping equipments which minimize the mill gate supply chain cost compared with more traditional cut-to-length systems.

Recently, main forestry players started to demand a new forest by-product: biomass and slashes recovery as a feedstock for co-generation at large scale [40-90 Mw/h and more]. Thus, forest residues and unmerchantable log sizes are collected as a post-harvesting operation in two ways: collecting from the field and forwarding the slashes to roadside and stocking in large piles roadside where grinders or chipping facilities will be installed. This approach is more suitable for recovering biomass from large harvesting areas.

The second approach is a compact design of slash bundlers, where the feeding table and packing units are mounted on a forwarder trailer. The roadside output are bales of pre-set lengths with a volume compaction ratio up to 80% compared with bulk residues, easier for handling and with similar logistic as traditional logs. This system, although slightly more expensive, has demonstrated being suitable for smaller areas and steep terrains, and trends to minimize biomass contamination with soil.

Finally, recent results from long term monitoring on fragmented ecosystems and forest plantations have evidenced that plantation are no longer "biological deserts". Instead of that, results on biodiversity, relative abundance, reproductive rates, biological interactions and other parameters suggested these "simplified ecosystems" as complementary habitats, where the functional behaviour of the species which occur in forest plantations become more important than species itself. This is the case of plantations understory density, where even invasive exotic species like "gorse" [Ulex sp.] or "broom" [Teline sp.] may play a key role as biological corridors in fragmented ecosystems, compared with clean understory plantations. Landscape and landuse planning, as well as the interaction with human neighbourhood are main components of the toolkit for conserving and enhancing biodiversity in forest plantations.

# Drought responses of Eucalypts in plantations – from physiology to management

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Rising concentrations of atmospheric greenhouse gases are affecting the earth's climate. While future changes are difficult to quantify and vary regionally, for the worlds *Eucalyptus* plantations they will include increases in mean temperatures and the frequency and severity of drought. Considerable research has been undertaken into plant responses to drought and temperature during the last 50 years (see for example recent reviews (Munns, 2002; Way and Oren, 2010). Despite this, understanding of the role that these two variables play in tree mortality remains limited and the subject of intense debate (McDowell et al., 2008; Adams et al., 2009; Leuzinger et al., 2009; Sala, 2009; Sala et al., 2010). McDowell et al.(2008) proposed a simple conceptual model that linked the principle mechanisms of climate driven tree mortality, hydraulic failure and carbon starvation, to the intensity and duration of drought. They argued that low to medium intensity droughts kill trees by carbon starvation while acute, high intensity droughts kill trees by hydraulic failure. In both situations attack by biotic agents may exacerbate mortality. Adams et al.(2009) provided some support for the carbon starvation hypothesis, demonstrating that drought induced mortality occurred earlier in *Pinus edulis* plants grown at elevated temperatures compared to *Pinus edulis* plants grown at ambient temperatures. However, evidence for the carbon starvation hypothesis remains limited and complete depletion of carbon reserves prior to mortality has thus far not been demonstrated (Sala et al., 2010).

In contrast, our understanding of the hydraulic constraints on gas exchange is much further developed. To maintain photosynthesis and hence carbon fixation plants must keep leaves well hydrated and to do so must co-ordinate the liquid and gaseous phases of transpiration (Sperry, 2000). Evaporation of water from the sub-stomatal cavities in conjunction with the cohesive properties of water molecules

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generates the pulling force for the extraction of liquid water from the soil matrix (Sperry, 2000) and creates a gradient in water potential from the soil to the leaf. Darcy's law predicts that transpiration is the product of the water potential gradient and the hydraulic conductance of the soil to leaf pathway. Hydraulic conductance decreases as the water potential gradient increases due to cavitation of some vessels so that for a given soil water potential there is an upper limit to transpiration that is associated with critical leaf water potential. Exceeding this rate can result in runaway xylem embolism within the water transport system, resulting in desiccation of leaves and ultimately death of the plant (McDowell et al., 2008; Sperry, 2000). Thus, stomata act as pressure regulators in the soil-plant-atmosphere continuum and play a pivotal role in regulating the water status of plants and drought induced failure of the hydraulic system is an attractive mechanism for explaining plant mortality.

The *E. globulus* plantation estate in Western Australia provides an ideal model for examining hydraulic regulation of water stress. *Eucalyptus globulus* occurs naturally in Tasmania, the Bass Strait islands and in southern Victoria (Kirkpatrick, 1974) where rainfall generally exceeds 800 mm per annum and is distributed uniformly throughout the year; days where air saturation deficits exceed 3 kPa are rare. This contrasts strongly with the Mediterranean environments in which this species has been extensively established in commercial plantations (Gentilli, 1989). Using measurements in *E. globulus* plantations over more than 20 years we have characterised the relationship between whole tree conductivity and water stress for a full range of water status from well watered to dead. Three main stages of water stress and hydraulic regulation were observed. We refer to these stages as (1) the normal operating range, (2) the water stressed range and (3) the severely water stressed range. We recognise that these stages are part of a continuum of responses from well-watered to critically water stressed. The main advance in this analysis is the capacity to define measurable thresholds that define the transition points between each of these water stress phases. These thresholds have been used to develop a modified version of the process based model CABALA (Battaglia et al. 2004) that incorporated some logic from SPA (Williams 1996) to predict hydraulic gradients in the tree.

Using *E. globulus* as a case study or benchmark species we will review variation in the responses of commercial plantation species to water stress, consider recent studies that test the effect of management (breeding and silviculture) on water stress and the productivity – risk trade-off. We will also use modelling to explore the potential to take advantage of this knowledge in developing future adaptation strategies.

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# Silviculture and management of *Eucalyptus* plantations in the frost occurrence areas of southern brazil

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The *Eucalyptus* plantations with species adapted to subtropical climate have gained prominence over the last five years in southern Brazil. There are great expectations regarding the wood production from these plantations, for these reasons it is necessary to use all the knowledge and technology available for the establishment of these forest stands. Different management systems are necessary for those areas, since they are subjected to subtropical climate, where subzero temperatures with frost formation are very common during the winter. The records show the occurrence of 15 to 40 frosts per year, usually causing considerable damage to *Eucalyptus* species, affecting the quality and productivity of those plantations.

Traditionally forest companies in southern Brazil, especially in Parana and Santa Catarina states, use pine as the primary species in most of their plantations, and *Eucalyptus* as a secondary species. This condition is highly favorable since the pine species present good growth and are considered more tolerant to climatic changes. However, few landowners visualize the need to use new silvicultural techniques quite different from those of pines, for establishment and maintenance of productive *Eucalyptus* plantations.

Since the 80's several *Eucalyptus* species have been introduced in southern Brazil. Among those species the *Eucalyptus dunnii* and *E. viminalis* showed good adaptability to freezing conditions. More recently, the *E. benthamii* trials also showed good frost tolerance for that species and rapidly it is being considered as the main species for the establishment of *Eucalyptus* plantations in the region. It is becoming apparent with time that to establish highly productive *Eucalyptus* forests in this region, it is necessary to adopt more advanced forestry technology, since the *Eucalyptus* species are much more demanding for resources and more sensitive to environmental changes than the pines, on the other hand they respond readily to forestry management and silvicultural practices.

A positive interaction between intensive silviculture and well adapted species

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need the complementary effect of some strategies before, during and after planting, such as edaphoclimatic zoning, planting season and silvicultural treatments.

#### Edaphoclimatic Zoning (planting)

For a correct zoning it is necessary to observe the variations in the relief, soil and microclimate, adjusting the species for each location, according to their tolerance to frost. Species more tolerant and/or resistant to cold are planted in the lower portions of the slope and on the south face exposure, where the intensity of frosts is higher. The upper portions of the slope are planted with species less tolerant. Plantations are consequently always composed by different species. On the lower slopes it is used *E. benthamii* and *E. viminalis*, both frost resistant, and on the upper slopes species less frost resistant, such as *E. dunnii* is recommended. At higher altitudes, usually above 1,200 meters on organic and humid soils, the establishment of *Eucalyptus* plantations is not recommended.

#### **Planting Season**

In frost occurrence areas, the period between the months of September through December, was defined as ideal for planting. At this time the occurrence of frost is minimum.

Plantations established during that period will develop enough height to survive the arrival of the next cold season which starts in April. The trees that are planted under those recommendations survive with minimum or no frost damage.

## Silvicultural Treatments

Complementary practices are recommended to promote the rapid growth of the trees to avoid risks of frosts.

After planting, the use of fertilizers and weed control are vital to promote a good start in the early development of the plants. *Eucalyptus* species growing in subtropical climate and soil conditions of southern Brazil have shown a high response to fertilization, and more specific to phosphorus.

## **Results and Future Prospects**

In the last five years, the planted forest areas in southern Brazil have increased by 12%, meanwhile the planted area with Eucalyptus grew by 53% (ABRAF, 2011). Much of the growth concentrated in the region subject to the frost occurrence, with negative temperatures up to (-8°C). Another characteristic of this region is that conditions the adaptation of the species is the daily temperature range, which in some cases can reach 30°C, with very cold nights (-5°C) and very hot days (20/30°C). However, those conditions has lead the selection of individuals tolerant and/or resistant and the development of silvicultural practices to minimize the possible damage caused by frost.

There are currently frost resistant clones of *E. benthamii* and *E. dunnii* planted with mean annual increment estimated of 35 and 42 m<sup>3</sup>.ha<sup>-1</sup>.yr<sup>-1</sup>, respectively. For the selection of new genetic material with higher pulp productivity, hybridizations are performed to increase also frost resistance, wood quality and resistance to pests and diseases.

Other lines of work in progress seek information on the physiological responses of *Eucalyptus* to high doses of fertilizers, such as potassium chloride. The hypothesis is that the higher uptake of nutrients by plants, prior to the period of stress, increases salt concentrations in cells and promotes acclimatization. In such condition the levels of frost damage would be minimized.

The success of reforestation activity with *Eucalyptus* in frosts occurrence areas of southern Brazil depends on the interaction between tree breeding and forest management practices on forests establishment. The result of this interaction will be highly productive forests able to tolerate the thermal stress caused by frost, very common in this region.

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## Plantation eucalypt species: recent trends in testing and planting

David Bush<sup>1</sup>

#### Background – the dominant plantation eucalypts

Eucalypts are the second most widely planted tree genus in the world, with commercially significant plantation areas is temperate, subtropical and tropical regions. Their success is due to rapid growth rates and adaptation to a wide range of sites. Many of the eucalypts have evolved in places that have a long dry seasons and soils with poor nutrient status. There are over 600 eucalypts, but it is estimated that 90-95% of the world's plantations are of only nine species from within the Eucalyptus subgenus Symphyomyrtus (E. camaldulensis, E. dunnii, E. globulus, E. grandis, E. nitens, E. pellita, E. saligna, E. tereticornis, E. urophylla), or are hybrids from among the nine. These species are adaptable to a wide range of environments and produce useful commodities including pulp, sawn timber, reconstituted wood products and fuel wood. Australian Tree Seed Centre records show that there is an ongoing demand for infusions of wild family seedlots to augment genetic diversity in breeding programs and that there is also a moderate level of trade in genetically improved material. Species from the Monocalyptus subgenus for which early-stage breeding programs were developed (e.g. E. delegetensis, E. regnans) are now seldom planted, probably because of their exacting site requirements and modest early growth rates making them less suited to shorter rotations.

Climate change, wood and energy security for the world's still-growing population also has a bearing on where and which eucalypts might be planted in future. During the last decade plantation estates have expanded to more difficult sites, as prime sites are planted up: a practice that seems likely to continue. Trends in utilisation and testing of some of the lesser-known species are reported here.

#### **Cold tolerant species**

Though temperate eucalypts such as *E. nitens* have a combination of good growth rates, wood properties and cold tolerance, there are few tropical species with these attributes. In the subtropics, particularly Brazil, Uruguay and southern China, where sudden frosts can cause severe injury, demand for cold-tolerant eucalypts

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such as *E. dunnii* and *E. benthamii* has resulted. Domestication and breeding of these species is now advancing in a number of programs. A limitation for *E. benthamii* is availability of germplasm, both for planting and breeding. The species has a very limited natural range and is listed as Vulnerable to extinction – there are only a handful of trees remaining in two of the remaining subpopulations. Collection of wild genetic resources is highly restricted. Other species that continue to be tested include *E. badjensis, E. macarthurii* and *E. smithii* which are actively being genetically improved for colder sites in southern Africa.

#### Pest and disease resistance

Pest and disease resistance may be the most important consideration in eucalypt forestry, particularly in the tropics, where foliar fungal diseases such as Kirramyces and Mycosphaerella can limit productivity. E. camaldulensis and E. grandis, though widely used as hybrid parents, are particularly susceptible to both fungi and insects. Another serious disease of the humid tropics is *Cylindrocladium* leaf blight which can affect E. camaldulensis and E. urophylla particularly severely. In Vietnam E. brassiana has shown relatively strong resistance to this disease, and it is now being successfully hybridised. An emerging pest epidemic in parts of the tropics including India and Thailand where E. camaldulensis and E. tereticornis are planted is gall wasp (Leptocybe invasa) that can destroy young plantations. Clones that don't involve *E. camaldulensis* are now being planted, sacrificing the typically good growth rates associated with hybrid combinations involving this species. Less susceptible pure E. urophylla, E. pellita or hybrids involving these are becoming more commonly deployed. In Australia hybridising Corymbia citriodora subsp. variegata (CCV) with C. torelliana has substantially increased resistance to Quambalaria shoot blight, increased cold and drought tolerance and improved cutting strike rate compared with pure CCV.

#### Water-limited sites

The shift towards more challenging sites also extends to those that are warmer and drier, often with lengthier dry season and low rainfall reliability. In countries including Australia, Chile and India, interest has increased in drought tolerant species. In the temperate zone alternative pure species such as *Corymbia maculata*, *E. cladocalyx*, *E. occidentalis* and red ironbarks, *E. tricarpa* and *E. sideroxylon* have been trialled quite widely. In the subtropics and tropics species such as *E. argophloia*, *E. longirostrata* and the *Corymbia* hybrid already mentioned are showing some promise. While many of these species have good sawn timber properties making them suitable for high value applications, growth rates are typically modest and pulp properties relatively poor. Hybrids involving *E. camaldulensis* (that confers good rooting ability and tolerance of drought and waterlogging) and *E. globulus* (good form and pulp properties) are also being investigated in temperate and some subtropical regions in Australia and South Africa.

#### **Bioenergy applications**

The largest use for eucalypt wood is energy, and the looming global energy shortage and need for renewable energy is causing the use of eucalypts for bioenergy to be revisited. A major issue in the bioenergy debate is the distortion of food prices where agricultural crops are diverted to energy production. There are many eucalypt species that are naturally well-adapted to planting on sites marginal for cropping and agriculture where displacement of food crops is not an issue. In the southern United States *E. benthamii* is being planted for dedicated bioenergy applications, and eucalypt wood is commonly used in the marginal cerrado region of Brazil for charcoal manufacture. However there is also a wide range of largely untested eucalypts suited to very marginal sites where agricultural production is low. These include the many mallees– species with a multistemmed habit and very strong coppicing ability that are typically found growing on difficult sites in semiarid Australia. Breeding of temperate species such as *E. horistes* and *E. polybractea* is now underway in Australia, and subtropical and tropical species from Sections Bisectae and Adnataria of Symphyomyrtus are in the early stage of speciesprovenance testing in Australia and India.

# Advances in eucalypt genetics: from genes to ecosystems

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The four years since our last meeting have seen enormous advances in eucalypt genetics on multiple fronts - from the genomic resources required to understand genome structure and function, through to the conceptualization and study of the effects of genes which extend beyond the individual to affect its neighbours, dependent biotic communities and even ecosystem processes. The International Eucalypt Genome Network's (EUCAGEN) 2006 proposal to the US Department of Energy's Joint Genome Institute (DOE-JGI) was successful and saw the public release of a *Eucalyptus* reference genome sequence in 2011 based on *E. grandis*, resequencing of the *E. globulus* genome and international co-operation in the development and public release of novel genomic and other resources (http://web.up.ac.za/eucagen/; Keller *et al.* 2009; Rengel *et al.* 2009; Neves *et al.* 2011; Paiva *et al.* 2011). As expected (Myburg *et al.* 2007), these initiatives have unleashed an international effort to explore the genome, and clearly placed *Eucalyptus* as a model forest tree system for genetic research.

One of the key molecular tools recently developed for *Eucalyptus* is the Diversity Array Technology (DArT) (Sansaloni *et al.* 2010). With wide transferability across *Eucalyptus* species, DArT provides an unprecedented level of resolution for linkage mapping, detection of quantitative trait loci (QTL), comparative mapping, population genetic, phylogenetic and evolutionary studies with thousands of genome-wide markers of known position available (Hudson *et al.* 2011; Kullan *et al.* 2011; Steane *et al.* 2011). Automation of genotyping and the reduced cost per marker have facilitated the development of high-density linkage maps (Hudson *et al.* 2011; Kullan *et al.* 2011). The transferability of DArT across species and pedigrees has allowed the transfer of QTL information between studies and, in combination with an increasing resource of microsatellites (Faria *et al.* 2011) and single nucleotide

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polymorphisms (SNPs; Paiva *et al.* 2011), has provided a high-resolution link between phenotypes in field populations and the *Eucalyptus* reference genome. Eucalypts are diploid and the number of chromosomes appears to be uniform across the genus (2n = 22; Oudjehih and Bentouati 2006). DArT markers form the foundation of a high-resolution comparative mapping study that has demonstrated only minor structural differences amongst three species studied (*E. grandis, E. urophylla* and *E. globulus*) (Hudson *et al.* 2011). This result argues for high overall genome similarity, and high transferability of the *E. grandis* genome sequence information and marker/trait associations amongst the lineages studied.

At the population level, our molecular and quantitative genetic studies have focused on the temperate species *E. globulus*, which is part of a complex of four related taxa, variously given species or subspecies status (Potts et al. 2004; Potts et al. 2007). Using a multi-species consensus map, genome scans of population parameters derived from DArT markers have located genomic regions associated with species differentiation, diversity deserts and introgression (Hudson 2011; R. Jones unpubl.). The natural introgression studied was between *E. globulus* and a rare Tasmanian endemic, *E. cordata* (McKinnon *et al.* 2010; R. Jones unpubl.). Despite evidence of introgression of chloroplast DNA affecting a large proportion of the E. *qlobulus* gene-pool and porosity of the *E. globulus* nuclear genome, such introgression appears to have only a minor effect on the nuclear gene pools of the two species as they maintain their morphological and overall molecular distinctiveness when the two species co-occur. Insights into the post-zygotic barriers that would maintain species integrity in the face of hybridisation have come from studies of artificial first (Volker et al. 2008) and advanced (Costa e Silva et al. 2011b) generation hybrids between E. globulus and E. nitens. These two species are well delineated genetically and differentiating DArT markers have been located on 8 of the 11 linkage groups (Hudson 2011). Significant outbreeding depression occurs in their hybrids and line-cross analyses argue that the development of favorable epistasis within species is a key mechanism underlying their differentiation (Costa e Silva *et al.* 2011b). Given the relatively high genome similarity observed to date, such epistasis is likely to be a key mechanism of speciation in Eucalyptus.

At lower taxonomic levels the distinction between recognized eucalypt taxa is often less clear and outbreeding depression is less obvious. This is seen no better than in the relationship of the four taxa comprising the *E. globulus* complex. While the cores of these taxa are geographically, morphologically and, in most cases,

genetically distinct, the phenotypic and molecular genetic variation between them appears continuous (Jones 2009). Intermediate populations occur over large geographical areas, and morphological and molecular affinities often do not correspond. Within *E. globulus* and its intergrades, there is increasing evidence for genetic differentiation in a wide variety of traits of ecological and economic significance, at both broad (Stackpole *et al.* 2010) and local (Foster *et al.* 2007) spatial scales. There is molecular and other evidence for contemporary and historic barriers to gene flow (Foster *et al.* 2007; Jones 2009; Jones *et al.* 2011), which could contribute to population divergence through drift. However, at this micro-evolutionary level there is increasing evidence that natural selection has played a significant role in shaping the geographic patterns of quantitative genetic variation observed in wood property (Hamilton *et al.* 2010; Stackpole *et al.* 2011), developmental (Hamilton *et al.* 2011), and stress-related (Costa e Silva *et al.* 2006; Tibbits *et al.* 2006; Dutkowski and Potts 2011) traits. A similar conclusion is reached from recent studies of other eucalypt species (e.g. Shepherd *et al.* 2010).

From a quantitative genetics perspective, understanding the potential for eucalypt populations to genetically adapt to diverse abiotic and biotic stresses (Myburg et al. 2007; Teulières et al. 2007; Guimarães et al. 2010), requires knowledge of the genetic architecture of the relevant traits, particularly their levels of additive genetic variance and co-variation. Of the wide variety of traits studied in *E. globulus*, the additive genetic control (and thus narrow-sense heritability) of growth and survival traits themselves is low and there is evidence for significant non-additive genetic control of growth under outcrossing which appears to be comparable to the additive genetic variation in some (Li et al. 2007; Volker et al. 2008; Callister et al. 2011), but not all populations (Costa e Silva et al. 2004; Costa e Silva et al. 2009\_ENREF\_7). However, growth and survival are also subject to severe inbreeding depression, which may vary with level of inbreeding, age, site and population. At the population level, this inbreeding depression does not appear to be due to epistasis effects (Costa e Silva et al. 2011a), but rather to dominance variation associated with a genetic load of rare and recessive deleterious alleles (Costa e Silva et al. 2010a, b). For diameter growth, the dominance variation expressed under inbreeding was estimated to be nearly 10-fold greater than the dominance variance associated with random mating and the additive variance (Costa e Silva et al. 2010b). With self-incompatibility, outcrossing rates and inbreeding depression varying amongst female parents (Mimura et al. 2009; Costa e Silva et al. 2010b; McGowen et al. 2010 ENREF 8), these adverse dominance effects expressed

under inbreeding appear to be a major driver of differences in growth amongst open-pollinated families (Costa e Silva *et al.* 2010a; see also Bush *et al.* 2011). Not until later ages, when substantial mortality of inbred progeny has occurred, do the additive genetic differences between parents appear to be manifest in the standing genetic variation in growth of their open-pollinated families (Costa e Silva *et al.* 2010b).

A current research frontier lies in linking phenotypic variation to the genome through association genetics using candidate-gene (Thumma et al. 2009; Sexton et al. 2010; Southerton et al. 2010; ; Kulheim et al. 2011; Thavamanikumar et al. 2011) or genome-wide (Grattapaglia and Resende 2011) approaches. There is also a new frontier in linking genetics and ecology, through community and ecosystem genetics (Whitham et al. 2006; Whitham et al. 2008). This research looks beyond the direct genetic effects on the tree phenotype to study the flow-on effects on neighbours, the associated biota and even ecosystem processes. Such extended indirect genetic effects and feedbacks are especially important in forest trees, which dominate many natural and artificial terrestrial ecosystems, provide habitat for numerous dependent organisms, and supply many important ecosystem services (Whitham et al. 2006). We have been studying these extended genetic effects in Eucalyptus *globulus*, following natural colonization of base-population trials by local fungi, insect and marsupial species. These Australian trials have provided a robust experimental system in which to study individual species and community level responses to genetic variation in a foundation tree species. In one field trial, E. *globulus* trees from different races supported different arthropod and fungal communities in their canopy (Barbour et al. 2009c), trunk (Barbour et al. 2009b) and litter (Barbour et al. 2009a); and there was even differences in soil nitrogen levels (Bailey et al. 2011). There is the potential for complex feedbacks between tree genetics and dependent biotic communities, with reciprocal and cascading effects likely (Whitham et al. 2006). We have been studying the drivers of these biotic responses (Miller et al. 2011; O'Reilly-Wapstra et al. 2011), the genetic covariance amongst dependent species, feedbacks to tree fitness (O'Reilly-Wapstra et al. 2011), potential correlated responses to selection and, more recently, the importance of indirect genetic effects in these artificial forests. Placing forest tree genetics into this broader community and ecosystem framework offers a more holistic approach to meet the challenges posed by human-induced global change and to sustainably manage the expanding area of artificial forests across the globe (Whitham et al. 2006; ENREF 49Whitham et al. 2010).

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# Twenty years of *Eucalyptus* molecular breeding: from discrete marker-trait associations to whole-genome prediction of complex traits

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#### Introduction

Marker Assisted Selection (MAS) has been the main justification underlying several large-scale forest tree genome projects worldwide in the last 20 years. The long generation times needed to complete a breeding cycle and the low heritability of most target traits were identified as the hurdles that MAS strategies could help overcome (1). However 20 years have gone by and no report exists on the actual use of QTL or association genetics (AG) information for operational tree breeding in general, irrespective of species, in spite of the large volume of QTL and AG mapping information published (2). Reasons for this derive mainly from the undomesticated nature of forest trees that leads to a rapid decay of LD in tree genomes, such that marker-trait associations detected in specific mapping families do not hold in unrelated pedigrees (3, 4). Furthermore, not surprisingly, several reports in recent years have shown that many more QTLs with small and variable effects across backgrounds and environments typically underlie complex traits in forest trees (2).

Although association genetics was presented as a way to develop more efficient methods of MAS in trees, experimental results based on candidate genes (5, 6) have captured very limited fractions of the genetic variance to be valuable to breeding, leaving a large proportion of trait heritability unaccounted for, i.e. the "missing heritability". Results for trees are not unexpected and in line with findings in much larger and significantly more powerful genome-wide association studies in humans (7) domestic animals (8) and crop plants (9) substantiating the complex nature of quantitative traits. These findings not only challenge the expectations of using a few marker-trait associations directly to the improvement of complex traits, but also question the value of perpetuating such mapping efforts for breeding practice. Unless genomic-enabled breeding can deal with a broad diversity and capture a large proportion of the phenotypic variation for many traits simultaneously, there

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is no doubt that conventional quantitative genetics approaches will be more efficient to advance populations.

#### Genomic Selection: a new breeding paradigm

With the advent of high throughput genotyping technologies the Genomic Selection (GS) or Genome-Wide Selection (GWS) approach was proposed a few years ago providing a new paradigm for the integration of genomics into breeding practice (10). Instead of the standard two-step MAS approach where marker-trait associations are first discovered and then used for selection, GS estimates all marker effects simultaneously precluding the prior search for significant markertrait associations. By avoiding prior marker selection the quandary of how to capture the "missing heritability" of complex traits (11) likely explained by large numbers of small effect QTLs is avoided. In GS, a "training" population involving several hundred individuals is genotyped for a genome-wide marker panel and phenotyped for target traits of interest. From these data sets prediction models are derived and validated in a "validation" set. This model is subsequently used to calculate the genomic expected breeding values (GEBV) of the selection candidates for which only genotypes are recorded (8). Although these GEBV, just as QTLs, do not say much about the function or identity of the underlying genes, they have provided accurate selection criteria in a number of validation reports (12, 13).

#### Genomic Selection in Eucalyptus: simulations

Just as in animal breeding, GS can be readily applied to forest trees where long generation times and late expressing complex traits are also a challenge. GS in forest trees would have additional advantages: large training populations can be easily assembled and accurately phenotyped for several traits, and the extent of linkage disequilibrium (LD) can be high in elite populations with small effective population size (Ne) frequently used in advanced forest tree breeding programs. We evaluated the prospects of GS in forest tree breeding by providing broadly useful guidelines regardless of the target species, recombinant genome size or breeding cycle length (*14*). Deterministic equations were used to assess the impact of LD, the size of the training set, trait heritability and the number of QTL on the predicted accuracy of GS. Results indicated that the benchmark accuracy of conventional BLUP selection is reached by GS even at a marker density ~2 markers/ cM when Ne d"30, while up to 5-10 markers/cM are necessary for larger Ne. Shortening the breeding cycle by 50% with GS would provide an increase e" 100% in

selection efficiency, and if a larger reduction can be achieved using flower induction, gains could surpass 300%.

#### Genomic Selection in *Eucalyptus*: experimental validation

To validate our simulation results we carried out a multi-population proof-ofconcept study of GS in tropical *Eucalyptus* (15). This study was carried out in two typical breeding populations. The first was a progeny trial developed by CENIBRA (CEN) with 43 full-sib families generated from 11 elite E. grandis x E. urophylla parents. The second was a progeny trial of FIBRIA (FIB) involving 51 elite parents, mostly F1 hybrids of *E. grandis, E. urophylla* and *E. globulus*. Genotypes at >3,000 DArT markers were obtained for 783 and 920 trees from CEN and FIB respectively (16). De-regressed phenotypes for height growth (HG), circumference at breast height (DBH), wood specific gravity (WSG), pulp yield (PY), were obtained. Accuracies of GS varied between 0.55-0.88, matching the accuracies achieved by conventional phenotypic selection. Substantial proportions (74%-97%) of trait heritability were captured by fitting all the genome-wide markers simultaneously. The estimated number of QTLs controlling the trait variation ranged between 142-263 for CEN and 97-276 for FIB. When a model developed for CEN was used to predict phenotypes in FIB or vice-versa, the accuracies declined drastically showing that populationspecific GS models will drive the initial applications of GS in forest tree breeding. GS brings a new perspective to the understanding of quantitative trait variation in Eucalyptus and forest trees in general and provides a revolutionary tool for applied tree improvement.

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## Environmental stress and growth of eucalypts

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There are more than 700 *Eucalyptus* spp. that encompass in their total geographic range, environments that range from the wet and dry tropics, through temperate and Mediterranean climates, to sub-alpine mountains. This remarkable range of species and environments ensures there are a great many traits and attributes that remain unexplored, and even unknown, physiologically and anatomically. Here I synthesize recent research into the responses of the genus to drought, high and low temperatures, and salinity, with special reference to the relationships between growth and physiological attributes.

Water stress has been a priority area for eucalypt research for many years, largely as a result of known tolerances of eucalypts to drought. Nevertheless, it is only recently, for example, that have we learnt that in the tallest of the eucalypts, E. regnans, that grows in sheltered locations on deep soils, xylem vessels narrow dramatically in the uppermost few metres but taper little in the rest of the stem (Petit *et al.* 2010). This is a significant feature in protecting tall trees from dehydration, excessive cavitation and embolism and drought death. The ability of *E. regnans* forests to survive is illustrated by their water use response to the extreme heat and atmospheric dryness that, in 2009, produced the worst bushfires in living memory in Australia. Under these extreme conditions, the normally close coupling of transpiration to atmospheric conditions broke down completely, yet trees recovered within days. In radically different environments, redgums (E. camaldulensis) often grow over shallow groundwater and may display almost complete coupling of their patterns of water use to atmospheric environments such that they desiccate and rehydrate as a matter of course, and with little risk of drought death (Pfautsch et al. 2011). Recently, Brodribb et al. (2010) noted that: "xylem vulnerability to cavitation as an index of drought tolerance is widely recognized and has stimulated the characterization of hundreds of plant species in terms of the water tension required to induce a 50% loss of xylem conduction." Their study differentiated between resilient species (e.g. those capable of recovering quickly from drought-induced xylem water potentials of -10 MPa, presumably with some capacity to refill xylem vessels in situ) and non-resilient species that recovered

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far more slowly and seemingly as a result of new growth of xylem tissue. This simple classification has potential with further research to help guide breeding of more drought-resistant eucalypts through better knowledge of the proximal causes of drought death and drought recovery.

In future, forest managers are likely to be confronted by a range of issues related to increasing temperatures, especially the need to understand mechanisms by which increasing temperatures could increase or decrease growth. Our recent research has focused on respiration (e.g. Kruse et al. 2011) as perhaps the most temperature-sensitive of all major plant metabolic processes. In a recent study we showed that across a range of 12 Eucalyptus spp. originating from a wide range of environments, 'growth capacity' acclimated and increased when plants were grown at increasing altitudes (and correspondingly lower mean temperatures). However this effect was counteracted in species adapted to high altitude habitats. The acclimation response was partly driven by changes in respiratory capacity and partly by enhanced electron partitioning towards the cytochrome pathway. This was also indicated by increased temperature sensitivity of O<sub>2</sub>-reduction with altitude. We observed an enhanced dynamic response of CO<sub>2</sub>-respiration to measurement temperatures, when plants were grown at high altitudes. Together, the respiration responses appear to indicate switching of carbon fluxes between different pathways. Adaptation to growth temperature included differences in respiration and growth capacities, but there was little evidence that Eucalyptus species vary in metabolic flexibility. These results suggest that breeding efforts to produce genotypes suited to specific thermal regimes, might consider that inherently slow growth of some species (e.g. those adapted to high elevations and cooler temperatures) is associated with lower respiratory capacity – a readily measureable trait.

Finally, a considerable proportion of the salt-tolerance of plants is due to their ability to accumulate solutes compatible with the cytoplasm. Foremost amongst such solutes are cyclitols and related compounds that carry little charge and that may accumulate to moderately high concentrations with no impairment of metabolism. Such capability has been suggested as a selection criterion in eucalypt improvement programs (Lemcoff, Guarnaschelli *et al.* 1994). Our research (Merchant *et al.* 2006, 2009, 2010) has highlighted that many such solutes have other roles in eucalypts, including C transport, and thus the importance of identifying truly adaptive responses to salinity stress.

Responses by eucalypts to variations in water availability and temperature are

likely to dominate research in coming years. New approaches to quantifying and elucidating major processes such as transpiration and respiration, especially their relationships to growth and survival, will help industry focus all of: breeding programs, site selection and management.

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# Measuring, modelling and managing water-use by *Eucalyptus* plantations: a South African perspective

Peter Dye

#### Background

Since the first forest plantations in South Africa were established in 1875, their total area has expanded to the present day total of ~1.4 million ha. The principal species are *Pinus patula*, *P. elliottii*, *P. taeda*, *Acacia mearnsii*, and various species of *Eucalyptus* (comprising approximately 39% of the total forest plantation area). Forestry is concentrated in the higher rainfall regions of South Africa. Tall, evergreen and deep-rooted forestry species have mostly replaced seasonally-dormant short grasslands and Fynbos (Macchia), and so evapotranspiration (ET) may increase substantially following afforestation, at the expense of catchment water yields. This is a major concern in South Africa, where water resources are very limited.

Complaints about the effects of forest plantations on surface water supplies were already being expressed by 1915. By 1935, a series of paired catchment hydrological experiments was initiated to investigate plantation forestry effects on catchment water yields in different regions. Results obtained over many years (Scott et al., 2000) confirmed that afforestation has a marked detrimental effect on catchment water yields.

#### **Evapotranspiration studies**

Research catchments represent only a small range of forestry sites, and so a wide range of forest hydrological process studies followed to explore impacts in more detail, at smaller scales, over a wider range of sites, and for different species and genotypes. These focused largely on ET, and especially sap flow patterns as transpiration is believed to be the major component of ET at most South African forest sites. Two case studies illustrate contrasting sap flow patterns and hydrological impacts.

#### Sabie district, Mpumalanga province

In a 3-year-old *E. grandis* plantation at a mist belt location (MAP = 1459 mm) with deep, permeable, granite-derived soils, sap flow measurements in four sample trees revealed an annual transpiration estimate of 1231 mm (Dye, 1997). No water

stress was observed over 12 months despite strongly seasonal summer rainfall, and more than half of this requirement was estimated to be taken up from depths greater than 8 m.

#### Carltonville district, North-West province

Sap flow was recorded in four trees in a 3-year-old stand of *E. dunnii* planted on deep soil downslope of a gold tailings storage facility in central South Africa, to assess potential for interception of contaminated groundwater. Soil water availability was severely limited by low rainfall (629 mm) and an inaccessible deep water table (14 m). The trees experienced severe dry season water stress, restricting annual sap flow to 673 mm. The trees recovered rapidly following the start of summer rains. The study highlighted the strong influence of rainfall distribution and amount on ET pattern, and the resilience of *E. dunnii* to drought stress. Under conditions of high soil water availability, atmospheric humidity played an important role in governing daily sap flow.

#### Spatial ET measurement

The above case studies revealed complex, fast-changing ET patterns and contrasting potential for reducing catchment water yields. There is great scope for improving our estimates of spatial patterns of water-use by *Eucalyptus* plantations beyond a simple extrapolation of research catchment results (Scott, 1998), or modelling site water balances at a catchment or sub-catchment scale (Gush, 2002). Sap flow studies cannot be replicated over a wide range of soils, species/clones, age classes and weather conditions. A range of micrometeorological systems (Bowen Ratio, Eddy Covariance, scintillometry) allow measurements of ET over larger scales. A major limitation, however, is the requirement to have sensors measuring above the tree canopy. Even with the use of scaffolding towers or telescopic masts, ET can only be measured over young trees before canopy height becomes too great. Such systems are very expensive and cannot be left out in the field for long periods without close supervision. Advances have been made in using remote-sensing imagery to estimate ET from forestry and other land-use types in South Africa, using models such as SEBAL. Accuracy is greatly enhanced if ET can be recorded over sufficient sample days to track rapidly changing growing conditions and physiological status of trees, but this is mostly severely constrained by costs and availability of cloud-free imagery. The use of simple regression models based on vegetation indices, temperature and net radiation has proved successful for such purposes (Wang et al., 2007), and may be a more practical solution for long-term ET estimation.

#### Managing plantations to reduce streamflow reductions

Plantation trees planted in riparian zones were shown to use more water than trees in non-riparian sites (Scott, 1995). A long-standing measure adopted in South Africa is to leave these zones unplanted to enhance catchment water yields.

The possibility of identifying and switching to clones with a high water-use efficiency (WUE) has long been a goal among South Africa hydrological researchers (Dye, 2000). A study of young *Eucalyptus* clonal plants (up to age 16 months) representing 6 commercial clones demonstrated that significant differences in WUE do exist (Le Roux, 1996) at this young age, but the persistence of these differences in older trees remains unproved. Stem growth was found by Olbrich (1993) to be intermittent and unsynchronized among replicate 4-year-old clonal trees, requiring annual increments and a sufficient sample size to characterize WUE. One-year studies of WUE in *Eucalyptus* trees (aged 3-6 years) based on sap flow measurements and manual stem diameter measurements at fixed heights showed WUE to vary from 0.79 to 5.51 g kg<sup>-1</sup>. Further progress in exploring the potential for selecting clones with improved WUE will require well-resourced and long-term comparative studies of sap flow and stem growth among clones on uniform sites.

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# Factors controlling carbon and water balances on fast growing Eucalyptus plantations

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#### Summary

Fast-growing *Eucalyptus* plantations contribute increasingly to the world wood supply. In Brazil they cover about 4.5 millions ha, and are among the most productive plantations in the world. Their high productivity (> 40 m<sup>3</sup> ha<sup>-1</sup> yr<sup>-1</sup> in Brazil) is often associated with high water-use, thus sometimes raising concern about their environmental impact. Good understanding of the key factors controlling tree growth and water-use (Actual Evapotranspiration, AET) will be important to continue productivity improvements without increasing plantation water-use. Levels of wood production and AET depend on the interactions between genetic, climate, stand-age, soil fertility and silvicultural practices, through their impact on a large number of physical and physiological processes determining resource acquisition by leaves and roots, conversion in carbohydrates, and allocation to the different tree compartments. Our main objective in this presentation is to illustrate different ways by which climate, genetic (clonal material), soil texture, stand age and fertilization impact on plantation wood production (PW), water-use, and wateruse efficiency (WUE) using results from recent or ongoing experiments in Congo and Brazil. Through these examples, including eight years of eddy-covariance (EC) measurements of water vapour and CO<sub>2</sub> fluxes above *Eucalyptus* plantations, we also aim at showing how our understanding of the key processes determining wood production and resource-use efficiencies can be fostered by multidisciplinary research conducted on heavily instrumented sites, and involving a large spectrum of observational scales and tools.

The influence of genotype on AET, PW and WUE is illustrated by the comparison of two clones in Congo that exhibit larges differences in annual wood increment

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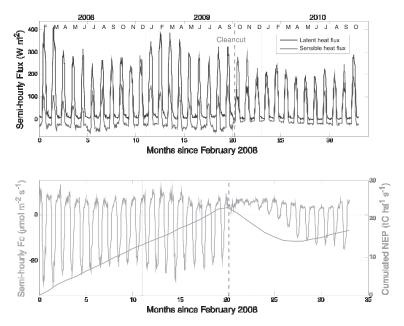
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(5.2 tC ha<sup>-1</sup> yr<sup>-1</sup> vs 10.3 tC ha<sup>-1</sup> yr<sup>-1</sup>). Leaf gas exchanges measurements did not show significant differences in photosynthetic capacities (Jmax and Vcmax), but large differences in stomatal conductance and intrinsic WUE (WUE<sub>i</sub>). The higher WUE<sub>i</sub> for the most productive clone was accompanied by much higher (about twice higher) LAI, and gross primary production (GPP, measured by EC), while AET (also measured by EC) was only marginally higher than AET of the less productive clone, resulting in large differences in WUE for GPP, observed at the canopy scale by EC. When considering WUE for PW, the differences in WUE between the two clones were amplified by differences in C allocation: PW was 65% and 81% of ANPP for the less productive clone and the more productive clone, respectively.

The effects of fertilization on key physiological processes determining PW was studied in an experiment conducted in Brazil comparing carbon (C) allocations in plantations with or without potassium (K) fertilization (Epron et al., 2011). K fertilization increased GPP and decreased the fraction of carbon allocated below ground. ANPP was strongly enhanced, and because leaf lifespan increased, leaf biomass and LAI was enhanced without any change in leaf production, and wood production was dramatically increased.

The effects of stand age, and clear-cut on the C and water-balance of *Eucalyptus* plantations was investigated using EC measurements of water vapour, energy and CO<sub>2</sub> exchanges between a plantation and the atmosphere (Fig. 1), measurements of water table depth, as well as measurements of LAI, soil moisture (till 10 m deep), and rooting depth over entire rotations in Brazil. EC measurements started 20 months before clear cut, thus covered the two last years of a 6 year rotation. During these two years, AET was 1290 mm yr<sup>-1</sup>, almost matching annual rainfall (1360 mm yr<sup>-1</sup>). Soil below 6 m was depleted in water. Percolation of recently infiltrated rain water never reached this depth due to the high AET. Sensible heat fluxes (H) were usually very low (Fig 1a), since most of the available energy was used for evapotranspiration (Latent heat fluxes, LE), except when soil moisture in the 0-10 m soil depth decreased below a threshold value, below which the bowen ratio (H/LE) increased sharply due to stomatal closure. Thus, at the end of the rotation, GPP and wood production were water-limited for large parts of the year. After clear-cut, AET decreased sharply, while H increased. These AET decreases allowed water to percolate deeply, reaching depths below 10m about 3 months after planting, and water-table rising of about 3 meters in the following months. After planting, AET increased with increasing LAI, and reached pre-clear-cut levels about 16 months after replanting. Root system expanded rapidly deeply in the soil reaching about



**Figure 1.** mean semi-hourly latent and sensible heat fluxes (top subplot) and net  $CO_2$  fluxes (Fc) measured at the eddy-covariance Itatinga site (Sao Paulo state, Brazil) from February 2008 to October 2010. Mean semi-hourly fluxes are computed for each month. The measurement period shown covers the two last years of a rotation, the clear-cut, and the first year after replanting. Negative Fc values mean a net C uptake by the *Eucalyptus* plantation, and positive values a net emission of C. Fc was positive after clear cut, due to the cessation of photosynthesis. Negative values were observed again five months after replanting due to LAI and GPP increases. Cumulated net ecosystem production (NEP=net C uptake) is also shown.

10 m, 20 months after planting. These results together with other soil moisture monitoring over the length of rotations showed that deep percolation (>10 m) occurs only the first year or 18 months after re-plantation, while for the rest of the rotation, most of the rainwater is recycled to the atmosphere, except during exceptionally rainy years. These results obtained in Brazil contrast with those obtained in less productive eucalypt plantations in Congo, where deep percolation occurred each year over the length of the rotation due to much lower AET, resulting from lower LAI, soil retention capacity, net radiation and atmospheric water demand. At the EC site in Brazil, GPP, ecosystem respiration (Re), soil respiration and net  $CO_2$  fluxes (Fc, Fig. 1b) exhibited large seasonal variations, mostly driven by incoming radiation and soil water availability. Ecosystem respiration remained high after

clear-cut, due to the abundant decomposition of root and surface residues (slash). Net ecosystem production (NEP=GPP-Re), which represents the amount of C sequestrated by the forest, was about 11 tC ha<sup>-1</sup> year<sup>-1</sup> before clear cut. It was negative (net C loss) thereafter due to the cessation of GPP, and returned again to positive values about 8 months after planting. For the first year after planting, increases in GPP and NEP were mostly driven by increases in LAI.

These examples show the large impacts of C allocations and rooting depth on wood production on these deep soils. Ecophysiological models should then be improved to better describe the evolution of rooting depth with stand age, and to account for nutrient effects on leaf life-span, LAI, GPP and C allocations.

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# Unsolved problems in whole-tree physiology: how to make progress for clonal Eucalyptus forests

Michael G Ryan<sup>1</sup>

Whole tree physiology studies the behavior of the whole organism, rather than viewing trees as a collection of independent leaves, roots, structure, flowers and fruits. Recent research, however, has focused on ecosystem-level fluxes at one end of the scale and on molecular biology at the other end—with little attention on the organismal physiology that links these scales. I review the evidence that whole tree physiology strongly regulates ecosystem-level fluxes of carbon, water and nutrients, and review the major unknowns in whole tree physiology. Changes in photosynthesis, transpiration, and carbon allocation with tree size and age are the most obvious impacts of tree physiology on ecosystem fluxes, but many other impacts remain unexplored. Some of the major unknowns include (1) the role of stored carbohydrates in sustaining physiology in lean times and the mechanisms that control storage input and release, (2) the regulation of phloem transport at different time scales, (3) the role of autotrophic respiration in regulating plant carbon balance and a mechanistic model for autotrophic respiration, (4) controls over processes that regulate plant 'sinks' for carbon and sink regulation of tree photosynthesis, (5) the mechanism(s) of size-related decline on wood growth, (6) the mechanisms that regulate carbon and nutrient allocation, (7) maintenance of water transport under water stress and the role of stored water in maintaining water transport, and (8) the molecular control of physiology and carbon allocation.

For *Eucalyptus*, probably the most important unknowns relate to drought (resistance, growth and survival) and the mechanisms that influence these. Other important unknowns include controlling partitioning of photosynthesis to wood production, the consequences to the tree and ecosystem, if any, of maximizing partitioning to wood production, and the maintenance of strong sink strength to maintain high photosynthesis. A mechanistic whole-tree understanding of physiology should allow us to predict what characteristics should be selected for and what the trade-offs at the whole-tree level might be for such selection.

Simply measuring something can lead to new insights, as the simple, inexpensive measurements of water transport (sapflux) have taught us. At the whole tree scale,

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measurements of photosynthesis remain problematic. Photosynthesis is often inferred from system-level measurements of eddy covariance, but these are measurements by difference, and major problems remain in measuring ecosystem respiration and in validating the technique by matching independent measurements of energy balance. We do not yet have the technology to measure phloem transport, but if we did, we would make rapid progress on carbon allocation. Techniques for making simultaneous measurements on different components of a tree are also poorly developed. Finally, measurements of root growth and biomass, and mycorrhizae are currently very labor intensive.

The size of trees has limited whole-tree studies, but we could also be more creative . Plant physiologists have settled on a very small flowering plant (*Arabidopsis*) as their model organism to link molecular biology and physiology or plant performance. If we had a similar, tractable tree model organism that we could easily manipulate and measure, whole-tree physiology could progress rapidly. Of course, to identify such an organism, we would have to understand the essential components of 'treeness', and what would be lost working with a small woody plant compared to a large tree.

#### Key Words

Stored carbohydrates, phloem transport, autotrophic respiration, sink regulation of photosynthesis, size-related decline in wood growth, carbon and nutrient allocation, water transport

# Well established as well as new technologies provide options to reduce increasing insect pest and pathogen damage to eucalypt plantations

Michael J. Wingfield, Jolanda Roux, Bernard Slippers, Brett Hurley, Jeff Garnas and Brenda Wingfield<sup>1</sup>

It is well recognised that insect pests and pathogens represent a serious threat to Eucalyptus plantation forestry. This is not only where these trees have been established as non-natives in plantations but also in areas where they occur in their natural habitats. The growing challenges to deal with diseases and insect pests of Eucalyptus arise from introductions into new environments, which are increasing in number annually. Interestingly, there are also growing numbers of unexpected new insect and disease problems that are emerging from new associations linked to host shifts. Adding to these problems, there are also emerging examples of new pest and disease problems arising from apparent climate change events. While the many new pest and pathogen problems in Eucalyptus plantations during even the recent past have raised much substantial concern amongst forest owners, there is reason not to be unduly pessimistic. This is assuming that plantation owners have not capitalised on limited genetic diversity such as via the planting of small numbers of clones or clones that are genetically very closely related.

There are many well-established options for disease and pest management that will continue to be useful in the future. Ensuring diversity of planting stock and thus avoidance of so-called "boom and bust" cycles will provide a relatively high level of long-term security. Biological control for insect pest management will be increasingly important as pests become established in new areas. Importantly, these approaches will rely increasingly on a much deeper knowledge of the planting stock and on biological control agents than has been required in the past. This new knowledge is emerging rapidly from the application of tools such as those linked to molecular genetics that were previously not available. For example, increased knowledge of the genetic makeup of Eucalypts will make it possible to more easily select planting stock resistant to damage. Molecular genetic techniques are already facilitating a deeper understanding of the populations of pests, pathogens and

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biological control agents, making it possible to understand for example the durability of resistance and the long-term sustainability of biological control programmes. Furthermore, the impact of knowledge arising from the rapidly increasing ability to sequence pathogen, pest, biological control agent and tree genomes will have a substantially positive impact on disease and insect pest management in the future. While immediate problems require rapid response, the key to long-term sustainable Eucalyptus forestry clearly lies in robust and wise investments in research and development underpinned by a long term vision.

## Minimizing disease risks in eucalyptus plantations

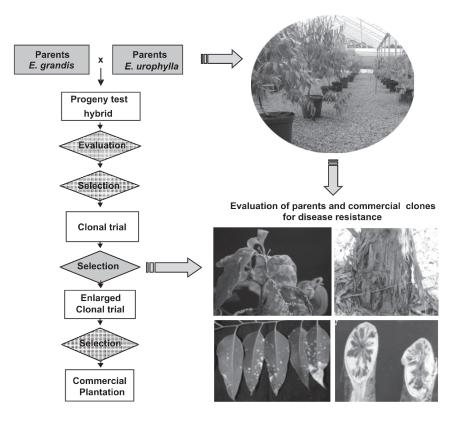
Acelino Couto Alfenas<sup>1</sup>, Lúcio Mauro da Silva Guimarães<sup>1</sup>, Rodrigo Neves Graça<sup>1</sup>, Rafael Ferreira Alfenas<sup>1</sup>, Talyta Galafassi Zaperlon<sup>1</sup>, Carla Cristina Gonçalves Rosado<sup>1</sup>, Edival Angelo Valverde Zauza<sup>2</sup>, Reginaldo Gonçalves Mafia<sup>3</sup> & Marcelo Magalhães Coutinho<sup>4</sup>

Until the 1970s, the area planted with eucalyptus in Brazil was relatively small and concentrated in the states of São Paulo and Minas Gerais. Until that time, the eucalyptus plantations were generally considered to be free of major diseases. Subsequently, the expansion of plantations to regions with favorable conditions for infection, the use of "more productive" genotypes without prior knowledge of their disease resistance, the implementation of new management techniques, and the successive plantation cycles in the same area have favored the incidence and severity of diseases caused by endemic or accidentally introduced pathogens (Alfenas et al., 2009). Among these diseases, the following are noteworthy: eucalyptus canker (Chrysophorte cubensis), eucalyptus rust (Puccinia psidii), leaf blights and defoliation, cause by fungi (Cylindrocladium spp., Teratosphaeria nubilosa, Rhizoctonia spp.) and bacteria (Xanthomonas axonopodis and Pseudomonas cichorii), Ralstonia wilt (Ralstonia solanacearum), Ceratocystis wilt (Ceratocystis fimbriata), eucalyptus die-back (Erwinia eucalypti), and Quambalaria stem girdling and leaf spot (Quambalaria eucalypti). The heterogeneity of seedling plantations and the incidence of canker in the early 1970's led to the deployment of the eucalyptus cutting technique, in Brazil, (Alfenas et al., 2009); presently used globally as a disease control method for many tree species. Currently within the approximately 4.7 million hectares planted with eucalyptus in Brazil, about one million hectares are planted with 362 clones, primarily inter-specific hybrids (Eucalyptus grandis x E. urophylla = "urograndis") or pure species that are established in areas ranging from 10 to 34,000 hectares/clone/company (TF Assis, assisteo@terra.com.br, personal communication). Despite the inherent risks associated with homogeneous plantations, cloning is a powerful tool for the commercial multiplication of superior genotypes that are resistant to diseases. A continuous generation of new clones with different genetic backgrounds is essential, incorporating species with diverse traits of commercial interest, such as growth, pulp yield, disease resistance, and frost or drought tolerance. Species such as E. globulus and E. dunnii (pulp), E. smithii

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**Figure 1.** Stages of the breeding program for which evaluations for disease resistance are necessary, based on the *Eucalyptus grandis* x *E. urophylla* hybrid. Adapted from (Fonseca et al., 2010).

and *E. benthamii* (frost tolerance), *E. camaldulensis, E. tereticornis, E. longirostrata*, and *E. brassiana* (drought tolerance), and *E. pellita* (disease resistance) should be inter-bred with *E. grandis, E. urophylla*, and their hybrids. For effective control of diseases affecting the eucalyptus plantations, it is essential to perform constant and systematic monitoring of diseases from the nursery to the field, including clonal and progeny trials. Because these forest breeding programs are typically long-term processes, it is essential to identify disease-resistant parents early in the process. Currently, most Brazilian forest companies are conducting selections for disease-resistant genotypes (Fonseca et al., 2010) (Figure 1). Furthermore, it is also critical to understand the genetic basis of resistance in relation to the variability in the pathogen populations, because new pathogen races can emerge and overcome resistance.

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## Breeding Eucalyptus for insect resistance

Carlos F. Wilcken<sup>1</sup>

Eucalyptus plantations in Brazil suffer with insect pest infestations since it has begun the commercial cultivation in the country. Most of pests are native insects, as leaf-cutting ants, termites, caterpillars and defoliator beetles and their control is made by use of chemical or biological insecticides, with good results. In last decade, it was verified the detection and establishment of exotic pests, as redgum lerp psyllid (Glycaspis brimblecombei), bronze bug (Thaumastocoris peregrinus) and eucalyptus gall wasp (Leptocybe invasa). For these insect pests it has been studied biological control methods, with introduction of natural enemies from Australia. To redgum lerp psyllid, the results of biological control are partially satisfactory. To other two exotic pests, the programmes are in the beginning yet. Other important control method do not used until now is the plant resistance, selecting Eucalyptus genotypes less susceptible to these insects. Until this moment, we do not have breeding programmes specific to pest resistance, as we have to Eucalyptus diseases. To G. brimblecombei it was developed a protocol to evaluate the susceptibility or resistance of Eucalyptus clones in laboratory conditions, it being one of the unique cases that forest companies incorporate this methodology and field evaluations to select resistant material. Considering subgenus Symphyomyrtus, all Eucalyptus from section Exsertaria (E. camaldulensis, E. tereticornis, E. brassiana, etc.) are considered susceptible to lerp psyllid, and *Eucalyptus* from other sections are resistant, except *E. urophylla*. The hybrid clones have showed a response gradient, with genotypes highly resistant and others considered susceptible. After pest detection in 2003, many forest companies have avoided planting susceptible genotypes but the pest has demonstrated to have high adaptation capability to new genotypes. For gall wasp, the plant breeding to resistance is the best option to control this pest, despite the time necessary to develop resistant clones, because chemical and biological methods have some restrictions, due the exigencies made by forest certification systems, considering use of chemical insecticides, and due scarce studies to rear natural enemies and difficulties to obtain official permission to introduce them in Brazil. However, the development of resistant genotypes will be hard, due the frequent introductions of new exotic pests in Brazil, causing distinct injuries in the trees. A new way to research will be the use of GMO techniques in next years, to incorporate resistant genes in eucalyptus to these new pests. Keywords: plant resistance to pests, eucalypt, exotic pests, *Glycaspis brimblecombei* 

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## Strategy of Eucalyptus breeding for disease resistance

 $\label{eq:Edival A. V. Zauza $^1$, Acelino Couto Alfenas$^2$, Lúcio Mauro da Silva Guimarães$^1$ and João Flávio da Silva $^1$}$ 

The *Eucalyptus* plantations cover about 1.5% of the agricultural area in Brazil and contribute to 4% of GDP and 3% in exports of forest products. Technological and research advances in silviculture and genetic improvement have increased productivity up to 80 m<sup>3</sup> of wood / ha / year, with an average of 35 to 45 m<sup>3</sup>/ha/year. However, the greatest challenge to the introduction and commercial use of exotic species is the adaptation of species to climatic conditions. Even when maladapted species are able to survive, they are subjected to continuous stress that limits the expression of their maximum genetic potential. The incidence of insects and diseases frequently represent another limiting factor, because the exotic tree species have not co-evolved with most of the local insect and pathogen pests. In recent decades, the expansion of *Eucalyptus* plantations in Brazil has been frequently associated with frequent disease outbreaks (Alfenas et al., 2009). Prominent diseases, such as ceratocystis wilt, ralstonia wilt, rust, bacterial fungal leaf blights, have limited the establishment and growth of plantations that contain susceptible species or clones in regions favorable to disease establishment. Plantations of hybrid clones or elite-resistant varieties represent the most efficient strategy for disease control. In general, fungicide application is restricted for the control of nursery diseases, and rarely for rust control in the field. The *Eucalyptus* clonal forests offer potential to establish homogeneous, disease-free stands of high yield. Currently, the hybrid clones of *E. urophylla x E. grandis* ("urograndis") are among the most planted material in Brazil because of their high adaptability, wood quality for pulping, and resistance to chrysoporthe canker. However, other interspecific hybrids have been developed in an attempt to incorporate specific traits from other species, such as drought and frost tolerance, disease resistance, and high pulp yield (Table 1). Primary breeding strategies are based on selecting species/provenances, coupled with individual genetic selection within populations to capture the natural variability among and within populations. The recurrent selection method and its variants are applied to genetic improvement of *Eucalyptus* to obtain new clones of interspecific and intraspecific hybrids. Backcrosses, mutation induction, and polyploidy have also

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Species	Positive Characteristics	Negative Characteristics*
E. grandis	Good adaptabilityGood fiber quality	Susceptibility to cancer Susceptibility to rust Susceptibility to leaf spot
E. globulus	High yield pulp High content of pentosans Wood with high basic density Low lignin content Low concentration of extract	Low adaptability Susceptibility to diseases
E. pellita	Resistance to cancer Resistance to rust Resistance to leaf spot Wood with high basic density Good adaptability	Susceptibility to SPEVRD** High content of extractives High lignin content Low content of pentosans
E. camaldulensis	Drought tolerance Wood with high basic density	High content of extractives High lignin content Low content of pentosans
E. urophylla	Resistance to cancer Good adaptability Wood with high basic density	Susceptibility to leaf spot Susceptibility to SPEVRD

 Table 1. Characteristics of some Eucalyptus species potentially important for inclusion in breeding programs for resistance to diseases

\*Negative impact on pulp and paper production.

\*\* Dry Eucalyptus Shoots of Vale do Rio Doce.

Source: Alfenas et al., 2009

been applied to develop new genotypes. These strategies help ensure the sustainability of commercial plantations and the continual contributions from the improvement program through the selection in advanced generations and superior clones. With interspecific breeding to incorporate contrasting traits, it is usually necessary to make continued backcrosses to the species of interest as the recurrent donor to recover the desired forest features and industrial properties (Alfenas et al., 2009). For each breeding generation it is essential to perform disease screening under controlled conditions to select disease-resistant genetic materials for commercial cloning or identifying sources of resistance for other crosses. For disease-resistance evaluations, reliable inoculation protocols are essential (Ruiz et al., 1989; Zauza et al., 2004; Alfenas et al., 2009; Graça et al., 2009). In recent years, approximately 90% of elite-clones were susceptible to at least one of the main diseases tested using artificially inoculations under controlled conditions. Determining the genetic basis and the mode of inheritance of disease resistance is an essential step to obtain disease-resistant plant material for the breeding program. The breeding strategy varies according to the inheritance model. To reduce losses caused by rust, efforts have been conducted to select and plant rust-resistant clones

and determine the mode of inheritance. In *E. grandis* the segregation pattern of resistance to rust (*Puccinia psidii*) is controlled by one locus with major effect, *Ppr-1* (*Puccinia psidii* resistance, gene 1), which is tightly linked to RAPD marker AT9-917 (Junghans et al. 2003). Controlled inoculations of other families allowed the identification of a homozygous, rust-resistant mother parent of *E. grandis*, currently used to obtain rust-resistant progenies, regardless of pollen source. Screen tests for resistance to Ceratocystis wilt showed a continuous variation in resistance, ranging from highly susceptible to highly resistant, which indicates a pattern typical of horizontal resistance. Additional studies are needed to determine the genetic basis, the new sources of resistance and the inheritance patterns of resistance of *Eucalyptus* spp. to other diseases. Furthermore, a better understanding of the population genetic structure of pathogens is needed to determine the role of pathogen variability in disease resistance that is targeted by the breeding strategies.

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## Clonal Forestry – is it always the best deployment option?

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The aim of commercial plantation forestry is to produce the highest possible value yield of harvestable product across the whole estate. This requires the use of physiologically robust planting stock of proven high genetic quality but the method of propagation does not *per se* add value.

Nursery stock may be raised from seeds or by vegetative propagation, with the choice dependent on species biology and factors such as maturity of the genetic improvement programme; and the technical capabilities of the grower. There is often no one "right" approach. Cloning is essential for hybrid eucalypt varieties, but for pure species the decision is particularly difficult where vegetative propagation is possible but not easy. Large scale production of quality planting stock is challenging and there is a strong risk of selecting for propagability rather than the production traits which are of real commercial interest. In such cases family forestry with or without vegetative propagation of seedlings (clonal family forestry) is a real option.

Case studies of *E.globulus*, grandis, *dunnii* and *nitens* are used to illustrate the pros and cons of cloning vs. the deployment of seedlings of tested families, with additional reference to other informative examples from other genera (Acacia, Abies and Pinus).

Key benefits of family forestry are reduced plant costs and reduced risk of suboptimal genotype/site matching in space and time because of the homeostatic benefits of within family genetic variation.

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## Hybridization and cloning eucalyptus

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#### Hybridization

Until the event of clonal forestry at the end of the 70s, intra-population recurrent selection was the principal breeding strategy for *Eucalyptus* in Brazil [6]. The cloning system introduced at that time by Aracruz [4], where superior spontaneous *Eucalyptus* hybrids were used to derive clonal forestry, served as a general model for most Brazilian companies. The recognition that hybrids could produce superior trees both in growth and disease resistance, strongly influenced *Eucalyptus* breeding programs in Brazil. Since then hybridization has been the principal breeding strategy used to improve *Eucalyptus* species in the country.

During the last decades the increasing demand of industries for process productivity and cost reduction, as well as of commercial areas for product quality, breeding objectives have been extended to wood quality, mainly pulp, charcoal and solid wood. Artificial hybridization has gained importance and several new crosses have been synthesized.

Currently for pulp production crosses have been oriented to combine the superior *E. globulus* wood properties with the growing of traditional and adapted species and clones. This species is considered one of the most important for this purpose due to its low specific consumption (high wood density and pulp yield), low lignin content, and good bleaching ability. Its excellent cooking and bleaching characteristics are considered to be related to the high S/G ratio, one of the highest among all woody species.

Hybrid production using *Corymbia* species, especially *C. torelliana*, *C. citriodora* and *C. maculata* is currently one of the principal strategies for charcoal production and these hybrids are being considered a new option to produce energy. These species have high wood density, low specific consumption, and superior mechanical resistance, all traits important for the steel mill industries. Also, the use of high density species like *E. paniculata*, *E. camaldulensis*, *E. pellita* and *E. resinifera* in crosses with hybrid clones has been an efficient short term strategy for increasing charcoal quality.

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Hybridization is also being used to improve frost, drought and disease resistance. The use of resistant or tolerant species in hybrid crosses is enabling significant risk reduction for commercial plantations.

The intensive hand labor costs involved in making hybrids led to the development of new and more efficient controlled crossing systems, with significant gains in productivity and reduction of costs. The development of OSP (One Stop Pollination) [5] and AIP (Artificially Induced Protogyny), and the inception of potted (indoor and outdoor) breeding orchards, were important factors that intensified the use of hybrids in breeding programs [2].

#### Cloning

Crossings between trees of heterozygous species frequently produce heterogeneous families. This aspect associated with the occurrence of abnormalities in many crosses makes the utilization of hybrids highly dependent on cloning.

The establishment of a functional and super intensive cloning system based on mini-cuttings represented an important advance in commercial scale *Eucalyptus* cloning [1,7].

While there has been significant progress in the *Eucalyptus* rooting methods, several studies on cloning are in progress. Propagule production methods have evolved from an extensive system with low productivity, progressing through an intensive system in clonal gardens reaching the present super-intensive systems, with high propagule productivity in hydroponics supported mini-clonal hedges.

The search for new advances in this field have found that cloning selected trees from sprouts obtained directly in the lignotubers has improved rooting ability in the current species and hybrids, and has especially enabled use of the clonal forestry concept in recalcitrant species for rooting like *Corymbia* and *E. cloeziana*.

#### **Clonal deployment**

The methodology currently used for clonal deployment comprises early selection for progeny and clonal trials, with the data being accessed mainly at age two. In general Pilodyn is used to rank individuals and later the clones in relation to wood density. Single tree plots with 30-40 replications on the field clonal experiments are currently used. BLUP analysis is performed for volume and wood quality. Usually one progeny trial is established by region and G x E studies in 2 or more localities depending on the environmental variability. NIRS analysis is used to access wood and pulp quality when a smaller group of individuals is defined. When a set of 20 clones is selected they are technologically characterized by laboratory analysis. After that, selected clones are screened for disease resistance using artificial inoculation and also tested on a commercial scale in nurseries for final selections. Demonstration areas are established in order to check the field performance before recommendation.

The experience of more than thirty years cloning *Eucalyptus* inter-specific hybrids, together with the evolution of silvicultural practices, have been the major drivers of the progress experienced in forest productivity in Brazil [3]. The evolution of pulp productivity obtained by cloning superior hybrids has been significant. While in 1970 the average MAI-Cellulose was 6 ton/ha/year it now reaches 12 ton/ha/ year. Current projections suggest the possibility of achieving a potential productivity of about 16 ton/ha/year by 2015 [3]. New projections of companies located in better sites are to reach 20 ton/ha/year after 2020. The current and the new projections are based on cloning interspecific hybrids that will be of fundamental importance on achieving these new levels of productivity.

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## State of the art of eucalypts breeding in the world

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Eucalypts plantations are currently an indispensable platform to fulfill human needs for wood products. As world population increases, improving eucalypt productivity and getting it adapted to less favorable environmental conditions become mandatory issues.

In this context, efficient traditional breeding programs as applied to eucalypt industrial plantations are required. Defining clear and relevant breeding objectives is a critical initial step in this process. Adaptation (survival/growth rate) is the universal major objective, but attention must be given to a restricted number of relevant wood quality traits impacting long term business. Knowing genetic control of the selected traits is critical.

Despite species most suitable to current productive areas are well known, there is evidence that genetic variability in Eucalyptus / Corymbia genus is far from being intensively explored. Multispecies programs will probably represent a key strategy to geographic expansion of sustainable forest plantations border.

Eucalypt breeding cycles are still too long. Traditional recurrent selection method, despite neglecting capture of non-additive effects in the recombination phase, should be preferred once this option saves time and money. This approach is valid also for multispecies programs, through development of synthetic populations (mixed landraces) in the long run. If the final objective is deploying superior clones, using half-sibs (pollen mix) is a good way to optimize resources and ensure results. It is also important to combine short and long term strategies. Short term narrow based programs involving elite material has proven to be efficient.

Evaluation phase requires huge attention. Progeny trials are a crucial step towards clonal trials (at least 100 individuals / progeny along locations and 20 ramets / clone / location). Lattice designs and Single Tree Plots shall be used in both cases. Trials shall not receive special care, but precision must be granted by environmental control. Phenotyping wood quality in scale is still a challenge. Genome wide selection may represent a shortcut to clonal trials and must be better evaluated in operational programs.

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BLUP is the most efficient approach for estimating genetic merit in the selection phase, but even mass selection works well. Clones shall be selected based on total genetic merit (additive and non-additive) while parents based only on the additive merit. G x E interaction is usually more significant for top clones selection than for breeding populations. Tandem selection is unavoidable so far (volume first, wood quality later) and half the rotation age seems to be an efficient and safe approach for early selection. Top clones deployment shall not happen every year for both operational and breeding efficiency reasons. Five to ten operational clones being partially renewed each three or four years seems to be good enough for providing long term sustainable gains, making feasible large scale production (nurseries and forests) and avoiding genetic vulnerability risks.

# Tree growth and stand production in mixed-species plantations of *Eucalyptus* and *Acacia mangium* in Brazil and Congo

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#### Summary

A general trend of increase in N fertilizer requirements over successive rotations is observed in commercial eucalypt plantations and planting N<sub>2</sub>-fixing species in eucalypt plantations might be an alternative to *Eucalyptus* monocultures. Tropical multi-species plantations may generate greater productivity through a more complete or more efficient use of natural resources. However some studies have shown no effect or a depressive influence of N<sub>2</sub>-fixing trees on the growth of *Eucalyptus* trees and on the overall stand productivity. It is then needed to gain insight into the influence of silvicultural practices and ecological conditions on inter-specific interactions (competition, facilitation or competitive reduction) in plantations mixing *Eucalyptus* with N<sub>2</sub>-fixing species.

The aims of this study were: i) to examine the effect of mixtures of *Eucalyptus* and *Acacia mangium* on stand growth, biomass allocation and net primary productivity, and ii) to assess the behaviour of the two species in contrasted pedoclimatic conditions.

The same experimental design was replicated at 4 sites in Brazil and on 1 site in the Congo (table 1). The treatments were:  $T_1$ : pure *Acacia mangium* stand (100A);  $T_2$ : pure *Eucalyptus* stand, without N fertilization (100E);  $T_3$ : pure *Eucalyptus* stand, with N fertilization (100E+N);  $T_4$ :  $T_2 + A$ . mangium planted in mixture at a density of 25% of the eucalypt density (25A:100E);  $T_5$ :  $T_2 + A$ . mangium planted in mixture at a density of 50% of the eucalypt density (50A:100E);  $T_6$ :  $T_2 + A$ . mangium planted in mixture at a density of 100% of the eucalypt density (100A:100E);  $T_7$ : Mixture at 1:1 proportion between *Eucalyptus* and *A. mangium* (50A:50E). Tree growth and stand biomass production were monitored up to the end of stand rotation. Site-specific

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allometric relationships were established by destructive sampling of 10 trees of each species in the 100A, 100E and 50A:50E treatments at the end of the rotation. Ten trees per species were also sampled in 50A:100E at the ESALQ and Congo sites. The allometric relationship for *Acacia* trees established in 50A:100E at the ESALQ site were used for all the treatments of the additive series in all the Brazilian sites. The allometric relationship established at each site in 100E were used for *Eucalyptus* trees in the additive series.

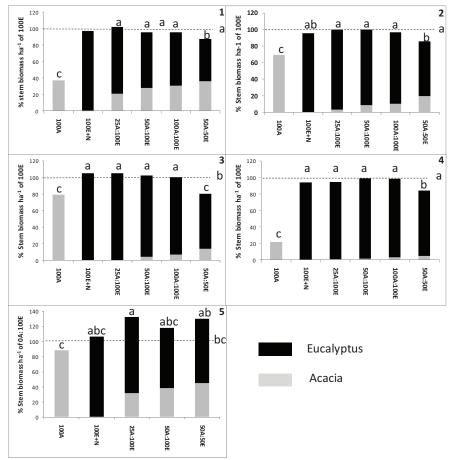
*A. mangium* mean heights at the end of the rotation ranged from 16.0 m to 19.4 m in Cenibra and Congo (at 66 and 77 months of age, respectively). In contrast *A. mangium* growth was much lower in the 3 other sites with tree mean height ranging from 8.4 m to 16.2 m in Suzano, Esalq and IP sites (at 63, 72 and 73 months of age, respectively). Increasing *A. mangium* stocking density led to a marked decrease in *Acacia* heights and circumferences whatever the sites. In replacement series, heights and circumferences of *Eucalyptus* trees were significantly larger than in pure stands (50A:50E > 100A). The opposite was observed for *A. mangium* trees (50A:50E < 100A), except at Cenibra where *A. mangium* growth was similar in both treatments. In additive series (T1 to T6), no effect of *A. mangium* neighboring on *Eucalyptus* growth was observed except in Cenibra where the increase in *A. mangium* density led to a significant decrease in *Eucalyptus* growth.

The effect of treatments on stem biomass was site-dependant (figure 1). N fertilization did not increase significantly stand biomass at the end of the rotation,

Company Site	Latitude Longit.	Soil type <sup>1</sup> C clay content <sup>2</sup> (%)	Organicmatter <sup>2</sup> (%)	Exc. bases <sup>2</sup> (cmolc kg <sup>-1</sup> soil)	Annual rainfall (mm)	Mean T <sup>re</sup> (°C)	Air humidity (%)	Length dry season
Cenibra Santa Paraiso Brazil	19°16S41°47W	Ferralsols 60	2.5-3.0	0.3-0.5	1240	24.7	70	6 months
Suzano Itatinga 1 Brazil	23°.11548.25W	Ferralsols 20	1.0-2.5	0.1-1.0	1310	19.9	60	2 months
International Paper (IP) Luiz Antônio Brazil	21°35S47.31W	Ferralic arenosols - 10	1.8-2.0	0.3-0.6	1250	23.0	70	4 months
USP EsalqItatinga 2 Brazil	23°02S48°37W	Ferralsols 20	1.02.5	0.1-1.0	1360	19.0	60	2 months
CRDPI Pointe-Noire Congo	4°41S12°01E	Ferralic arenosols - 5	0.5-1.0	0.2-0.3	1200	25.0	85	5 months

Table 1. Main site characteristics of the mixed-species eucalyptus plantations trials.

even though a 5-7% increase in production (100E + N > 100E) was observed at Itatinga and in the Congo. The lowest stem biomass was always found in the pure *Acacia* stands. Rates of fallen trees > 35% in 100A from 5 years after planting onwards accounted for the low productions at Cenibra and IP sites. Adding *A. mangium* trees in 100E stands led to an increase in stem biomass by 5-20% over the first half of the rotation, except at the Suzano site where the effect was null and at the IP site where a negative effect was observed (data not shown). At the end of stand rotation, there were no more significant differences between treatments in



**Figure 1.** Percentage of total stem biomass per ha in the different treatments, relatively to stem biomass per ha in the 100E treatment (represented by a dotted line). 1= Cenibra (age 66 months); 2= Suzano (age 63 months); 3= Esalq (age 72 months); 4= IP (age 73 months); 5= Congo (age 77 months). Different letters indicate significant differences between treatments (P < 0.05).

the additive series, excepted in Congo where the mixtures exhibited total stem biomasses 18 to 33% higher than in 100E. The 50A:50E treatment was always less productive than the treatments in the additive series except in Congo where stand stem biomass was 30% higher than in 100E.

The biomasses of tree components left on-site at the harvest (*i.e.* bark, leaves and branches) were 10-96% higher in the mixtures than in monospecific stands depending on the sites and treatments.

The early increase in stem biomass in the additive series in Brazil may result from the higher stocking densities enhancing light and water capture by stands. A strong competition for water between the 2 species might be involved in the lack of significant difference in production between treatments at the end of the rotation. Other studies in similar eucalypt plantations have shown that all the rainfall is evapo-transpirated from 2 years after planting onwards in Brazil. The higher production of mixtures in Congo might result from an improved N status of *Eucalyptus* trees in N-depleted soils, as well as a reduction of the amount of water lost by deep drainage in the mixture (H" 500 mm year<sup>-1</sup> in 100E).

These results show the interest of a network of experiments to get a general picture of the potentialities of tropical mixed-species plantations. Mixtures of *Eucalyptus* and *A. mangium* trees might substitute for *Eucalyptus* monocultures in areas with adequate conditions for *Acacia* growth (high temperature and air moisture...) and/or marked soil N deficiencies. In the other sites such associations might be also attractive when taking into account other potential positive effects: better weed control, reduced N fertilisation, enhancement of C sequestration and N cycling, reduced risks of tree diseases or diversification of wood products. However, socio-economical studies are required to assess the profitability of such plantations and the conditions of their establishment at a large scale.

# Quantifying the effects of species interactions in mixed species plantations

David I. Forrester<sup>1,2,3</sup> and Jürgen Bauhus<sup>3</sup>

It is not unusual for the productivity of mixed-species plantations to exceed that of monocultures of the component species, as long as they are growing on appropriate sites. For example productivity is often enhanced by mixing eucalypts with a nitrogen-fixing species (Forrester et al., 2006) but on sites where nitrogen is not a major limiting resource, the addition of any "fixed" nitrogen may have little influence on growth and instead the nitrogen fixing species may compete for other resources, or be outcompeted by the fast growing eucalypts.

#### Quantifying the effects of species interactions

Comparisons of mixtures relative to monocultures have often been described using concepts such as facilitation, complementarity and competitive reduction (Vandermeer, 1989). However, these concepts can be difficult to quantify and separate. An alternative way to examine growth, also in mixed stands, is the Production Ecology Equation, where

- Gross primary production = resource supply x fraction of resources captured
  - x efficiency with which resources are used

This equation can be used to describe woody biomass production (Mg<sup>-1</sup> ha<sup>-1</sup> year<sup>-1</sup>) (minus C allocated to respiration and non-woody tissues) as a function of the supply (Mg<sub>resource</sub> ha<sup>-1</sup> year<sup>-1</sup>), capture and use efficiency of water, light and nutrients (Mg<sub>biomass</sub> Mg<sup>-1</sup><sub>resource</sub>). Each of these factors can be quantified and this concept was applied to agricultural crops by Monteith (1977) in the 1970's. Species interactions in mixed-species stands can influence each of these variables. The Production Ecology Equation provides a framework for quantifying the allocation of resources between species, the efficiency with which they are used, and how the interactions between species influence these variables, as has been shown by

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Richards et al. (2010). When examining mixtures it is also important to make the distinction between (i) comparing different species growing in a given mixture versus (ii) comparing the growth of a given species in mixture compared to its monoculture. Although the comparison of species (i) is certainly useful, it is the former (ii) that is required to determine how species interactions have influenced the growth of a given species, and will be focused on here.

## Nutrient availability, uptake and use efficiency

A review of the literature reveals a wealth of studies that have examined the influence of mixing species on nutrient availabilities, nutrient uptake and nutrientuse efficiencies (Richards et al., 2010). The former two often increase in mixtures, particularly, and not surprisingly, if one of the mixed species is an N-fixing species, and N is often not the only nutrient that changes. There are several mechanisms responsible including N fixation, accelerated rates of litter decomposition and nutrient cycling, and temporal, spatial or chemical stratification of nutrient uptake. Changes in nutrient-use efficiencies are much more variable, such that increases, decreases and no changes have been reported.

### Water use, light capture and their use efficiency

There are far fewer examples of the effects of species interactions on light or water availability, use, and use efficiency. Light interception by trees and stands is the foundation for their growth and an increase in total light interception and/or a reduction in the competition for light experienced by the upper canopy species due to canopy stratification is often put forward as a reason for increased productivity in mixtures (Forrester et al., 2006). That is, the upper canopy species may be more efficient at higher light levels, while a more shade tolerant species in the lower canopy intercepts light that penetrates the upper canopy, thereby increasing total stand light interception while reducing competition for light for the upper canopy species.

A major argument that is often used in favour of mixtures is their greater productivity, however, the faster trees grow the more water they are likely to use (Law et al., 2002), which may in turn make them more susceptible to periods of low rainfall. Despite this potential problem, very few studies have compared the water use of mixtures to monocultures, and those that have, found that mixtures do use more water (Forrester et al., 2010). Nevertheless, at the tree and stand levels, mixed stands of *Eucalyptus globulus* and *Acacia mearnsii* were also more water-use efficient, thereby producing 43% or 76% more aboveground biomass per unit of water transpired than *E. globulus* or *A. mearnsii* monocultures, respectively (Forrester et al., 2010). This is in agreement with work in *Eucalyptus* plantations, which shows that increases in resource availability and changes in stand structural diversity can increase water- and light-use efficiencies (Stape et al., 2004). It implies that the interspecific interactions in mixtures, which improve resource availability, could be manipulated to improve forest water-use efficiency. More water-use efficient forests may be more resilient and resistant to predicted effects of climate change.

In conclusion, the production ecology equation provides a useful framework for examining the species interactions that occur in mixtures, at both the tree and stand levels. Many studies have revealed the influence mixtures have on nutrient dynamics in mixtures, however, relatively little is known about water use and water-use efficiencies in mixtures. Lastly, since species interactions are likely to change as stands develop and with climatic fluctuations, studies that incorporate these temporal aspects (Forrester et al., 2011) could help to refine silvicultural treatments and sites most suitable for mixtures.

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## Soil Organic Carbon Dynamics in Eucalyptus Silvopasture in Brazil: A Review

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#### Abstract

Interest in studying organic C in soils has increased recently because of its well-known beneficial effect on nutrient dynamics and soil structure, as well as its potential role as a sink for carbon dioxide (CO<sub>2</sub>) (IPCC 2000). The increase in atmospheric concentrations of CO<sub>2</sub>, as well as other greenhouse gases (GHGs), is the major cause of global climate change, or global warming. Carbon (C) sequestration is a mechanism for reducing the CO<sub>2</sub> concentration in the atmosphere and depositing it in long-term pools of C through afforestation, reforestation, and restoration of degraded lands. Agroforestry systems (AFSs) are a land management practice where trees and crops (with or without the raising of animals) interact in combination on the same unit of land for environmental and economic benefits. Such systems are well suited to new paradigms aimed at the integration of resources and production factors and the promotion of processes driving soil-plant (-animal) relations (Gama-Rodrigues 2006). In Brazil, deforestation and forest burning account for about 75% of the GHG emissions, and fossil fuel use accounts for the remaining 25% (Lal et al. 2006). In this scenario, AFSs play an important role as land-use systems that mitigate GHG emissions, help reduce deforestation and restore degraded soils and also play an important role in soil C sequestration because of the high input of organic material into the soil. Agrosilvopasture systems of eucalyptus tree plantations are becoming popular in Brazil and can be more efficient than the traditional eucalyptus and forage monocultures for C fixation because they involve a larger number of species in the system and benefit from their interaction. Soil organic matter is a large and dynamic reservoir of C and is a major part of the global C cycle. Restoring soil C is essential in enhancing soil quality, sustaining and improving food production, maintaining clean water, and reducing increases in atmospheric CO, (Lal et al. 2004). The world soil C pool is estimated to be around 1300 to 1500 Gt C, which is about twice the pool in the terrestrial plant biomass and three times the atmospheric pool; however, studies

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regarding C accumulation in soils under agrosilvopasture systems are scarce in Brazil.

Aggregates, which are secondary particles formed through the combination of mineral particles with organic and inorganic substances, represent a significant pool of soil C. This is because the inclusion of organic materials within soil aggregates reduces their decomposition rate, improves C sequestration, and reduces both the rate of increase in CO2 concentration in the atmosphere and the associated global warming (Bronick and Lal 2005). Density fractionation divides soil into a small number of mutually exclusive soil organic matter fractions using density and ultrasonic dispersion. Key fractions include the following: (1) free light organic matter (free light fraction—FLF), which is isolated before the breakdown of stable aggregates; (2) intra-aggregate organic matter (intra-aggregate light fraction— ILF), which is isolated after ultrasonic dispersion to break up aggregates; and (3) organomineral material, which is recovered as the residual material (heavy fraction—HF).

The goals of this review are to present the impact of the introduction of eucalyptus on the soil organic matter and soil enrichment of pasture land (Neves et al. 2004, Wendling et al. 2008, Vergutz et al. 2010, Tonucci et al. 2011). The systems studied with respect to their historical use are as follows: (1) native forest (savannah), (2) eucalyptus + rice (zero year-old), (3) eucalyptus + soybeans (1-year-old), (4) eucalyptus + pasture (2-year-old), (5) eucalyptus + pasture + cattle raising (8-yearold), (6) old eucalyptus plantation (25-year-old) and (7) old pasture (30-year-old). The agrosilvopasture systems have a relatively high stock of organic C in the soil; however, this system induces a decrease in the soil organic C (SOC) when compared to native forest, due to the soil disturbance involved in various tillage operations that accelerate the oxidation process and loss of C to the atmosphere. The total SOC stock to a 1 m depth ranges from 144 to 414 Mg ha<sup>-1</sup> under native forest, from 131 to 462 Mg ha<sup>-1</sup> under old pasture, from 138 to 392 Mg ha<sup>-1</sup> under old eucalyptus, and from 133 to 408 Mg ha<sup>-1</sup> under eucalyptus agrosilvopasture (10-year-old). On average, 59% of the entire C stock in the soil is in the macroaggregate fraction (2000 to 250  $\mu$ m), 22% in the microaggregate fraction (250 to 53  $\mu$ m), and 18% in the silt + clay fraction (< 53  $\mu$ m) under eucalyptus agrosilvopasture. The time needed to recover the original C stock thus transcends the 10 years devoted to the implementation of the system. The light - fraction organic C and the quickly oxidizable organic C are the most sensitive indicator of change in organic C quality. The light – fraction organic C decreases from 15 Mg ha<sup>-1</sup> (zero-year-old) to 5 Mg ha<sup>-1</sup>

<sup>1</sup> at 10 years, and the labile organic C decreases from 6 Mg ha<sup>-1</sup> (zero-year-old) to 4.5 Mg ha<sup>-1</sup> at 10 years. No-disturbance soil management practices should be employed to increase the C storage potential in eucalyptus agrosilvopasture systems. These studies indicate that more research is need to understand the precise relationship between the soil organic matter and soil enrichment processes under eucalyptus agrosilvopasture systems and their potential for soil C sequestration.

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## Coverting Eucalyptus biomass into ethanol

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Biofuels have taken an important role in the future of the alternative energy sources as a substitute for petroleum-based energy sources. Biomass has been identified as part of a long term plan to reduce the dependency on imported energy, as a national security strategy as well as a more sustainable and environmental friendly source of energy. Past and ongoing research have shown several ways to convert biomass into different types of energy, as well as some cost estimates for the production, transport and use. Cellulosic ethanol has caught the attention of research and industry as a promising petroleum-based energy sources substitute, yet several development and improvements in the technology and the industry are required. This presentation shows the current and future trends in cellulosic ethanol as well as the key variables and barriers that the industry needs to overcome to successfully become a competitive product.

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## Effect of climate and soil on the quality of Eucalyptus wood

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In Brazil, as in many other countries, significant advances and increasing wood production have been observed on fast growing clonal eucalypts plantations. Higher eucalypts wood yields have been reached using fertilization associated with irrigation. Results have shown that irrigation can increase productivity in 29% (49 to 63 m 3 /ha/year) and irrigation-fertilization in 38% (49 to 68 m 3 /ha/year) in relation to traditional management system. Experimentation in Northeastern Brazil has provided the opportunity to study the effect of continuous fertilization and irrigation-fertilization on wood quality of 7.11 years old *Eucalyptus grandis x urophylla* clones. Clones were planted with heights varying from 25 to 35 cm in a 3.0 x 3.0 m grid and fertilized with NPK twice, a first time during plantation and a second time 6 months later. The treatments were installed when the plantation was 3 years-old and the trees were 10 cm diameter and 15 m tall. The trees were harvested and wood samples were collected at DBH and other 5 different commercial heights. The wood properties were evaluated: sapwood/heartwood content, longitudinal wood basic density, DBH radial density by X-ray densitometry, fiber and vessel dimensions. There were significant differences in sapwood content between fertilized-irrigated and rainfed trees. The differences in wood basic density were restricted to the base and apical region of the trunk of eucalypt trees. The X-ray densitometry analysis provided a detailed radial wood density profile with the delimitation of stem tissue formed before and after the treatment application: the internal core not presenting significant differences between rainfed and irrigated-fertilized trees. The external wood core expressed the effect of treatments and irrigation lead to lower wood density and alterations on fiber and vessel dimensions. The physiological and technological aspects of eucalypts wood due to the treatments are also discussed.

**Keywords**: *Eucalyptus. grandis* x *urophylla*, wood density, fertilization, irrigation, X-ray densitometry

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# Technologies and uses of *eucalyptus* as raw-material for solid products

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The vast majority of intensive plantations of *Eucalyptus* in Brazil are intended for single use, particularly charcoal and pulp. This means that genetic selection, silvicultural methods and forest management aimed at those applications. Although these *Eucalyptus* eventually be used for solid products, only about 15 years some plantations began to be established for solid wood. To date, the possible uses are based on old plantations, established without proper planning had been adopted.

Besides presenting good silvicultural performance, for that particular genetic material of *Eucalyptus* be well suited to solid product, it is important to investigate aspects such as: i) the shape of the stem, the tendency to the formation of growth stresses and tension wood; ii) the wood characteristics such as color, density, dimensional stability and mechanical properties; iii) the performance of the timber in different methods of logs breaking-down and drying; iv) the performance of the timber in the machining and surface finishing, and v) the product performance in service.

Thus, the objective of this work is mainly present results of research carried out in Brazil on fast growth *Eucalyptus* wood. Results that characterize the *Eucalyptus* wood are presented, comparing it with other species, as well as the potential uses for solid products and technologies, and tendencies of research on timber for these products. Importantly, the proposal to use the *Eucalyptus* wood to solids is closely related to the idea of multiple uses of forest plantations, which is still relatively new and unusual in Brazil.

Several published results demonstrate that the *Eucalyptus* wood presents physical and mechanical properties similar to the tropical woods commercially used in Brazil (Lima *et al*, 1999, 2000, 2005). These works also show that this wood has a high variation in their properties mainly towards the pith to bark, but also between different genetic materials and sites. Caixeta *et al* (2003) evaluated the linear and volumetric shrinkage of 44 genotypes of *Eucalyptus*, which allowed them to deduce that many of these materials could be suitable for flooring, decking, window frames and structures for buildings.

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Growth stresses represent a limiting factor for the use of fast-growing *Eucalyptus* as a producer of logs for sawing. Lima *et al* (2004) measured the longitudinal residual strain (LRS), a measure of growth stress, on five trees of *Eucalyptus* clones and correlated them with other characteristics of both the wood and the size of the trees. The results showed that LRS varied depending on the clones and between wood traits. Only the density was significantly and positively correlated with the LRS when the clones were analyzed together. However, for this condition, among the growth traits, only the total height of the tree resulted in no significant correlation with the LRS.

As for the quality of lumber, Caixeta *et al.* (2002) distributed 44 phenotypes of *Eucalyptus* in three classes of cracks, curving, bowing, knot and kino presented by the lumbers after air drying. Twelve phenotypes of the upper class were considered the most suitable for the establishment of a breeding program, since it showed lower percentage of defects compared to the other.

The yield of three tangential methods of conversion of logs into green boards of ten *Eucalyptus* clones were evaluated by Ferreira *et al.* (2004). They found that the logs in general showed low taper, low bowing and high circularity, while the average yield in the conversion in lumber was around 35%. The bowing of the boards was small for all clones and with little difference among the three methods of breakdown; this was also observed for crooking the boards. Working on the same ten clones, Barbosa *et al.* (2005) measured the defective boards dried in conventional dryer. The authors developed a drying schedule common to the ten clones and verified that the boards showed low susceptibility to cracking, but high susceptibility to collapse.

Silva *et al.* (2007) compared the machinability of *Eucalyptus grandis* wood, sampled at different radial positions of the stem, with the timbers of mahogany and imbuia. Although the cutting conditions were not suitable for making a better surface quality, the results were satisfactory.

For the use of *Eucalyptus* in objects that have the exposed surface, it is also important to consider the color of wood. Mori *et al.* (2005) evaluated the color of wood samples of 11 clones of *Eucalyptus* by quantitative colorimetry (CIELAB, 1976). All colorimetric parameters, represented by the chromaticity coordinates L \*, a \*, b \*, C \* and h varied between clones and radial position of sampling indicating wood of different colors, tending to yellowish and reddish.

*Eucalyptus* have also been investigated for making veneers and particles for panels; also for the confection of mechanical structures of both round wood and

sawn wood, furniture, barrels, floors, small objects and other. The results of these studies show that the wood of *Eucalyptus* has a high potential for use. However, it is important to emphasize that the successful application of this wood to a solid product depends on proper planning and a strict control on every link in its supply chain.

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# The genome of *Eucalyptus grandis*: A resource for genetic improvement of eucalypts

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#### Background

Eucalypt genetic improvement is characterized by extensive tree breeding programs linked to forestry, pulp and paper industries. Excellent progress has been made in the genetic improvement of tree growth, form and, to some extent, wood properties. World-wide, eucalypt plantations exceeded 20 million ha in 2009 (http://www.git-forestry.com/) and continue to expand reinforcing the status of Eucalyptus as a global fibre crop. The release of the draft genome sequence of *Eucalyptus grandis* in 2011 (http://www.phytozome.net/eucalyptus.php) has been a major milestone for the eucalypt research community promising to generate new genomic and genetic resources to accelerate eucalypt tree improvement. Only the second reference genome for a forest tree genus after that of *Populus* (Tuskan et al. 2006. Science 313:1596-1604), the E. grandis genome, together with other woody plant genomes recently completed (e.g. Vitis, Cacao, Prunus, Citrus and Malus), offers exciting new opportunities to unravel the unique biology of large woody perennials and wood fibre development in particular. *Eucalyptus* is a foundation tree genus in Australia with over 700 species comprising 70% of the native forest estate. The genome sequence will therefore also empower many new avenues to study the ecology and evolutionary biology of eucalypts. In this paper we report on the sequencing, assembly and annotation of the *E. grandis* genome and highlight opportunities for research and application of genome information in eucalypt tree improvement.

#### Sequencing and assembly of the *E. grandis* genome

Genome sequencing was performed by the Joint Genome Institute (JGI) with funding from the US Department of Energy (DOE). Members of the *Eucalyptus* Genome Network (EUCAGEN, (http://www.eucagen.org) supported the effort by contributing

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resources such as genetic linkage maps, expressed gene sequences (ESTs) and bioinformatics capacity. To circumvent perceived problems with genome assembly of a highly heterozygous genotype, we selected a partially inbred (S1), 17-year-old tree of *E. grandis* (est. genome size 640 Mbp, n = 11), called BRASUZ1 (Suzano, Brazil). The JGI produced 7.7 million Sanger reads (5.4 Gbp, 8X total coverage) from plasmid, fosmid and BAC libraries of the BRASUZ1 genome. Approximately 25% of the genome assembled into two separate haplotypes of 3-4X coverage in contrast to the single haplotype of 6-7X coverage obtained for the rest of the genome. The high residual heterozygosity may be ascribed to viability selection in the S1 family from which it was derived and was confirmed by genome-wide genotyping of BRASUZ1 and its seed/pollen parent. The chromosomal context of the genome sequence scaffolds was determined by anchoring the scaffolds to high-density genetic linkage maps with over 2400 molecular markers. The draft (V1.0) assembly comprises 11 large chromosome scaffolds (605 Mbp total) and 4941 smaller, still unlinked scaffolds (85 Mbp). Approximately 96% of expressed gene loci are included in the 11 main chromosome assemblies.

#### Annotation of protein-coding loci

Teams from the JGI and the University of Ghent performed parallel annotations of the BRASUZ1 genome. *Ab initio* and homology-based annotation efforts were supported by over 4 million EST reads produced by the JGI and large amounts of EST data provided by collaborators. More than 90% of the predicted protein-coding loci (total 44,974 - JGI, 47,974 - UGent) are located on the 11 chromosome scaffolds.

#### Evolution of the *E. grandis* genome

The *E. grandis* genome is the first reference for the Rosid order Myrtales. Together with the genome of *Vitis* (Jaillon et al. 2007. *Nature* 449:463-467) representing an earlier diverging Rosid lineage (Vitales), the *E. grandis* genome is providing novel insights into Rosid genome evolution. Besides the ancient hexaploidization event shared by Rosids and Asterids, preliminary analyses suggest that the *Eucalyptus* genome has undergone at least one more recent genome-wide duplication event. Collaborators at INRA have shown that the modern eucalypt genome (n = 11) can be reconstructed from seven chromosomes predicted for the paleo-hexaploid ancestor of all eudicots (Salse et al. unpublished). There is also evidence of expansion of key gene families through tandem duplication of individual gene loci. These include genes involved in wood formation and secondary metabolism.

#### Genome variation in eucalypts

The JGI has resequenced (>20X) the genomes of the parent and six living siblings of BRASUZ1 and analyzed more than 600,000 heterozygous sites in the inbred pedigree. This revealed several regions that appear to remain heterozygous in the selfed progeny possibly due genetic factors contributing to inbreeding depression. Genome-wide resequencing (>30X) has also been performed for an *E. globulus* clone (X46, Forestal Mininco, Chile). *E. grandis* and *E. globulus* are in different sections (*Latoangulatae* and *Maidenaria*) of the subgenus Symphyomyrtus and differ in genome size (*E. globulus* - 530 Mbp, *E. grandis* - 640 Mbp, Grattapaglia &, Bradshaw. 1994. *Can J For Res* 24:1074-1078). Analyses of repetitive DNA elements in the *E. grandis* genome performed by collaborators in Brazil suggest that the difference in genome size may be related to recent transposable element activity in regions dispersed throughout the *E. grandis* genome (Pappas et al. unpublished).

#### Ongoing analyses and future perspectives

The extensive transcriptome sequence data produced by the JGI and EUCAGEN collaborators for BRASUZ1 (E. grandis), X46 (E. globulus) and other eucalypts (Novaes et al. 2008. BMC Genomics 9:312. Mizrachi et al. 2010. BMC Genomics 2010, 11:681) now provide the *Eucalyptus* research community excellent transcriptome resources (e.g. Eucspresso, http://eucspresso.bi.up.ac.za/) for gene function analyses. EUCAGEN collaborators are performing genomic analyses of the predicted proteome of E. grandis, including key gene families involved in biotic and abiotic stress tolerance, wood formation, flowering and secondary metabolism (www.eucagen.org). At the same time, high-throughput marker genotyping platforms such as Diversity Arrays Technology (DArT, Sansaloni et al. 2010. Plant Methods 6:16) and single nucleotide polymorphism (SNP) analysis are being developed for eucalypts. These resources link phenotypic variation in breeding populations to genomic regions and genes, which together with recently proposed genomic selection approaches (Grattapaglia &, Resende 2011. *Tree Genetics and Genomes* 7:241-255) will deliver powerful tools for the application of genomics in eucalypt breeding programs. Furthermore, the availability of next-generation genomics technologies will fast-track *Eucalyptus* research towards the integration of "omics" data with trait variation data at the population level to yield systems genetics models of tree growth and development.

### Transgenic Technologies for Eucalyptus

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To meet the demand of the commercial forestry industry needs trees that can do more --- produce more wood, on less land. We combine our leading technology with our well-established forestry expertise to develop trees that grow faster, yield more wood, are more resistant to cold and disease and can be processed more efficiently. All these traits are being tested in different projects. To improve growth we are testing genes to improve allocation of carbon, stress tolerance and nitrogen use efficiency. To increase processing efficiency we have Eucalyptus plants with increased production of cellulose or plants that are able to release of fermentable sugar easier by using genes that regulate cell wall biosynthesis. We have tested plants with an increased cellulose yield by reducing total lignin content. Reduced lignin enhances sugar release because lignin prevents cellulose from being broken down into sugars so it is converted more easily by fermentation technologies into biofuels. Chemistry of lignin can also be engineered by manipulating the amount of syringyl and guaicyl lignins. With a higher syringyl lignin pulping efficiency is increased and costs in a pulp mill are reduced with chemical savings. For the frost resistance we developed a freeze tolerant tropical Eucalyptus product, currently under USDA review, that combines the fast-growing characteristics of the tropical Eucalyptus grown extensively in Brazil with the ability to grow in geographic areas further north than conventional tropical Eucalyptus can grow. While not currently widely grown in the Southeastern United States, Eucalyptus has the potential to be a ready source of biomass for the biopower and biofuels markets as well as a source of high quality wood for the pulp and paper market. The advanced and technology-enhanced products are designed to improve growth rates, yields, stress tolerance, uniformity, wood quality and processing efficiency of trees. Combining superior nursery management practices and methods, conventional tree breeding, species selection, advanced tree improvement and biotechnology to produce traitspecific superior trees, ArborGen is working to ensure that our customers get more out of every tree they grow.

### Biosafety, environmental and health risk assessments of genetically modified eucalypts

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#### Abstract

Genetically modified (GM) eucalypts are produced in laboratories worldwide. Main genetic traits being assessed include wood properties for cellulose pulp and paper production, growth ratio, insect and disease resistance, and abiotic stress tolerance. In Brazil and the USA, a number of field tests were and are being conducted in order to evaluate GM eucalypt trees. Proposals for the commercial release of GM eucalypts are therefore expected in the coming years. Motivated by the Brazilian Ministry of Agriculture, Farming and Supply (MAPA), we created in 2009 the "Collaborating Center in Agriculture Defense Relative to the Biosafety of Genetically Modified Eucalypts" (Project "CDA Eucalyptus") in order to collect information and conduct research to assess the biosafety of GM eucalypts in the Brazilian context. The Normative Resolution Nr. 5 of the National Biosafety Technical Commission (CTNBio) is the official document presenting all the information needed to propose the commercial release of GMOs in Brazil. Based on this document and along with the personnel of FuturaGene Ltd., we conducted a series of experiments with GM and non-GM eucalypts planted in a test field in the state of São Paulo to start collecting the necessary information. Two independent groups of transgenic plants, harboring two different transgene constructs along with non-GM control plants are being assayed. The genetic traits, the identity or names of the transgenes as well as the identity of each tree individual will not be revealed due to intellectual property request still pending. Each group of plants was represented by four independent events in triplicates (2 groups x 4 events x 3 clonal trees + 3 non-GM clonal trees), therefore totaling 27 individuals under analysis. Samples were identified by random numbers and all assays were conducted in a simple-blind or

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a double-blind fashion. Tests concluded until now included (i) the detection of transgene regulatory sequences in purified DNA samples by conventional PCR and RT-qPCR, confirming the expected sampling conducted; (ii) extraction, chemical characterization and analysis of the antifungal effects of essential (volatile) oils extracted from leaves; (iii) pollen germination *in vitro*; (iv) flower morphology; (v) seed production; (vi) initial seedling development; (vii) leaf allelopathy; (viii) measurements of total phenolic compounds in leaves and roots; and (ix) effects of leaf extracts on the viability of human colon cells. All results obtained from experiments (ii) to (ix) revealed no statistical differences between GM- and non-GM-derived samples. A second round of experiments will be conducted to confirm these results. Proteomic and transcriptomic profiling of GM and non-GM trees are under analysis, as well as a series of experiments that include the chemical, nutritional and biological analysis of honey samples derived from bee hives located in fields of GM *versus* non-GM plants; and bee (*Apis mellifera*) population dynamics.

#### Keywords

Biosafety; environmental risk assessment; eucalypt; genetically modified organism; transgene; transgenic plant.

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# Forestry for rural development and poverty alleviation in the tropics : can we do better?

Sadanandan Nambiar<sup>1</sup>

The International Year of Forests 2011 is dedicated to the cause: 'Forests for **People'**, for celebrating people's actions towards sustainable forest management around the world. Is this an enlightened path to a new future or another slogan? I explore some issues that are important in the discussions on this question. We have much at stake in the answers.

#### Tragedy of poverty and failed hopes

One out of four of the poorest people on earth are dependent on forests and forests products for survival. Several countries are progressing towards the monumental task of reducing poverty, but even there, the people at the end of the long queue, yet waiting to see the rays of economic growth, are those living in the forest-rural landscapes. For those forgotten people, "our forests" have become poverty traps. The only way out of their poverty may be to walk away from forests to other ways of life, if they can be found. Tropical forests are being lost at a rate of 13 million hectares every year, mostly due to clearing for agriculture. The riches of the forests include timber, non-timber forest products, much promoted "eco-system services" and "spiritual values". Have we been able harness these riches to help the poor to take the first step on the ladder out of poverty? The answer is no. There is little evidence that more than two decades of "pro-poor" forest strategies (mostly based on native forests), policies and research by several national and international organisations have advanced the life of the poor people in an enduring way. Timber logging (with the notoriously overlapping boundary between legal and illegal) had unleashed wealth for some, but they shared little with the poor. Several "pro-poor" forestry programs have not worked because in their design some values were over stated (e.g. prospects of non-timber forest products) and, romantic notions and "donor values" may have been given more weight over some of the fundamentals required for initiating economic growth in those conditions. They include, for escaping from poverty, first the people need to get few dollars in their empty pockets

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on a regular basis and any forest based solutions (through the business of forestry) should be nursed within a framework of local economic and social development and proper governance.

#### Sustainable forest management for multiple benefits?

Several global "forest dialogues" including the various UN Forums, have been reduced to ideological, national and political battlefields. Bureaucrats, donors, politicians and other delegates in these gatherings often missed sight of a seminal issue. That is, *forestry*, the science, art and the complex package of practices of managing the forests, is essential for delivering any or all values from forests to people, poor or rich. And every action towards those goals requires investments and returns, innovation, technology and robust economic base and governance for progress. Those of us devoted to forest management wonder: what has happened in recent years to the much discussed (and hoped for) *sustainable management of forests* for multiple benefits? If it is still valid, why was forestry and forestry business left out of the many global agendas for economic development during the last decades, until the new dawn of REDD+? Today, much of the attention about the future of forests, even planted forests, has shifted from multiple values to enlarging the carbon sinks. Yet, many serious questions loom over the expectations of REDD+ and the destiny of and hope for a better deal for the forest dependant people.

#### What can plantation forestry and products sector offer?

We have encouraging evidence, from selected regions that this sector, small and large, can augment sustainable solutions for the rural poor to enable them to step on to the economic ladder. Effectiveness is greater, if they are well connected to the national/regional development efforts and with the active participation by the private sector. Farming systems which integrates trees for wood production and other values with food production offer the prospects for *food and wood security* in arid - tropical regions, ravaged by endemic poverty.

There are clear examples of successful plantation forestry in the world. Productivity of *Pinus radiata* plantations in South Australia, measured over the last 100 years, show that today the third rotation stands are growing at rates faster than they were in the second rotation and thus site quality have been upgraded in most sites across the region. This resource is the economic backbone for that region.

The opening line in this Conference Web site is "We all know that the productivity

of each rotation of Eucalyptus plantation worldwide has increased by 10 to 20%, as a result of major advances in silviculture and genetics". This claim may be well founded for Brazil, and perhaps in few other places. But it is a questionable global claim. It has been estimated only few years ago that about 50% of the plantation forestry in the tropics may be economic failures.Large areas of eucalypt plantations are failing to meet sustainability in countries as diverse as Australia (the first home of eucalypts!), India, Vietnam, China and Ethiopia. Eucalypt plantation development faces recalcitrant opposition from environmental groups, especially based on the potential impacts on water. It has been banned in a part of India in response to the pressure from political environmentalism, despite India's urgent need and huge demand for more wood. We should remember that at least 50% of the wood harvested in developing countries ends up as fire wood, the only source of cooking fuel, for the poor people. Entrenched environmentalisms help neither rural communities, nor the environment.

#### We need a refreshed narrative on forestry-we can make a difference

Poverty can be and should be eliminated from our world. Economic growth is not a zero sum game. Forestry business should rise up to this challenge. We need a renewed vision to pro-actively direct a part of the forestry efforts towards poverty alleviation goals, as indeed all our economic, social and political endeavours should. This should be a goal if forestry sector (business) in the tropics is to gain the due status in the global agenda for human development. As the first step towards such a goal, the combined voices of the *forestry sector* need to articulate a refreshed narrative on forestry in which the legitimate economic aspirations- profit- are in balance with binding environmental responsibilities. For that narrative, the *purpose of forestry*, I propose following interrelated aspects (among others) for your consideration.

- The purpose of forestry for society should be placed and fostered in the context of forests as a continuum of the natural resource base linking conservation forests at one level through a range of forest types including plantation forests and farms at other levels. Each component of this continuum should be managed to provide different sets of primary and secondary values to society but none should be expected to serve all values at all times .
- Promote renewed efforts, building on forestry and forest-based industries, for poverty alleviation. These efforts should be disentangled from the unsuccessful "poor-poor forest strategy" followed in the recent decades.

- Articulate ways (investments and policy framework) to meet the rising demands for wood and wood products for serving peoples' needs, particularly in the developing world. Food security will always remain a supreme need for a growing population but trees and forests can enhance both food and wood (fibre) security from a landscape. A new generation of engineered wood products can contribute greatly to construction which can house families in rapidly urbanizing societies.
- Strengthen and foster effective linkages between forests and wood based sectors, livelihood, and activities aimed at sustainable development (e.g. improved education, better wages and infrastructure) in rural communities, who are left out of the national economic growth in many countries. This is more than the traditional and passive "Corporate Social Responsibility" which some companies sponsor. It has to be a more *symbiotic and enduring partnership* between the members of the all inclusive *forestry sector*. The outgrower wood production schemes developed by companies in South Africa and in parts of India are positive examples of such strong *symbiotic relationships*.

#### Go global or local or both?

Forestry can contribute to our efforts to partly contain and mitigate climate change, and deliver other key "ecosystem services", the value of which can not be measured adequately in economic terms. Forests can be managed for wood production and environmental benefits as complimentary values. The alleged conflict of values should not deter us from addressing the challenges pointed out here- capturing the economic opportunity offered by forestry. This new narrative will not be effective if we let it float "global", as is often the case. It has to be focused on the priority needs and opportunities of specific regions within countries. It is time for a new awakening and leadership to build and communicate a brighter narrative of forestry, backed by actions, which will advance the forestry sector and its business in the agenda for economic development with environmental care.

We can do more and better with *forestry for people*.

## Role of plantation forests in biodiversity conservation and the provision of biodiversity-related ecosystem goods and services

Eckehard G. Brockerhoff<sup>1</sup>, Hervé Jactel<sup>2</sup>, John A. Parrotta<sup>3</sup>

#### Extended abstract

Forests are vitally important as habitats for a major part of the world's biodiversity, and the ongoing loss, fragmentation and degradation of forests (FAO 2006) cause much concern about the resulting decline in biodiversity (Brook et al. 2003, Barlow et al. 2007a, Brockerhoff et al. 2008, Lindenmayer 2009). As biodiversity is an important driver for the provision of many *ecosystem goods and services*, such as ecosystem productivity, nutrient cycling, and stability (e.g., resistance to consumption or invasion) (Balvanera et al. 2006, Thompson et al. 2009), the decline in biodiversity is likely to have far-reaching consequences. The effects of plantation forestry on biodiversity have been debated vigorously over the last two decades, and there is a growing recognition that the consideration of biodiversity issues is an important element of planning and management of plantation forests as well as wider considerations of biodiversity conservation across landscapes (Carnus et al. 2006, Brockerhoff et al. 2008).

Plantation forests currently represent less than 4% of the total forest area world-wide. Contrary to the area of natural forests, which has been declining, plantation forests are expanding (FAO 2006, FAO 2010). Forest loss varies considerably by region, with Europe and eastern Asia recording net afforestation, while Africa, South America, Oceania and southern Asia have lost forest at a rate of 0.2%-0.5% annually, particularly in tropical and sub-tropical climates (FAO 2010, see also Achard et al. 2002, FAO 2006). The main driver for forest loss is development for agriculture (Achard et al. 2002, FAO 2010). In Australia, fire and drought were important drivers for forest loss in the last decade (FAO 2010). However, plantations have replaced natural forests in some countries (Cossalter and Pye-Smith 2003, Lindenmayer 2009), and the development of oil palm plantations in particular has been a major cause of forest loss in Malaysia and Indonesia (Koh and Wilcove 2008, Wilcove and Koh 2010). Plantation forests are usually highly productive

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and, by providing an alternative source of timber and other forest products, can contribute, indirectly, to the protection of natural forests (Sedjo and Botkin 1997, Brockerhoff et al. 2008, Paquette and Messier 2010). The conservation value of plantations depends on the identity of tree species and their origin (i.e., native vs. exotic) and to what degree conservation aspects are considered in their management. Plantations of native tree species are generally preferable for forest biodiversity but exotic plantation forests can also provide habitat to native plants and animals (e.g., Brockerhoff et al. 2003, Brockerhoff et al. 2005, Eycott et al. 2006, Pawson et al. 2010). Methods for conserving biodiversity in plantation forest have been suggested for the stand and landscape levels (Hartley 2002, Lindenmayer and Franklin 2002, Fischer et al. 2006, Brockerhoff et al. 2008).

Biodiversity is important for forest ecosystem functioning. No fewer than 25 different provisioning, cultural, supporting, and regulating ecosystem services are sensitive to changes in biodiversity (MEA 2005). Plantation forests, which are typically characterized by lower levels of biodiversity than mixed semi-natural or natural forests, are likely to be less capable of providing ecosystem services that are linked to biodiversity (Kelty 2006, Thompson et al. 2009). There is evidence, for example, that mixed stands are less prone to insect herbivory than forest 'mono-cultures' (Jactel & Brockerhoff 2007).

Of all countries, Brazil recorded the greatest loss of forests with nearly 2.2 million ha, or about 40% of the global forest loss, for the period 2005-2010 (FAO 2010). For the same period, Brazil recorded an increase in plantation forest area of ca. 330,000 ha per year, the second highest (after China with about 2 million ha) (FAO 2010). This contrast of significant forest loss and substantial establishment of plantation forests is intriguing and represents a valuable case study to examine the role of plantation forestry in biodiversity conservation and the provision of biodiversity-related ecosystem goods and services. Brazil's plantations are dominated by non-native (exotic) trees, mainly eucalypts of Australian origin (ca. 4,26 million ha) and pines from North America (ca. 1.87 million ha). Consequently, there are concerns that the substantial loss of natural forests in Brazil contributes significantly to the decline in biodiversity. While the establishment of plantation forests in Brazil partly compensates for the loss of natural forests, plantations, and even secondary forests, contain only a small proportion of the flora and fauna of primary forests (e.g., Barlow 2007a) and may be inferior in their provision of some ecosystem services (Barlow et al. 2007b). These and other examples will be explored to review measures that benefit biodiversity in plantations and natural forests.

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#### Trends in FSC forest certification

Freitas

FSC is a multi-stakeholder organization created in 1993 to promote the responsible management of the world's forests. In order to achieve its mission FSC uses forest certification as a tool to promote positive change in the way forests are used and perceived.

One of the key features of the organization is its balanced multi-stakeholder governance, in which economic, social and environmental interest groups have all the same decision making power. In practice, this means that solutions have to be built together between industry, environmental groups and social movement representatives, with no single interest group being able to impose its views on the others.

After 18 years of existence, FSC has now become a truly global organization, with more than 144 million hectares certified in 79 countries and almost 22,000 chain of custody certificates in 106 countries. Using FAO's data on the world's wood production and taking a conservative estimate regarding the volume of wood produced in FSC certified forests, it is estimated that they account for approximately 12.5% of the global wood production.

The global area which is FSC certified has grown continuously over the years and this is a trend that is expected to continue. Over 60% of the FSC certified forests are considered natural forests, with the rest being composed of semi-natural forests (approximately 30%) and plantations (closed to 10%). However, when looking at the production of wood fiber, a better indicator for some purposes, the picture is a more balanced one between the different types of forests.

Much of the FSC certified area is in developed countries like Canada, United States and Sweden. But there are also bright spots in terms of emerging economies, with significant areas certified to FSC standards also in Russia, Poland, Brazil, Chile and South Africa.

Products coming from FSC certified forests are linked to the consumer through what is called a chain of custody certificate. This assures consumers that the products they are buying are linked to an FSC certified forests. FSC chain of custody certification has grown in double digits for the past several years, which indicates a strong demand from the market and ultimately represents the pull force that is necessary to encourage forest producers to achieve certification.

Most chain of custody certificates are also in the developed world, with Europe taking the lead as a continent. Given the global nature of the forest products trade, this type of certification has also grown quite strongly in places like China and Brazil, which are expected to continue to have an increasingly prominent role in FSC certification.

The strong growth in FSC certification, both at the forest and supply chain levels, can be credited to a few main drivers. The most important one so far has been the demand generated by private procurement policies from retailers, manufacturers, publishers, banks and others, which specify and buy FSC products both from a corporate social responsibility point of view, but increasingly also for risk management purposes of their supply chains.

Another important driver in some countries has been the development of public procurement policies, that reference certification as a tool to demonstrate compliance with public sustainability sourcing requirements. These public policies often have an effect on the private sector, as they are seen as a benchmark for smaller and medium sized companies.

Demand from final consumers is still nascent though it is a driver that is becoming increasing important for FSC certification and is expected to play a much stronger role in the future. FSC has invested significant efforts in consumer related research in the past couple of years and this has shown that consumers are interested in buying responsible products and that there is great potential for increasing consumer demand for FSC certified products.

As a mission based organization, FSC continuously works to monitor the impacts of FSC certification. There is increasing evidence that FSC certification is resulting in positive change on environmental issues such as restoration of degraded areas, lower impact of forestry operations on soil and water, protection of endangered or threatened species, minimizing unauthorized activities such as fires and poaching and others. There is also growing information on how FSC certification has contributed to improve working conditions, from health and safety issues to the quality of camp sites and protection of worker's rights. In addition, FSC certification has been effective in protecting the rights of local communities and indigenous peoples.

Looking ahead, there are also a few challenges that FSC will need to overcome to remain successful in the pursuit of its mission. One of them is related to the fact that most forests in the world are not yet managed to the level of FSC's standards and that the low hanging fruit are most likely gone. FSC is working to establish new mechanisms to support the move from conventional to certified forestry.

Another challenge is ensuring that FSC certification is a relevant and applicable tool for small forest owners. In certain parts of the world, small producers are extremely relevant for the forest sector and FSC is working to provide better adapted systems and support mechanisms to ensure that it delivers well to their reality.

FSC has undergone a process to revise its main international standard, the FSC Principles and Criteria (P&C), which has now been submitted for decision by the FSC membership. This new version better incorporates plantations into the whole FSC standard, emphasizes the need for a more active role of forest operations with regards to social issues and clarifies aspects related to natural forests, plantations and conversion.

As a growing system that has increasing market value, FSC is continuously working to ensure a high level of credibility in FSC certification, which ultimately results in the confidence and support from economic, social and environmental stakeholders. Key elements of this are FSC's balanced multi-stakeholder governance, a strong focus on transparency and performance of FSC certification and a recognition that certification is an ever evolving mechanism.

## Forest plantations landscape management for water conservation

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Forest plantation areas in Brazil are increasing in last years reaching about 6 million of hectares in 2009 (ABRAF, 2010), due to internal economic growth and the worldwide demands for fiber and paper. Forest management plans are in the seek of environmental sustainability of those planted areas, guided by crescent restrictions enforced by Brazilian environmental law and also Environmental Certification (Lima, 2011). Current competition for world markets, a greater pressure of government and society and environmental restrictions are resulting in the improvement of environmental planning methods in order to reach a more sustainable forest management. As proposed by Lima et al. (2008), a sustainable forest management should observe different scales: macro, meso and micro (Figure 1), in order to attend different planning aspects inherent to each scale.

The most important attribute observed at macroscale is the regional climate, mainly the aspects of water availability, considering the total annual precipitation, potential evapotranspiration and also the soil water balance along the year. As proposed by Calder (2007), a regional hydrological classification could point out areas where water availability allows greater flexibility on decisions about forest

Macro	Meso	Micro
Regional aspects • Water availability • Precipitation • Potential Evapotranspiration • Climatic water balance • Environmental legislation	Catchment health • Water balance • Stream flow regime • Aquatic ecosystem Land-use planning • Road design • Riparian area • Soil hydrology	Adaptive forest management practices • Species • Spacing • Rotation length • Surface soil protection • Harvesting techniques

Figure 1. Scales that are needed for the consistent analysis of the potential water impacts of forest plantations (Lima et al., 2008).

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plantations management, while other regions, with lower water availability and high potential evapotranspiration, could represent environmental risks regarding the greater water consumption by forest plantations. Other important aspect at this scale refers to physical attributes of landscape like geology and relief, indicating potential water behavior regarding infiltration, runoff and problems related to erosion, soil water retention and nutrient losses (Hewlett & Hibbert, 1967).

Observing these two main aspects: climate and relief/geology, Table 1 shows the most important actions at macro and mesoscale that could be considered for a better planning seeking a more sustainable forest management.

Macroscale actions – the regional hydrological zoning based on total annual precipitation and potential evapotranspiration as proposed by Calder (2007) would allow to identify regions more sensitive to water consumption by forest plantations where it is expected environmental and social impacts by the significant reduction of water flow in the streams (Farley et al., 2005). In this case, those areas could be submitted to specific actions at meso and micro scale in order to compensate the low water availability, increasing the "blue water" at managed areas (Falkenmark & Folke, 2002) and avoiding conflicts. The hydrological response is another important aspect that could be assessed by discharge/precipitation ratio, or specific indices like Flashness (Baker et al., 2004). This regional characterization is based mainly on soil and terrain attributes and allows the land classification considering water behavior that is very useful to guide forest management in order to reduce environmental impacts. For example, high hydrological response areas are more suitable to extreme events of droughts and floods, and also erosive

Macroscale	Actions	Mesoscale	Actions
- Climate	- Hydrological zoning	- Land-use planning	<ul> <li>Forest plantation proportion at landscape</li> <li>Mosaic clear-cut management</li> <li>Landscape structure of forest plantations</li> <li>Hydrological sensitive areas mapping</li> </ul>
- Relief / Geology	- Hydrological response	- Forest management - Roads	<ul> <li>Hydro forest management units</li> <li>Road system redesign</li> </ul>

**Table 1.** Management actions for water conservation in forest plantations at differentscales.

98 - Invited Speakers

processes.

Mesoscale actions – one of most important actions at this scale is the definition of forest plantation proportion at landscape level. Actually, forest plantations in Brazil occupies a proportion varying between 10% and 40% of landscape, according to regional environmental law and the existence of conservation areas. The protected area could be increased at low water availability regions, not only by creation of reserves, but also by the alternative forest management considering longer rotations where the plantation effect (Rosoman, 1994) would be diluted in time. The same way, the mosaic management of clear-cut presents high potential of water consumption impacts reduction since it consider a mix of stands with different ages (and water consumption), avoiding the large clear-cut areas and diluting in time and space the effects of forest plantation water use and its management operations. The spatial structure of plantation areas in relation to streams and runnof channels also could be a management tool seeking to attenuate impacts and decrease water consumption, shifting plantations from saturated areas that have important water yield function at catchment scale, as hydrological sensitive areas (Agnew et al. 2006). The identification of those areas could help the local land-use planning, indicating protected areas to occupy them, and modifying forest management in potential saturation areas in order to reduce impacts on water resources. The definition of management units considering the water soil dynamics could be an alternative to reduce impacts of forest management operations in function of natural sensibility of those units, indicating specific management to units according to their hydrological characteristics. Also, the road design could be modified considering the water dynamics at landscape, removing roads in more sensitive areas, reducing density and also redesigning some roads in order to avoid effects of water and soil carrying to streams (Ferraz et al., 2007).

At this paper, we will show examples of effective actions on forest management modifications based on technical criteria at macro and mesoscale that could contribute to improve management in order to increase water conservation at forest plantation landscapes.

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ORAL SESSION 1. FOREST ESTABLISHMENT AND REGENERATION

### Harvest residue management in *Eucalyptus* plantations established on a tropical sandy soil largely influences soil CO<sub>2</sub> effluxes and tree growth without modifying C mineralization in the mineral soil

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#### Summary

The management of organic residues (OR) at harvesting plays a key role for the sustainability of plantations managed in short rotations in tropical regions. A network of tropical plantations coordinated by CIFOR showed that OR management greatly influenced tree growth on poor soils, and the strongest response in this network was found for *Eucalyptus* plantations established on Arenic Arenosol soils in the Congo (Saint-André et al., 2008). Litter manipulation can largely influence soil CO, effluxes through soil water content, temperature and substrate modifications in the top soil (Sayer, 2006). Soil carbon is a major component of the fertility for highly weathered tropical soils, as a support of cations and source of nutrient through mineralization (Feller and Beare, 1997). Controversial effects of OR management on soil organic matter (SOM) contents have been reported but some *in situ* studies have showed that increasing OR amounts was likely to enhance soil C mineralization (Crow et al., 2009, Chemidlin Prévost-Bouré et al., 2010). We hypothesized that i) contrasted amounts of OR deposited at the soil surface at the clearcut of Eucalyptus stands in a C-depleted sandy soil largely influence soil CO, effluxes, ii) tree response to OR management was positively correlated to soil CO, effluxes over the 2 first years after planting, and iii) OR management does not modify significantly SOM mineralization and thus C sequestration in mineral soil layers.

Soil CO<sub>2</sub> effluxes were measured in a complete randomized block design where OR were either removed (R) or added with a double slash inputs (DS) and compared to a control treatment where the residues of stemwood harvesting (SWH) were deposited at the soil surface. The size of individual plots was 1250 m<sup>2</sup> and 3 blocks were installed. Cumulative soil CO<sub>2</sub> effluxes for each treatment were calculated

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from 53 measurement dates over the first 2 years after planting. Tree growth was inventoried at 6 dates over the study period. We estimated the mineralization of SOM by deducting the contributions of OR, old roots and autotrophic respiration to total soil  $CO_2$  effluxes. The contributions of OR and old roots were estimated monitoring the decomposition of residues in litter bags and assuming a microbial C-use efficiency of 0.2. Root biomass was measured in each treatment 12, 18 and 24 months after planting for several root diameter classes. The contribution of the autotrophic component to total soil  $CO_2$  effluxes was thus estimated from the biomass of each root class and diameter-specific root respiration rates measured at the same site.

Before OR manipulation, SWH and R plots exhibited no difference in soil  $CO_2$  efflux (R<sub>s</sub>), and values were slightly but significantly lower in DS plots than in SWH plots (Tab. 1). Soil respiration was characterized by high seasonal variations. During dry seasons, RMANOVA showed that soil  $CO_2$  effluxes were not significantly different between the two treatments with OR but were systematically lower in the R treatment (Tab. 1). Treatments had a strong effect on R<sub>s</sub> during rainy seasons. Mean R<sub>s</sub> values during the first rainy season were 2.3, 4.5 and 6.4 µmol m<sup>-2</sup> s<sup>-1</sup> for the R, SWH and DS plots, respectively, and 3.3, 5.1 and 6.1 µmol m<sup>-2</sup> s<sup>-1</sup> during the second rainy season (Tab. 1). These large responses of R<sub>s</sub> to OR managements were not linked to soil water content variations between treatments (Tab. 1) and were

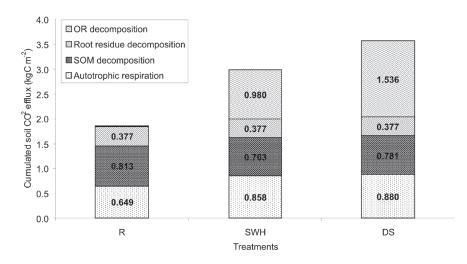
<b>Table 1.</b> Mean values of soil $CO_2$ efflux ( $R_s$ ) and volumetric soil water contents ( $W_s$ )
for each block and treatment. The studied Eucalyptus stand was harvested on day 0
and treatments were established during the first 60 days after clearcutting. The
results over the study period were split in three periods to examine the temporal
variability of $R_s$ : before harvesting (day -65 to -11), the first 330 days after clear-
cutting, and from 331 to 838 days after clearcutting. Treatments R (all residues
removed), SWH (stemwood harvest) and DS (double slash) were studied. Different
letters in the same column indicate significant differences between treatments (P
< 0.05).

		Day -65 to -11		Da	Day 38 to 330				Day 331 to 838			
				Dry sea	Dry season		Wet season		Dry season		Wet season	
Rs	Bloc 1	5.6	а	1.4	а	3.9	а	2.5	а	4.6	а	
(µmol m <sup>-2</sup> s <sup>-1</sup> )	Bloc 2	5.8	ab	1.8	b	4.7	b	2.8	b	5.0	b	
	Bloc 3	5.9	b	1.6	ab	4.5	b	2.6	ab	4.7	а	
	R	5.3	ab	1.0	а	2.3	а	2.0	а	3.3	а	
	SWH	5.5	b	1.6	b	4.5	b	2.9	b	5.1	b	
	DS	5.0	а	1.7	b	6.4	С	3.0	b	6.1	С	
w <sub>s</sub>	R	12.9	а	5.7	а	11.3	а	6.4	а	10.7	b	
(%)	SWH	13.0	а	7.9	b	12.3	b	6.3	а	9.6	а	
	DS	12.7	а	7.8	b	12.2	b	6.3	а	9.8	а	

thus attributed to OR amounts. For most of the treatments, soil  $CO_2$  effluxes rose the second year after planting both during dry and rainy seasons as a result of root growth and increasing autotrophic respiration.

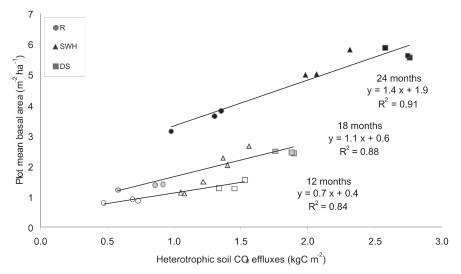
A tentative partitioning among compartment of total soil CO<sub>2</sub> effluxes showed that OR decomposition had the largest contribution for the treatment representative of commercial plantations (SWH in Fig. 1). Together, OR and root residue decompositions accounted for 46% of total soil CO<sub>2</sub> efflux for the same treatment. In the DS treatment, OR decomposition contributed to 43% of total soil CO<sub>2</sub> effluxes and the contribution of OR+Root residues was 54% (Fig. 1). In the R treatment, SOM decomposition was the first contributor to total soil CO<sub>2</sub> effluxes with a relative value of 44%. However, absolute contributions of SOM remained not significantly different between treatment with values of  $0.813 \pm 0.204$  kg C m<sup>-2</sup>,  $0.763 \pm 0.173$  kg C m<sup>-2</sup> and  $0.781 \pm 0.101$  kg C m<sup>-2</sup>. The variability was mainly a consequence of interblock variability. our results suggest that OR management did not largely modify the C stocks in the mineral soil, contrarily to other studies in temperate and boreal areas (Crow *et al.*, 2009, Chemidlin Prévost-Bouré *et al.*, 2010).

OR management had strong effects on tree growth (Fig. 2, Photo 1). Basal areas were significantly lower in the R plots than in SWH plots representative of the commercial silviculture in the Congo (41% lower on average at age 2 years) while values in the DS plots were enhanced in comparison to SWH plots (8% higher on average at age 2 years). Differences in tree growth between treatments remained



**Figure 1.** Cumulated contributions of each soil compartment to total soil  $CO_2$  efflux for the three treatments over the 2 first years after planting.

106 - Oral Session



**Figure 2.** Relationships between mean basal area in each plot at 12 (white-filled symbols), 18 (grey-filled symbols) and 24 months of age (black-filled symbols) and heterotrophic  $CO_2$  effluxes estimated by our partitioning method and cumulated on the first 12, 18 and 24 months after planting in R (circles), SWH (triangles) and DS treatments (squares).



**Photo 1.** Illustrative effect of organic residues management on tree growth. R treatment on the left and DS treatment on the right side of the photograph.

stable over the first two years after planting. The strong relationships observed between cumulated heterotrophic  $CO_2$  effluxes and tree growth over the study period supports the hypothesis that tree growth variations were dependant on the mineralization of organic matter within harvest residues (Fig. 2). Although SOM was an important contributor to total soil  $CO_2$  effluxes in this sandy tropical soil, our results suggest that large inputs of OM at the soil surface at planting did not lead to a strong priming effect in the mineral soil.

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### Monitoring silvicultural quality using uniformity coefficients

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#### Introduction

The productivity is resulted of selection of superior genetic materials, correct silviculture management and the environment it is inserted (Gonçalves et al, 2004). To measure it, is a common practice in planted forests the execution of continue forestry inventory, that monitor the stock of forest resources, mainly the wood stock (Campos and Leite, 2006). In case of *Eucalyptus* planted forests this surveys begin at juvenile forest, between 1 and 2 years, which is when occurs the canopy closure and the intraspecific competition becomes stronger (Binkley et al, 2010). This competition can be detected through the stand uniformity, which has a strong relationship with the productivity, using different coefficients, like Coefficient of Variation (Weiner, 1986) and PB50 (Stape, 2006).

The juvenile inventory has been used to estimate the wood stock and to project future productivity (Campos and Leite, 2006), but there has done a few works using it to do a qualitative evaluation. So, the objective of this work is to propose an alternative use to the juvenile inventory, applying it to monitor the silvicultural quality using uniformity coefficients.

#### Material and methods

The work was divided in two steps: i) development of a range using clonal tests and ii) validation of this range in wide commercial area.

To create the range of uniformity, five clonal tests with different site index were chosen (Table 1). The clonal tests were chosen because of i) genetically identical, making the competition between plants and, consequently, the uniformity, only the result of management applied to the tests and ii) the silvicultural rigor applied to them, which ensure that this can be considered a great range of uniformity for *Eucalyptus* clonal plantations. We choosed tests with different site index to evaluate the effect of site quality in the uniformity.

The experimental design consisted in randomized blocks with 3 repetitions. Plots were composed by 49 plants (7 x 7) in spacing ranging from 6 to 9,0 m<sup>2</sup> per plant. Were measured the height and DBH at 2 years and calculated the volume

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using specific equations to each genetic material. The uniformity was determined by the percentage of volume of 50% thinner trees (PB50). This ratio was chosen because it has limits (0 à 50) and, due of this, it is possible to create a finite range. The stand uniformity and productivity of each site at age two were compared using ANOVA followed by test of means (Tukey 5%). As there was no difference in relation to uniformity among the sites, the envelop was determined using the confidence interval (1% probability) of all sites together.

To validate the range, the optimal envelop created in step 1 was applied in the commercial plantation areas, planted between 2001 and 2007, totaling 428 plots. In order to exclude the historical genetics gains and difference in site quality, those plots were composed by one clone (IPB1) planted in Oxisoils. The validation was analyzed statistically using Pearson's correlation to verify the relationship of three variables over time: i) productivity; ii) PB50 and iii) the number of plots within the optimal envelop of PB50.

#### Results

Although there are differences in productivity between the clonal tests, there was no difference in stand uniformity. This shows that for *Eucalyptus* clones planted in the present region, there is a standard of uniformity, which is reached when there is a good silvicultural management. Aspinwall et al. (2011) studied the difference in uniformity between *Pinus taeda* genotypes with different levels of genetics improvement and found similar results. They observed that the influence on uniformity was not caused only by the level of genetics improvement but the silvicultural quality applied to the forest.

Once there was no difference between those five clonal tests, was prepared the PB50 optimal envelop using all of them, which was between [39.4 to 42.3%].

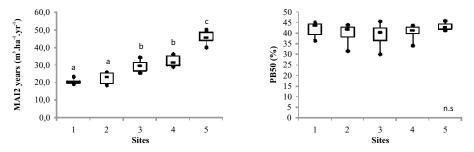
Applying the range in the commercial area, the Pearson correlation between the variables and planted year was highly significant (P <0.001) (Table 1). In 2001, 55.1% of the plots were assessed within the optimal range of uniformity. In 2007 this figure raised to 100%, showing clear progress in the quality of silvicultural

Site	Location (City)	Soil	Site Index (m)	Number of clones
1	Mogi Guaçú	LV2-5	32,2	11
2	Brotas	NQ1-1	29,5	10
3	Aguaí	LV3-4	27,0	7
4	Mogi Guaçú	LE1-3	32,7	7
5	Luis Antônio	NQ1-1	25,5	5

 Table 1. Informations about the clonal tests.

**Table 2.** Evolution of productivity, uniformity (PB50) and number of plots within the envelop. The significative (P < 0,001) Pearson correlation between the years and the variables has shown the evolution of quality of silvicultural management.

	Variable					
Year	MAI	PB50	Plots within the envelop PB50			
	m³.ha <sup>-1</sup> .year <sup>-1</sup>	#	%			
2001	33,4	39,1	55,1			
2002	39,1	39,3	61,0			
2003	43,0	40,2	67,8			
2004	39,9	39,0	54,1			
2005	42,5	40,9	73,6			
2006	45,6	38,8	58,1			
2007	49,7	44,6	100,0			
Pearson correlation (P<0,001)	0,92	0,61	0,65			



**Figure 1.** (Left) MAI at year 2 differed within the sites and (right) the uniformity has a pattern independent of the productivity, showing there is an optimal range of uniformity if a good silviculture is done.

management. This can be confirmed also by increasing the PB50 from 39,1 to 44,6% in the same period and increased productivity of 33.4 m<sup>3</sup>.ha<sup>-1</sup>.year<sup>-1</sup> to 49.7 m<sup>3</sup>.ha<sup>-1</sup>.year<sup>-1</sup>.

#### Conclusion

- There is no difference in stand uniformity in different site index plantations;

- There is an optimal pattern of uniformity among clonal plantation in the studied area;

- The uniformity can be monitored through juvenile continuous inventory and serve as a tool for monitoring the quality of silviculture

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## Thinning, pruning and fertiliser application interact to influence the growth, structure and resource-use of *Eucalyptus nitens* plantations

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#### Background

Silvicultural treatments such as thinning, pruning and fertiliser application are often used to increase growth or wood quality in forests or plantations. The structural and physiological responses to each of these treatments usually vary and since these treatments are often applied simultaneously they may interact in ways that are difficult to predict from studies that examine a single treatment. This not only has implications for growth and wood quality, but also for the management of other resources such as water, and the susceptibility of trees to periods of water stress.

#### Methods

This study examined whether thinning, pruning and nitrogen fertiliser application at age 3 years, interact with each other to influence the growth, canopy architecture and photosynthesis, as well as the light and water use and useefficiencies of *Eucalyptus nitens* trees up to 3 years after treating. Two levels of each treatment were applied in a factorial design replicated three times in a plantation near Carrajung, Victoria, Australia. Treatments included: unthinned (T<sup>-</sup>), or thinned (T<sup>+</sup>) from ca. 900 to 300 trees ha<sup>-1</sup>; unpruned (P<sup>-</sup>), or 50% of the live crown length (75% of leaf area) pruned of the largest 300 potential sawlog crop trees ha<sup>-1</sup> (P<sup>+</sup>), and nil (F<sup>-</sup>), or 300 kg ha<sup>-1</sup> N fertiliser (F<sup>+</sup>). Thinning to 300 trees ha<sup>-1</sup> resulted in the retention of 33% of the trees, 40% of the basal area, 42% of the volume and 45% of the leaf area.

#### **Results and Discussion**

Thinning had the strongest influence such that even  $P^+F^-$  crop trees (largest 200 ha<sup>-1</sup>; SCT<sub>200</sub>) had larger aboveground biomass and leaf areas in T<sup>+</sup> stands than  $P^-F^+$ 

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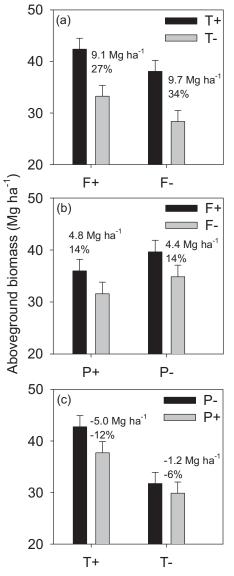
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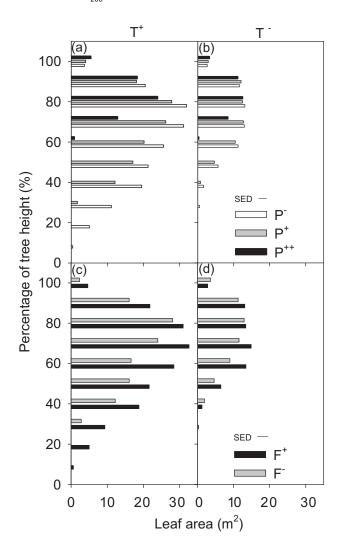
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trees in T<sup>-</sup> stands (Figure 1). By this age 6 years, pruning had reduced aboveground biomass of SCT<sub>200</sub> by 12% in T<sup>+</sup> but by only 6% in T<sup>-</sup>, where the lower (pruned) foliage was shaded, inefficient and contributed less to tree growth than foliage at the same position in T<sup>+</sup>. Pruning and fertiliser application also interacted, such that pruning



**Figure 1.** Aboveground biomass of the largest 200 crop trees ha<sup>-1</sup> at age 6.3 years, showing the interactions between thinning, pruning and fertiliser application (applied 3 years earlier). The text inside the graphs shows the absolute (Mg ha<sup>-1</sup>) and relative (%) effect of the treatments. Error bars are standard errors of difference.

reduced SCT<sub>200</sub> leaf area by 12% in F<sup>-</sup> and 28% in F<sup>+</sup>. This is consistent with the Resource Limitation Model (Wise and Abrahamson, 2007) that suggests that this interaction could result when pruning directly influences light capture or carbon fixation, and while in F<sup>+</sup> these are the major limiting resources, in F<sup>-</sup> nitrogen is more limiting and pruning did not have the same influence on N use or its acquisition. Thinning increased SCT<sub>200</sub> aboveground biomass by 4-5 Mg ha<sup>-1</sup> regardless of



**Figure 2.** The influence of pruning (a,b) and fertiliser application (c,d) on leaf area distributions in thinned and unthinned stands at age 6 years. Leaf area is divided into 10% sections of tree height, which averaged 18.3 m for all treatments. SED = standard error of difference.

fertiliser treatment, however the relative thinning response was higher in  $F^{-}$  (34%) than in  $F^{+}$  (27%). This interaction is likely results because competition is more size-symmetric on lower quality sites (c.f.  $F^{-}$ ) where belowground resources are relatively scarcer than above-ground resources (i.e. light) (Pretzsch and Biber, 2010).

Most of the growth response could be explained by structural changes in terms of increased canopy size, a higher partitioning of aboveground biomass to leaf mass at the expense of stem mass (Figure 2) and associated changes in branch size distributions and mortality. That is, linear regressions between annual aboveground biomass increment and leaf area of SCT<sub>200</sub> gave Adj. R<sup>2</sup> of > 0.9 with small but significant increases in intercepts due to thinning, pruning and fertiliser application. These small increases in intercepts indicate physiological responses and improvements in efficiency.

Significant increases in leaf-level rates of photosynthesis were observed up to about 20 weeks after treating, however these effects were relatively short lived and disappeared after 80 weeks. Thinning reduced total stand transpiration by 48%, but increased the water-use efficiency (WUE; kg wood per m<sup>3</sup> water transpired) by 23%. Pruning reduced both the volume and the WUE of SCT<sub>200</sub> by 10% and 17%, respectively. Fertiliser application increased transpiration by 12% but did not influence WUE.

# Conclusions

Pruning, fertilising and especially thinning significantly influenced growth as well as the amount of leaves and their display within the canopies. The effect of each treatment was usually modified by the other factors, illustrating the value of considering density, nutrition and defoliation conditions when interpreting the effects of individual treatments. These treatments improved growth largely via structural changes in terms of increased canopy size and architecture, and to a smaller extent by increasing the resource-use efficiency of the trees. This shows the benefit of creating space for canopy expansion as opposed to resource availability *per se*, and that these treatments could be used to design more efficient (as well as faster growing) plantations.

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# Restoration Treatments for *Eucalyptus* Plantations Infested by Introduced *Paspalum* to the Development of Native Forests in an Australian National Park (Ph.D. Thesis)

Dr. Benjamín Villa-Castillo<sup>1</sup>

#### **Background and Objectives**

Sixty hectares of 36-year old *Eucalyptus saligna* and *Eucalyptus pilularis* plantations with the understorey invaded by the introduced pasture *Paspalum wettsteinii* in Bongil Bongil National Park, NSW, Australia. In the 1950s, the area was cleared for cattle or dairy pasture, and in 1971 for eucalypt plantations. Until 1999, this area was used for dairy farming and forestry. Natural regeneration of native pioneer, secondary or mature forest species is scarce in most of the plantations which appear to be in an arrested state of plant succession. The Park's Plan of Management states that these plantations will be restored to determine appropriate techniques for initiating succession towards endemic communities.

Potential biotic barriers were identified to the succession of these plantations towards more natural forest: (1) competition and suppression by the dense *Paspalum* sward and associated litter of the regeneration of native species, (2) competition from the unthinned eucalypts, (3) the absence of a native seed bank, (4) lack of germination cues for any *in situ* or persistent native seed bank, and (5) wallaby browsing of woody seedlings in the event of any regeneration.

#### Methods

In 5.6 ha, a completely randomised strip plot experiment was established. The experiment consisted of 14 relatively uniform plantation blocks (80 x 50 m), 12 of *E. saligna* and two of *E. pilularis*. A. Overstorey treatments. For *E. saligna*, three canopy reduction treatments, (i) 0%, (ii) 50% and (iii) 75% canopy reduction. For *E. pilularis*, the two blocks were both logged to 75% canopy reduction. B. Burning treatment. Two treatments, (i) burnt after logging and (ii) not burnt after logging. C. Herbicide, chisel plough and control treatments. In unburnt blocks, three understorey treatments, (i) *Paspalum* sprayed with glyphosate herbicide, (ii) cultivated with a chisel plough, and (iii) do nothing to the undestorey (control). D. Wallaby browsing exclusion fencing. Two treatments, (i) fenced to exclude *Wallabia bicolor* using 1.3 m

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high netting and (ii) unfenced. E. Revegetation treatments. Four treatments were applied in 10 x 10 m subplots in each plot in both the fenced and unfenced sites in each block: (i) no planting and no weed control, (ii) no planting with weed control, (iii) planting treatment 1 with a mix of native pioneer shrubs and small trees; and (iv) planting treatment 2 with a mix of native pioneer and late successional shrubs and small trees. Post-planting weed control consisted of spot spraying with glyphosate herbicide in a 50 cm radius around each planted seedling, as required.

# Results

Burning had most effect on topsoil chemistry in both experimental plantations and changes due to burning were detected in most response variables (pH, electrical conductivity, organic matter, available phosphorus, total nitrogen, ammonium, nitrate, sodium, potassium, calcium, magnesium and cation exchange capacity). Canopy reduction had less effect, with only calcium responding significantly. Chisel ploughing and herbicide spraying did not alter topsoil chemistry. Logging and burning had positive impacts for regenerating vegetation in terms of soil pH changes, nitrification and other nutrient gains from burnt *Paspalum* and eucalypt slash.

Logging and burning had a marked effect on the composition and structure of trees and shrubs in both experimental plantations. Changes were detected in eight of nine response variables (plantation tree and other woody species density, canopy density, photosynthetically active radiation, and structural indices of distance, mingling, diameter, height and uniform angle). Logging decreased tree density with 557 *Eucalyptus* stems/ha in control plots, 179 stems/ha in the 50% and 137 stems/ ha in the 75% canopy reduction treatments, respectively. Canopy density was reduced to 51% and 29% in the 50% and 75% canopy reduction treatments. Controls had a canopy density of 84%. Photosynthetically active radiation increased to 1378 imol and 1640 imol in the 50% and 75% canopy reduction treatments, respectively. Controls had 363 imol.

The groundcover of plant species other than *Paspalum* was not significantly affected by treatments (7% on average), although the number of plant species (mainly herbs) increased in burnt plots subjected to 50% and 75% canopy reduction. Regeneration of native species in the chisel plough and herbicide plots was poor. The *E. pilularis* plantation showed greater cover of other plant species due to differences in disturbance history. *Paspalum* cover recovered almost to pretreatment levels in all treatments after 1 year, but its recovery was slower in the herbicide plots. The *Paspalum* seed bank was abundant (27 040 seeds/m<sup>2</sup> on

average), while the seed bank of other plant species was poor (2380 seeds/m<sup>2</sup> on average).

One year after planting 2750 planted seedlings of 11 native woody species, 48% of the seedlings were alive, 26% were dead due to wallaby browsing and 26% were dead due to other factors. More seedlings were browsed in unfenced than fenced areas, despite the fact that some of the fences were initially substandard. The type of planting (pioneers versus a mix of pioneer and late successional species) had a significant effect on planted seedling mortality, the pioneers being more browsed. On the other hand, in the absence of browsing, the pioneers (particularly the *Acacia* species) grew the fastest. The growth of the majority of live seedlings was reduced by wallaby browsing, although , notably not three rainforest late successional species.

# Conclusions

The hypothesised restoration barriers, mainly competition from *Paspalum* and the absence of an abundant, diverse native seed bank, appear to be the main limitations to restoration by natural regeneration. Management resources should be focused on post-logging burning, fencing of planted seedlings or use of advanced seedlings, and spot spraying with a grass-specific herbicide. Research needs to test various burning, slashing, grazing and herbicide application strategies, targeted at weaknesses or vulnerable stages in the life cycle of *Paspalum*. The long-term control of *Paspalum* could be problematic if plants continue to emerge from long-lived seeds in the soil. A detailed economic analysis of all costs and returns should be made to determine whether *Paspalum* infested plantations can be restored at or near cost-neutrality, using the revenue from logging.

# Comparing the accuracy of a process-based (3PG), a hybrid (Glob3PG) and an empirical (Globulus 3.0) model against long term permanent plot data from *Eucalyptus globulus* Labill.

Josep Crous<sup>1</sup>, Susana Barreiro<sup>1</sup>, José Tomé<sup>1</sup> and Margarida Tomé<sup>1</sup>

# Background

In Portugal, Eucalyptus globulus Labill. plantations cover around 21% of the total forest area of the country and is an important source of raw material for the pulp industry. In Portugal some empirical growth models have been developed for eucalyptus such as Globulus 3.0 (Tomé et al. 2006) while the most commonly used physiological model is 3PG (Sands and Landsberg 2002) which was parameterized for Portuguese data (Fontes el al. 2006). A hybrid model, Glob3PG (Tomé et al. 2004), was first developed by linking 3PG and Globulus 2.1 (Tomé et al. 2001) through the stand level allometric relationship between basal area and woody biomass. Recently, Glob3PG was updated with Globulus 3.0. Three models were integrated in two stand level simulators: the empirical model (Globulus 3.0) was integrated into GLOBALL simulator while the hybrid (Glob3PG) and the processbased model (3PG) into 3PGout+ simulator. Both tools were used to simulate the evolution of each plot. The main objective of this study is to compare the performance of the two simulators including the three types of growth models to forecast first rotation production of *Eucalyptus globulus* Labill. against longterm permanent plot data in Portugal.

# Methods

**Data:** A large amount of information on eucalyptus' growth has been collected as a result of combined efforts from Pulp and Paper Companies and Universities. The database (integrating inventory data, permanent plots and experimental trials) represents the diversity of these stands over the country. The 22 plots' selection was based on three restrictions: 1) stands must have been measured at least 6 measurements for first rotation; 2) their geographical distribution should cover coastal and inland areas; 3) average stand densities should be between 950 and 1500 trees/ha.

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**Model-inputs:** Dendrometric inputs came from the eucalyptus database. Climatic information came from the Portuguese Meteorological Institute. The altitudes were calculated using a Digital Elevation Model. The soil type information was obtained from the Portuguese Soil Map (www.ihera.min-agricultura.pt/cartografia/solos.htm) while soil characteristics, i.e: depth and texture came from the Portuguese Ministry of Agriculture (www.dgadr.pt/ar/cartografia/notaexplisolo.htm). Soil information was also used to calculate the minimum and maximum Available Soil Water (ASW) and fertility rating (FR).

**Validation:** The estimates coming from the simulation output runs were compared to the data observed for the selected plots. Residuals and absolute residuals were calculated as the difference between the observed values and the simulated values.

The stand variables considered were quadratic mean DBH, basal area, number of trees, volume under-bark and aboveground biomass.

# **Results and Discussion**

First results obtained were plotted against stand variables expressed as classes in order to detect possible relationships, namely with geographical location, stand density, site index (SI), starting age and projection intervals.

With regard to geographical location, results do not indicate a strong influence of continentality for any of the models, although there is a slight tendency for higher residuals on coastal plots. This tendency needs to be further investigated.

For all the variables analyzed, intermediate SI classes' present smaller residuals. The three models have similar behavior even if Globulus seems to perform better for lower and intermediate SI classes and Glob3PG for higher ones. In relation to density classes, and similarly to what has been observed for SI classes, smaller residuals were obtained for the intermediate class. In general terms Globulus performs better for the lowest density class and Glob3PG for the highest class. 3PG seems to be inconsistent for the different variables showing the smallest residuals for some of them and highest for others.

None of the models show a clear evidence of a relation between increasing initial simulation ages and the residuals values. However, when analysing the residuals for the increasing projection intervals, all three models present good results up to 13-year projection interval. After which, Glob3PG and 3PG' residuals increase with the projection interval class with 3PG presenting clearly worse results. Nevertheless, the results presented may be influenced by the number of observations considered on each class.

# Conclusions

In general Glob3PG predictions seem to be between the other two models' predictions.

This might be due to the fact that it results from the combination of Globulus 3.0 and 3PG.

The results reported here are provisional and need to be analyzed in more detail. It is important to emphasize that these results are directly affected by the model inputs and that an amelioration of inputs for 3PG and Glob3PG will be positively reflected on future predictions.

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# Evaluation of 3PG and Glob3PG models to predict growth of *Eucalyptus globulus* coppice stands

Susana Barreiro<sup>1</sup>, Josep Crous<sup>1</sup>, João Palma<sup>1</sup>, José Tomé<sup>1</sup> and Margarida Tomé<sup>1</sup>

#### Background

Over the last decade the application of 3PG process-based model (Landsberg and Waring, 1997) has been studied for *Eucalyptus globulus* Labill. in Portugal. However, the model's performance for coppice stands has never been assessed for Portugal.

Recently, the calibrated version of 3PG (Fontes et al., 2006) and the hybrid growth model (Glob3PG) (Tomé et al., 2004) were integrated into 3PGout+ stand simulator.

The first model is composed of a set of sub-modules for predicting: biomass production, biomass allocation, stem mortality, soil water balance and stand characteristics. The last module uses allometric relationships to predict variables of interest for forest managers. Mean diameters, basal area, standing volume and increment are computed from the biomass pools and number of stems. The second model, Glob3PG, predicts biomass growth with a version of the calibrated 3PG model, while other basal area, dominant height, merchantable volumes are calculated with the empirical prediction functions of Globulus 3.0 growth model (Tomé et al., 2006).

This study's main objective is to evaluate the ability of the two growth models integrated in the simulator to predict the growth of coppice stands. Given the need to simulate several consecutive rotation cycles for wide planning horizons, the ability of the models to simulate the transition between rotations as well the process of shoots selection is also analyzed.

# Methods

**Data**: A complete database integrating permanent plots representing the diversity of eucalyptus stands over the country was queried. A set of plots was selected based on some restrictions: a) average stand densities for the first rotation around 1140 trees/hectare; b) a minimum of 4 measurements in coppice stands

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and c) coppice stands must have a measurement made before they were harvested. A total of 22 plots were selected.

**Simulation inputs**: Initial stand inputs came from the eucalyptus database, while weather data were obtained from the Portuguese meteorological institute. A digital elevation model (DEM) was used to get the plot altitudes. Soil characteristics needed as input for the simulator were derived from soil type maps: soil depth, texture and soil type/classes. With this information, the maximum available soil water was calculated for each plot. Plots were tunned for different fertility rating (FR) values and the FR that best mimicked stand growth per plot was selected. Different management prescriptions were defined in order to allow simulating each plot according to the management practices observed.

**Simulation**: 3PGout+ was used to simulate the evolution of each plot using two growth model variants: 3PG and Glob3PG. Because most of the coppices started being measured after the shoots selection operation was performed, simulations made from the first coppice measurement onwards miss representing not only the transition between rotations but also the thinning of the shoots. In order to counterbalance this, another set of simulations starting with the input data of the last measurement of the 1<sup>st</sup> rotation was considered. As a result of combining the two model variants with the two different inputs, 4 simulation sets were produced.

**Validation**: Prediction errors were calculated based on the differences between observed and estimated values for each plot. Simulations and residuals were computed for the 4 output datasets. In an attempt to illustrate the differences between the growth models, basal area, under-bark volume and aboveground biomass predictions were analyzed.

# **Results and Discussion**

The graphical analysis between the models shows a wide diversity of situations: with Glob3PG performing much better than 3PG, producing similar results or being slightly better. For some plots one of the models produced better results for basal area and worse results for volume, while the other performed in the exact opposite way.

However, the residuals computed for the simulation outputs obtained with the two models show that Glob3PG presents smaller residuals than 3PG. As it was expected, the difference in residuals is much more evident for the stand variables calculated using different methodologies (allometric functions for 3PG against Globulus 3.0 prediction functions).

In terms of the initial simulation age applied, the end of the 1st rotation against the beginning of the 2<sup>nd</sup> rotation, results show smaller residuals for simulation runs that were started under the coppice regardless of the model. Stand density, though is considerably higher than under simulations started before the stands were harvested, which might be one reason for the poorer results obtained for the 1<sup>st</sup> rotation datasets.

Moreover, forecasts could be improved in general if better density estimates were produced.

#### Conclusions

The work presented here results from an ongoing study. The results obtained so far indicate that: in general and except for stand density, simulation output values draw near or match measured values for most of the plots tested. The comparison of simulation results for the 4 output datasets show that Glob3PG estimated stand variables quite realistically for coppices, which is an advantage over the currently used growth model in Portugal: Globulus 3.0. In this way, Glob3PG offers the possibility of long-term simulations accounting for changing management and/or climate combined with the reliability of empirical predictions. Overall, results demonstrate that more efforts should be put into finding how to improve the transition between rotations as well as density estimates.

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# Harvesting impact on forest soil productivity. The "Extended Use Sampler", a new tool to estimate it

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#### **Key Words**

Soil compaction, penetrometers, forest harvest impact.

#### Background

Changes in soil physical properties is one of the main factors impacting agricultural soils. Forest machine traffic and operations increase soil bulk density (BD) and consequently the mechanical resistance to penetration (MRP). The magnitude of this process depends on multiple variables and its interactions. In general, increments in BD are considered detrimental to plant growth in mild climate soils.

Penetrometers and gravimetric determinations are widely used to measure these impacts. There are static penetrometers available commercially, consisting in a rigid metallic axle with a cone at one end, and a pressure measurement device at the other. The instrument is connected to a data collector. Dynamic penetrometers are based on the application of a constant rate kinetic energy to a penetrating cone. On the other hand, static penetrometers work with a known amount of kinetic energy given by the fall of a mass that runs through a steel rod down to a collar attached to the rod ending in a cone that penetrates the soil.

Among the various methods to evaluate this impact Jones and Kunze (2004) found the dynamic cone penetrometers (DCP) to be a cheap, efficient, and practical methodology that can be used in a wide range of conditions.

The DCP result is usually presented as the Cone Index (CI) which is a measure of the resistance to penetration expressed in pressure units, and calculated as the strength needed to introduce the cone into the soil divided the cone base surface. This CI is a complex parameter reflecting the cutting, compression, tension and soil-metal friction characteristics which are difficult to separate in its influence on the results. All four factors are influenced by the soil humidity content, so the

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MRP is consequently affected by the water content of the soil. Different studies found MRP vs water content strong relationships that range from linear to logarithmic, exponential and negative potentials with good R<sup>2</sup>. The equations and its coefficients vary depending on soil conditions, depth, and cultural treatments, with soil texture being a main driving force.

The maximum strength that plants use to expand their roots vary according to many factors (soil and plant factors), with values ranging from 0.9 to 3 MPa, using 2 MPa as a reference value (Eavis et al, 1969; Camargo Corrêa and Daniluk, 2006). Micucci and Taboada, 2006 deduced that root elongation of many crops decline with values above 2.5-3 MPa.

The objective of this study was to use the Extended Use Sampler (EUS) to assess the soil mechanical resistance at different time intervals in a *Eucalyptus grandis* plantation, in order to understand the natural recovering of the soil properties.

#### Methods

The study was located in northeastern Argentina, province of Entre Ríos, 31º 41´ S; 58º 07´ W, on fluventic Haplumbrept soils (USDA taxonomy), with loamy sand texture down to the sampling depth.

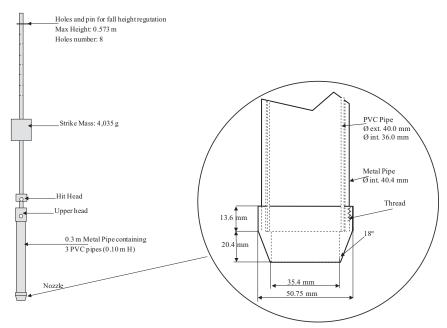


Figure 1. Diagram of the "Extended Use Sampler".

The EUS is a new instrument. It was presented by Micucci and Menardi (2004) and was slightly modified and built by the authors of this paper. Figure 1 gives a schematic presentation of the EUS.

The main advantage of the device is that it can be used to estimate MRP together with the collection of soil samples in the hollow tube, down to 30 cm, to perform analysis of soil texture, moisture and chemical substances later in the laboratory. The instrument was used on sites with no superficial disturbance, and on 0.1 and 0.2 m depth tracks, produced by an *Eucalyptus grandis* plantation clearcut. Twelve samples from each treatment were taken six months after the clearcut and six samples from each track depth treatment were collected again thirty months after the harvest.

# **Results and Discussion**

A significant increase in BD was related to track depth (Table 1), with the first 10 cm of soil being mostly impacted, though significant increases were measured at least up to 30 cm depth. Two years after the first evaluation, the sampling showed certain soil recovery, especially in the first 10 cm of soil. Lower bulk density values also showed up on sites with no visible disturbance, indicating that they should have had compaction during the harvest, probably due to machine traffic with no signs or tree felling or dragging.

Correcting to 40% of relative water content (RWC), results showed that the deeper the track the more pressure is needed to penetrate the soil, with 144% and 98% increments for the 20 cm and 10 cm track treatments, respectively, at the 30 cm sampling depth and six months after harvest (Table 1). This effect lasted up to 30 months, but the values decreased, showing certain amount of "natural recovery" of the soil. This reduction has also been detected in the non-disturbance treatment,

			Bulk density (Mg/m³)	1	Mechanical resistance to penetration (MPa)				
Time after	Track			Sampli	ng depth				
harvest	depth	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm		
Six months	20 cm	1.543 (a)	1.760 (a)	1.720 (a)	3.869 (a)	6.689 (a)	11.688 (a)		
	10 cm	1.427 (b)	1.700 (a)	1.713 (ab)	2.825 (b)	6.061 (a)	9.494 (b)		
	0 cm	1.331 (c)	1.571 (b)	1.681 (b)	1.103 (c)	3.536 (b)	6.048 (c)		
Thirty months	20 cm	1.281 (a)	1.613 (a)	1.752 (a)	1.751 (a)	6.301 (a)	N/A		
	10 cm	1.263 (a)	1.525 (ab)	1.684 (b)	1.091 (b)	5.593 (a)	N/A		
	0 cm	1.069 (b)	1.497 (b)	1.663 (b)	0.717 (c)	3.705 (b)	N/A		

Table 1.	Bulk density	and Mechanical	l resistance t	to penetration	under	different
wheel tra	ick depth.					

similar to the BD measurements, suggesting that compaction was also produced in the non-disturbance sites.

The MRP values increased with the track depth but this increment is not linear. The 0.1 m depth track accounted for 65% and 68% of the bulk density and MRP increase of the 0.2 m track, respectively. This result agrees with the bibliography in that 0.1 m depth wheel tracks produced two thirds of the 0.2 track impact.

Similarly to the BD results, the relative increments of MRP decreased with depth (at six months the average of the two track treatments were 204% for the 0-10 cm soil depth and 80% for the 20-30 depth, both compared to the non-disturbance site) being the MRP more sensitive than the BD.

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# Developmental Homeostasis in the Leaves of *Eucalyptus* camaldulensis (Dehnhardt)

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# Introduction

Development is a complex process involving progressive change from single cell to a multicellular complex organism. Such development and differentiation occurs in an orderly fashion with little variation. Hence, developmental pathways are highly resistant to genetic and environmental perturbations and exclusively designed to ensure phenotypic constancy in a given set of genetic and environmental conditions (Waddington, 1953). However, such homeostasis divided into two components: canalization and developmental stability since lesser asymmetric values enhances phenotypic stability proportionately (Watson and Thornhill, 1994). However, both these conclusions unaccounted for occurrence of higher phenotypic variation.

An *E. camaldulensis* plantation was established at Midnapore, West Bengal and remained unthinned for 18 years that leads to formation of different heights and canopies due to creation of stress and non-thinning. The understorey trees possess symptoms of stress like nutrient deficiency in the form of pale leaves, reduced growth. Hence, an attempt was made to test whether stress created in the genotypes has disrupted the developmental process and caused developmental instability thus weakening the homeostatic mechanisms in the leaves and the results were reported here.

# **Materials and Methods**

A *Eucalyptus camaldulensis* plantation was established during 1985 in an area of three hectares at Midnapore, West Bengal from few Australian provenances and planted at a spacement of 3 × 2M but remained unthinned for 18 years. Due to nonthinning competition sets in and the trees developed different canopies. Besides, the soil was severely eroded. The plantation was divided into 1) top canopy (Candidate Plus Trees); 2) Middle canopy and 3) understorey trees. From each of top canopy trees and understorey trees, 10 trees were selected and from each tree

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20 leaves were collected. Petiole length, lamina length, total leaf length, total leaf width, right half, and left half were measured. Fluctuating asymmetry was estimated through Log L- Log R (left-half minus right-half (Palmer and Strobeck, 1986)). To estimate the intra- genotypic variability, one- way ANOVA on GLM was performed. Spearman's correlation was conducted to test the interrelationships among the leaf components (Vanclay, 1994).

# Results

The inventory of data showed no skewness (95% CI of  $g_1$  including 0) for dominant trees ( $g_1 = 0.849$ ; n= 200) and understorey trees ( $g_1 = 1.21$ ; n = 200). Estimates of kurtosis was nonsignificant in dominant trees ( $g_2 = 0.818$  at 95 % CI including 0) where as it reached some what significance level in understorey trees ( $g_2 = 2.08$ ; including 0 at 95 % CI) suggesting a narrower peak than expected for normally distributed data (Fig.1 & 2). One-way ANOVA revealed highly significant intraspecific variation in all leaf components (p = <0.001). Strong positive correlations (p = <0.001%) were observed between all leaf components in both the groups

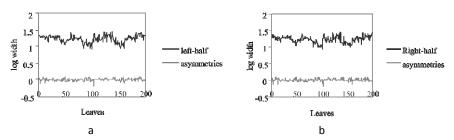


Figure 1. Fluctuating asymmetries in the leaves of low yielder: a) left- half; b) right-half.

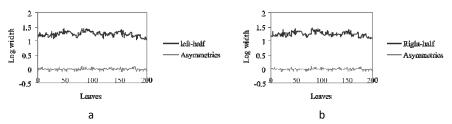


Figure 2. Fluctuating asymmetries in the leaves of high yielder: a) left- half; b) right-half.

indicating development and differentiation of those leaf components through a set of genes except petiole length, which correlated only with leaf length (Table-1). The mean values of leaf parameters indicated that understorey trees produced smaller leaves suggesting that the genotypes were growing and surviving under stress.

# Discussion

Judging from data on leaf size, the understorey trees exhibited significant levels of stress. However, despite the evidence of nutrient stress, we found no support for the idea that adverse growth conditions disrupt developmental stability in foliar characteristics (Fig.1 & 2). Although the mean asymmetry was significantly affected by adverse growth conditions, there was no significant difference in mean asymmetry between high yielding dominant trees and understorey trees. Based on these observations, there is no evidence to suggest that developmental stability in Eucalyptus leaves is related to the adverse growth conditions experienced by each individual in contrast to the earlier reports (Wilsey *et. al.* 1998) who found genetic and environmental stress induced significant levels of leaf asymmetry in inter specific hybrids between *Betula pendula*, *B. pubescens* and *B. nana* had more asymmetric leaves than the parental species. In a study of *B. pubescens* seedlings Nitrogen increased developmental stability in leaf morphology (Lappalainen *et. al.* 2000).

Variation in plant organs may occur due to environmental variations and plastic responses between components in the mechanisms responsible for character expression represent major determinants of variance in the leaves (Palmer and Strobeck, 1986).

Strong correlation coefficients among leaf components indicated that stress did not alter the development and differentiation of the leaf components suggesting that the mechanisms responsible for buffering leaf components against both external and internal environmental variation are same or interrelated and assumed that the developmental pathway in these genotypes was better buffered both with respect to the micro environmental and macro environmental variations.

Hence, it can be concluded that these genotypes are versatile in their adoptedness and hence, able to live successfully in a broader range of environments. The height growth in *E. camaldulensis* was a complex outcome of physiological and biomechanical forces and deterministic factors determine the growth and to its extent than stochastic rules.

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ORAL SESSION 2. ENVIRONMENTAL STRESS

# Carbon allocation and leaf gas exchange of *Eucalyptus* hybrid clones under different climates

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# Background

Hybrid clones of *Eucalyptus grandis* x *E. urophylla* are most widely planted and used for pulp production in Brazil. This country provides an excellent climate for the development of species of this genus. However with the growth of the forestry sector, the plantations have expanded to new regions with different water regimes. The supply of water strongly influences wood production, but predicting how forest growth and ecosystem carbon storage respond to changes in resource supplies, remains a challenge, particularly because the controls over carbon allocation are poorly understood (Landsberg, 2003). Thus, joining the understanding of the genetic behavior of materials under conditions of stress with physiological variables will provide important drivers for breeding programs.

# Objectives

This study was done to evaluate the response of different hybrid clones cultivated under distinct climates and to establish correlations between growth performance and physiological traits.

# Methods

Net photosynthetic rate (*A*), stomatal conductance (*Gs*), transpiration (*E*) and the water use efficiency (*WUE*) were evaluated for four *Eucalyptus grandis* x *E*. *urophylla* hybrid clones (C1, C2, C3, C4) in two regions with different rainfall conditions. The experiment was conducted in four campaigns, two sites in southeast Brazil: Aracruz (Espírito Santo State) (S1) and Montezuma (Minas Gerais State) (S2) with 1300 and 700 mm average annual rainfall, respectively, and in two periods: October 2008 (dry season) and April 2009 (wet season). *A*, *Gs* and *E* were measured in nine fully expanded leaves per clone, selected from the top of the canopy, with

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infrared gas analyser (LI-6400) at 8:00 am, 12:00 pm and 4:00 pm. The **WUE** was calculated by dividing **A** by **E**. Biomass of stem, bark, branches, and roots >5mm were measured using three trees per treatment in the two periods (dry and wet season). The total wet biomass of stem, bark, branches, and roots per tree was weighed in the field and after that a sample of biomass of each component was weighed and taken to the laboratory to be dried and obtain the final dry weight/ component/tree for each treatment.

Statistical analysis were done using the SAS System (SAS Institute, 1993).The data were analyzed by pearson correlations, and the carbon allocation data were compared by t-Test with 5% of significance.

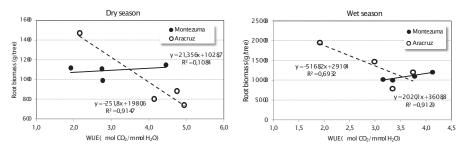
#### **Results and Discussion**

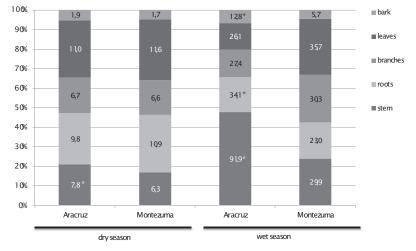
Α

High correlations were verified between gas exchange variables and root biomass, specially with WUE (Table 1). Eucalyptus clones grew more in Aracruz and were more water use efficients, allocating less carbon to roots (Figure 1). In Montezuma, clones grew less than in Aracruz and allocated more biomass to roots.

**Table 1.** Correlations between biomass of stem, bark, branches and roots and net photosynthetic rate (A), stomatal conductance (Gs), transpiration (E) and the water use efficiency (WUE). (A) Dry season - Aracruz data located in the bottom diagonal e Montezuma data located in the top diagonal. (B) Wet season - Aracruz data located in the bottom diagonal.

						-	-	-	
	leaves	stem	bark	branches	roots	Α	Gs	E	EUA
leaves	1	0,34	0,31	0,81	-0,26	0,43	0,44	0,23	0,18
stem	0,91	1	0,78	0,35	0,37	0,34	0,23	-0,07	0,36
bark	0,91	0,96	1	0,44	-0,10	-0,02	0,02	-0,23	0,12
branches	0,91	0,74	0,73	1	0,42	0,04	0,00	-0,05	-0,01
roots	0,89	0,88	0,66	0,78	1	-0,17	-0,55	-0,58	0,84
Α	-0,16	-0,23	-0,06	-0,27	-0,65	1	0,80	0,39	0,69
Gs	0,00	-0,07	0,10	-0,18	-0,14	0,95	1	0,74	0,22
E	0,05	0,02	0,13	-0,22	0,51	0,77	0,89	1	-0,37
EUA	-0,35	-0,42	-0,26	-0,24	-0,98	0,72	0,51	0,11	1
_									
В									
В	leaves	stem	bark	branches	roots	А	Gs	E	EUA
B leaves	leaves 1	<b>stem</b> 0,82	<b>bark</b> 0,82	branches 0,93	<b>roots</b> 0,30	<b>A</b> -0,30	<b>Gs</b> 0,03	E -0,43	<b>EUA</b> 0,07
leaves	1	0,82	0,82	0,93	0,30	-0,30	0,03	-0,43	0,07
leaves stem	1 0,74	0,82 1	0,82 0,80	0,93 0,77	0,30 0,21	-0,30 -0,03	0,03 0,15	-0,43 -0,40	0,07 <b>0,50</b>
leaves stem bark	1 0,74 0,90	0,82 1 0,90	0,82 0,80 1	0,93 0,77 0,82	0,30 0,21 0,78	-0,30 -0,03 -0,27	0,03 0,15 -0,09	-0,43 -0,40 -0,52	0,07 <b>0,50</b> 0,32
leaves stem bark branches	1 0,74 0,90 0,93	0,82 1 0,90 0,67	0,82 0,80 1 0,76	0,93 0,77 0,82 1	0,30 0,21 0,78 0,12	-0,30 -0,03 -0,27 -0,23	0,03 0,15 -0,09 0,11	-0,43 -0,40 -0,52 -0,35	0,07 <b>0,50</b> 0,32 0,07
leaves stem bark branches roots	1 0,74 0,90 0,93 0,09	0,82 1 0,90 0,67 0,17	0,82 0,80 1 0,76 0,59	0,93 0,77 0,82 1 0,26	0,30 0,21 0,78 0,12 1	-0,30 -0,03 -0,27 -0,23 -0,34	0,03 0,15 -0,09 0,11 <b>-0,74</b>	-0,43 -0,40 -0,52 -0,35 -0,77	0,07 <b>0,50</b> 0,32 0,07 0,33
leaves stem bark branches roots A	1 0,74 0,90 0,93 0,09 -0,54	0,82 1 0,90 0,67 0,17 -0,47	0,82 0,80 1 0,76 0,59 -0,62	0,93 0,77 0,82 1 0,26 -0,53	0,30 0,21 0,78 0,12 1 - <b>0,96</b>	-0,30 -0,03 -0,27 -0,23 -0,34 1	0,03 0,15 -0,09 0,11 <b>-0,74</b> 0,83	-0,43 -0,40 -0,52 -0,35 -0,77 0,82	0,07 <b>0,50</b> 0,32 0,07 0,33 0,36
leaves stem bark branches roots A Gs	1 0,74 0,90 0,93 0,09 -0,54 -0,08	0,82 1 0,90 0,67 0,17 -0,47 0,05	0,82 0,80 1 0,76 0,59 -0,62 0,00	0,93 0,77 0,82 1 0,26 -0,53 -0,28	0,30 0,21 0,78 0,12 1 - <b>0,96</b> - <b>0,93</b>	-0,30 -0,03 -0,27 -0,23 -0,34 1 0,51	0,03 0,15 -0,09 0,11 <b>-0,74</b> 0,83 1	-0,43 -0,40 -0,52 -0,35 -0,77 0,82 0,77	0,07 <b>0,50</b> 0,32 0,07 0,33 0,36 0,04





#### Percentage of Carbon Allocation (kg/tree)

Figure 1. (A) Correlations between biomass of roots and water use efficiency (WUE) in both sites (Aracruz and Montezuma) during the dry season; (B) Correlations between biomass of roots and water use efficiency (WUE) in both sites (Aracruz and Montezuma) during the wet season and (C) Percentage of carbon allocation in *Eucalyptus* hybrid clones divided in five components: stem, roots, branches, leaves and bark. \* P<0,05; t-Test.

In the dry season, there was only significant difference between sites for carbon allocation in stem, while in the wet season, the differences were significant between sites for stem, bark and roots (Figure 1).

The clones used in this experiment were obtained from populations adapted to Aracruz. Explicit consideration of genotype differences in water acquisition and efficiency of use may be a valuable component of genetic improvement programs, with clear attention to interactions with silviculture and site factors.

For example, it is not clear if higher growth rates would be achieved by genotypes that maximize water use, maximize efficiency of water use, or some optimal combination of both features (Blum, 2008). These issues may be particularly important for the environmental impacts of intensive plantation forestry, especially for issues of stream-water yields in landscapes receiving less than 800 or 900 mm/ yr of rainfall (Jackson et al., 2005; Little et al., 2009).

# Conclusions

The variability found in this experiment allows the company to continue researching this issue to determine a physiological parameter to enhance profit in its genetic improvement program.

Water supply is critical when rainfall varies among years, and the responses to these variations differ substantially among clones. Fibria is already examining these interactions with a variety of clones and with rainfall exclusion treatments, providing detailed experimental manipulations of water supply.

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# Environmental drivers of eucalypt productivity

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# Introduction

The availability of highly productive land for production forestry has been decreasing in the subtropics of Australia and many other regions of the world. Plantation expansion has consequently developed in environments subjected stress from climatic and edaphic factors. Australian government agencies and private industry have invested in the establishment of extensive field trial networks designed to evaluate alternative eucalypt populations in these conditions (Lee *et al* 2011). These trials have been useful for estimating potential productivity in these poorly characterised areas and providing insights into the differential impacts environmental drivers have on taxa productivity.

# Methods

Plot-level data on individual tree growth and survival was compiled to estimate mean annual increment (MAI) for taxa within 38 trials ten years after establishment across the tropics of Australia. When a species was represented by multiple taxa or provenances, each was classified as a different observation within trials and separate least square means (LSMeans) were estimated for each taxon in a single across-trial analysis. Environmental variables were collated for each trial from the point of trial establishment to trial assessment (Figure 1 footnote). LSMeans from a selection of 16 species that were well-represented across the trials were associated with 11 environmental variables for each trial. Simple linear regression was then used to estimate Pearson correlation coefficients, which related the trial specific MAI estimate (dependent variable) for each species to the 11 environmental characteristics. The correlation coefficient for any pair of species and environmental variable was thus interpreted as the response of the species to the environment and represents the extent of change in MAI that can be expected across

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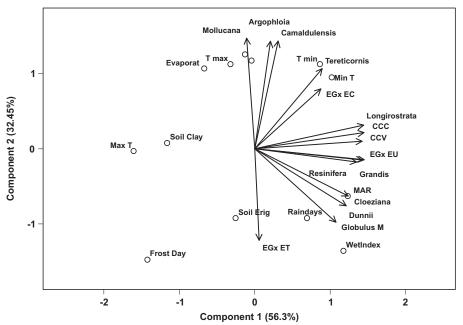
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that environmental gradient. A principal components analysis (PCA) based multidimensional preference analysis was then completed using the resulting twoway table, which included genotype by environment estimates, to generate a biplot for developing hypotheses about species specific environmental drivers of productivity.

Within the biplot of Figure 1, the length of the species vectors indicates PCA fit and the direction of the vector indicates environmental preferences by the individual species, with preference increasing as the vector moves from the origin. Each environment point can be orthogonally projected onto any species vector and the environment that projects farthest along a vector in the direction it points is that species' most preferred environment. While points that are tightly clustered in a



**Figure 1.** Biplot depicting relationships between eucalypt taxa<sup>1</sup> response (vectors) to environmental variables<sup>2</sup> (open circles).

<sup>&</sup>lt;sup>1</sup>EGxEU= *E.grandisXE.urophylla*, EGxET= *E.grandisXE.tereticornis*, EGxEC = *E.grandisXE.camaldulensis*, GlobulusM= *E.globulus* subsp. *maidenii*, CCC= *Corymbia citriodora* subsp. *citriodora*, CCV= *Corymbia citriodora* subsp. *variegata* <sup>2</sup>Environmental variables and species abbreviations included: Tmax= Average daily maximum temperature, Tmin= Average daily minimum temperature, Q= Net daily radiation, MAR= Mean annual rainfall, Evaporat= Mean annual evaporation, WetIndex= Annual rainfall / Annual evaporation, Rainday= Average annual rain days, MaxT= Extreme high temperature, MinT=Extreme low temperature, SoilWate= Soil water holding capacity, SoilBrig= Buntley & Westin soil brightness index, SoilClay= Soil clay percentage,

region of the plot represent environments that have the same preference patterns across the species, vectors that point in a similar direction represent species that have similar sensitivity to environmental variables.

# Results

Some highlights that can be drawn from figure 1 follow: 1) *E.dunnii*, *E.cloeziana* and *E.globulus* respond positively to water related variables such as Wetness Index (most influential), MAR and Raindays while EGxEC and *E.tereticornis* are relatively unresponsive to water and *E.mollucana* responds most negatively to increased water, 2) while no species respond positively to soil clay content or extreme maximum temperature *E.mollucana*, *E.argohloia*, *E.camaldulensis* and EGxET are show little response with the latter responding very differently to the former 3 species, 3) *CCC, CCV E.pellita* and *E.longirostrata* appear to be broadly adapted and show generic responses to environmental variables (following PCA1); higher extreme minimum temperature and MAR are only laterally influential environmental variables.

# Discussion

While these experiments were targeted to sample a range of climates and soil types, the utility of the empirical models that have been derived from this trial network is generally limited to the environments in which the stands are measured. Forestry process-based models (PBMs) offer an alternative for predicting productivity and are particularly useful when predictions are required for conditions that differ from those in which the models has been calibrated. Nevertheless, PBMs generally require extensive calibration to ensure they are properly parameterised for a species or genotype of interest and validation requires existing stands in the area of interest. Using information such as that derived from the biplot based on this taxa trial network to facilitate the development and adoption of PBMs rather than for post-hoc verification of 'calibrated' models could be an effective way to focus resources on key physiological traits and accelerate model application.

The reported generalisations on the responses of eucalypts to environmental drivers leads to the concept of plant functional traits; quantitative traits related to the fitness and success of individuals in a given environment which provide an indication of species' ecologies and are often related to competitive status (Nicitona et al 2010). The clustering of species around environmental drivers would point to similarities in functional traits and potentially similarities in PBM

parameterisations. For example, using the 3PG species parameters detailed by Almeida et al (2004), it would appear logical to focus on traits other than response of canopy to vapour pressure deficit or stomatal conductance if developing a new parameterisation for species within the water associated clade (highlight 1 above). Alternatively, it would appear prudent to focus on adjusting minimum, optimum and maximum temperatures for growth when extending PBMs to species like *E.mollucana* and EGxET. While empirical growth model development has traditionally been a major output of species trial evaluations, the meta-analysis presented herein should be useful specifically for PBM development and more generally for plant functional research, which aims to generate general rules of species assembly and species reactions based on plant functional traits.

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# Drought tolerant *Eucalyptus globulus* provenances have thick bark, high basic density and grow poorly on wet sites

Gregory W. Dutkowski<sup>1</sup> and Brad M. Potts<sup>2</sup>

#### Introduction

Reduced rainfall and increased temperature is predicted to increase drought risk and affect planted eucalypt forests in Australia (Battaglia *et al.* 2009). Significant genetic variation in drought damage in *E. globulus* at both the provenance and family level has been reported in Australia (Dutkowski 1995) and Spain (Toro *et al.* 1998) and is targeted in breeding programs. Costa e Silva *et al* (2006) have indicated that provenance level genotype by environment interaction for growth is related to drought susceptibility. This study looks at the provenance and additive genetic correlation of the drought damage reported by Dutkowski (1995) with later age survival, growth and other traits on the South Australian and Tasmania sites reported by Costa e Silva *et al* (2006) which were not sublined.

#### Materials and Methods

Open pollinated seed from parent trees from the natural range of *E. globulus* and its intergrade populations were collected (Gardiner and Crawford 1987; Gardiner and Crawford 1988) (Table 1) and localities allocated into 22 geographic subraces (adapted from Dutkowski and Potts, 1995).

The drought trials were established as 4 sublined trials in close geographic proximity in Western Australia with 10 common families. In these trials drought damage was assessed on a 9 point ordinal categorical scale (DRY) following an extended dry period at around age 4. In the trials without drought deaths, survival, height, DBH, bark thickness (% of DBH), pilodyn penetration (inversely related to density) and core basic density were measured at a variety of ages.

The data were analysed for all of the DRY data and for each trait on each other trial in turn using a linear mixed model:

$$y = X_{t}t + \frac{\frac{X_{t}t}{m}}{m} + \frac{\frac{Z_{t}t}{r}}{r} + \frac{\frac{Z_{t}t}{i}}{i} + Z_{T,s}T,s + Z_{T,f}T,f + \frac{t}{\xi} + \frac{t}{\eta}$$

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	Subrace					Nu	mber o	of Fami	lies					
Code	Name	Collection	D 1	D 2	D 3	D 4	Any	S9	S10	T11	T12	T13	T14	T15
							D	BA	HE	WO	WR	MG	SO	EX
21	Far West Otways	6		6			6			3	4	6	5	5
1	Western Otways	124		119			119	*57	*57	98	89	120	113	113
2	Cape Patton	21		20			20	3	3	9	9	18	18	15
3	Eastern Otways	31		24			24			24	24	23	24	24
4	Strzelecki Ranges	59			58		58	26	26	57	55	58	59	58
5	Strzelecki Foothills	9			9		9	7	7	7	6	8	8	8
22	Foothills	4			3		3	1	1	3	2	3	3	3
6	Coastal Plain	14			8		8	3	3	9	7	13	10	9
7	Wilsons Promontory Lighthouse	16			13		13	7	7	14	12	16	15	15
8	Flinders Island	66	19			44	63	4	4	46	45	61	54	53
9	Southern Furneaux	50				43	43			46	43	50	50	48
10	St. Helens	13								8	6	11	10	10
11	North-eastern Tasmania	21	12	2	2	2	12	13	14	16	13	19	19	18
12	Inland North- eastern Tasmania	15	10	1	1	1	10	10	10	*16	*13	*15	*19	*18
13	Dromedary	4								4	4	4	4	4
14	South-eastern Tasmania	63	32	4	4	4	32	24	23	49	46	61	58	53
15	Southern Tasmania	28	12	2	2	2	12	7	7	21	19	27	27	25
16	Tasman Peninsula	5								4	3	5	5	5
17	Recherche Bay	5	3				3	3	3	2	2	4	4	4
18	Port Davey	6	6				6	4	4	3	4	6	6	5
20	King Island	50	13			17	30	9	10	28	28	32	32	32
Total		643	112	179	101	114	476	182	183	493	457	589	571	553

Table 1. Subrace representation in the droughted (D), South Australian (S) and Tasmanian (T) trials.

Common families in the drought trials are indicated in bold type. The S and T trial number follows Costa e Silva *et al* (2006). \* extra families from another collection.

The data vector **y** includes all of the DRY data and the other trait for each trial (**t**), augmented to facilitate spatial analysis;  $X_t$  are the fixed effect design matrices for the mean for each trial (**t**), and  $X_{t/m}$  for the missing values for spatial analysis in each trial (**t**/m);  $Z_{t/k}$  are random effect design matrices within each trial for the design features (**k**) for replicates (**r**), incomplete blocks (**i**) and plots (**p**);  $Z_{r,g}$  are the random effect design matrices across the traits (**T**) for the genetic terms (**g**) for subraces (**s**) and families (**f**);  $t/\xi$  are vectors of spatially auto-correlated residuals; and  $t/\eta$  vectors of independent residuals within each trial. Henderson's (1984) formulation of these equations was used:

$$\begin{bmatrix} 2 \end{bmatrix} \begin{bmatrix} \mathbf{X}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{X}'\mathbf{R}^{-1}\mathbf{Z} \\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{Z}'\mathbf{R}^{-1}\mathbf{Z} + \mathbf{G}^{-1} \end{bmatrix} \begin{bmatrix} \hat{\mathbf{b}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{R}^{-1}\mathbf{y} \\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{y} \end{bmatrix}$$

where **G** is the direct sum of the variance–covariance matrices of each of the t/k design effects each of the form  $\sigma_k \begin{bmatrix} 2 \\ k \end{bmatrix}$  and the **T.g** genetic effects, each of the form  $\sigma_k \begin{bmatrix} 1 & r_s \\ r_s & 1 \end{bmatrix} = \sigma_{kel}$  where  $r_g$  is the genetic correlation, and **R** is the variance–covariance matrix of the residuals comprising an independent term and a separable first-order autoregressive correlation matrix with autocorrelation  $\rho$  in each direction (Dutkowski et al. 2002). The poorly growing subraces of Wilsons Promontory and Port Davey were eliminated from the estimates of subrace correlations.

Preliminary analyses using a simplified model were used trial to determine if spatial analysis improved the model fit – and to verify that the DRY additive variance was similar for all of the four drought trials to allow data pooling.

The variance parameters were estimated by REML using ASReml 3.0 (Gilmour et al. 2009).

# Results

The subrace effects for DRY showed a distinct spatial pattern (Fig. 1). King Island and the west coast of Tasmania were the most susceptible to drought damage, grading through intermediate susceptibility in south-east Tasmania to low susceptibility in the north-east. There was a similar cline from west to east in the Otway Ranges and from the Gippsland coastal plain to the Strzelecki Ranges. Wilsons Promontory did not conform to this trend in Gippsland. Flinders Island had moderate to low susceptibility.

The subrace correlations indicated drought damage was generally associated with low survival on drier sites (negative correlation) grading through to the opposite effect for high rainfall sites. Trial S10 was not consistent with this trend, as was also found by Costa e Silva *et al.* (2006) for growth, perhaps related to waterlogging on this site. Growth correlations broadly followed the same trend, but there was no relationship on low rainfall sites with drought damage. Correlations decreased

	D1	D 2	D 3	D 4	<b>S</b> 9	S10	T11 WO	T12 WR	T13 MG	T14 SO	T15 EX
Year planted	1988	1989	1989	1989	1988	1988	1989	1989	1989	1989	1989
Latitude (S)	33º34	33º34	33º34	33º34	37°54	37°35	40º49	41º08	41º05	41º17	41º17
Longitude (E)	116º3	116º3	116º3	116º3	140°49	140°55	144º53	145º48	145°54	146º27	146º52
Altitude (m)	240	180	170	240	60	75	60	180	120	100	120
Rainfall (mm)	818	818	818	818	763	715	1161	1249	1065	955	969
Replicates	5	9	9	9	4	4	5	5	5	5	5
Incomplete blocks per replicate	: 11	13	13	13	29	29	25	23	25	24	28
Plots per block	10	13	7	8	7	7	20	20	24	24	20
Trees per plot	5 or 10	5	5	5	5	5	2	2	2	2	2

Table	2.	Trial	information.
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with age for survival for the highest rainfall site, but increased with age for growth across all sites. Additive genetic correlations for survival were mildly negative across all sites, and positive only at later ages for growth at the high rainfall site. The subrace correlations strongly indicated thin bark and low density (high pilodyn) for drought damaged subraces, but a much weaker relationship between these traits at the additive level.

# Discussion

The pattern of geographic variation in drought susceptibility across the geographic range of *E. globulus* observed is broadly in line with studies of drought survival (Toro et al. 1998) and damage by the cerambicid beetle *Phoracantha semipunctata* (eucalyptus longhorned borer) (Soria and Borralho 1998) following drought in Spain.

These results indicate that there is likely to be substantial genotype-by environment interaction at the subrace level for survival and growth, with our results suggesting that subraces with low drought damage would actually be

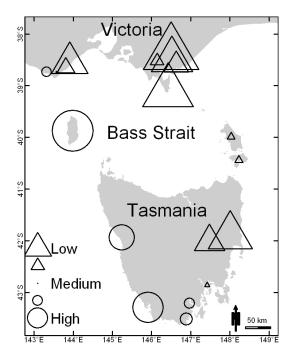


Figure 1. Subrace means for drought damage.

**Table 3.** Subrace and additive correlations between drought damage and traits in other trials, with trials ordered in decreasing rainfall. The superscript indicates 10 times the t-value (Estimate/Standard error). B indicates an estimate at the boundary.

Trait	Age	T12 WR	T11 WO	T13 MG.	T15 EX.	T14 SO	S9 BA	S10 HE
			Su	brace Correl	ations			
Survival	2			0.0401				
	4/5	0.45 <sup>19</sup>	0.0401	0.0502	-0.17 <sup>04</sup>	-0.47 <sup>08</sup>	-0.18 <sup>05</sup>	0.3909
	8	0.2911	0.1806	0.1504	-0.14 <sup>05</sup>	-0.53 <sup>10</sup>		
	11	0.2409						
	15/16	0.3012				-0.39 <sup>13</sup>		
	19/22	0.2308	0.19%					
Ht	2			-0.25 <sup>09</sup>				
	4	0.3714	0.38 <sup>15</sup>	-0.07 <sup>02</sup>	-0.17 <sup>05</sup>	0.1204		
DBH	2			-0.32 <sup>12</sup>				
	4/5	0.2007	0.3011	-0.08 <sup>03</sup>	-0.13 <sup>04</sup>	-0.07 <sup>02</sup>	-0.18 <sup>05</sup>	$0.18^{04}$
	8	0.4519	0.4821	0.2308	-0.01 <sup>01</sup>	0.0401		
	11	0.58 <sup>27</sup>						
	15/16	0.58 <sup>29</sup>				0.0000		
	19/22	0.55 <sup>25</sup>	0.49 <sup>23</sup>					
Bark %	6/7	-0.97 <sup>285</sup>	-1.00 <sup>в</sup>	-0.87 <sup>105</sup>	-0.97 <sup>285</sup>	-0.92 <sup>158</sup>	-0.89 <sup>107</sup>	
Pilodyn	6/7	0.8470	0.7752	0.7651	0.94168	0.7855	0.7238	
Basic	12/17				-0.87104	-0.68 <sup>39</sup>		
Density								
			Ac	ditive Correl	ations			
Survival	2			-0.1815				
	4/5	-0.1210	-0.2216	-0.18 <sup>15</sup>	0.1410	0.0101	0.0906	-0.2314
	8	-0.2324	-0.22 <sup>19</sup>	-0.05 <sup>05</sup>	-0.05 <sup>06</sup>	-0.11 <sup>09</sup>		
	11	-0.2023						
	15/16	-0.13 <sup>15</sup>				-0.18 <sup>19</sup>		
	19/22	-0.1212	-0.25 <sup>25</sup>					
Ht	2			0.1012				
	4	-0.22 <sup>23</sup>	-0.0910	0.1518	0.05%	-0.06 <sup>07</sup>		
DBH	2			0.0809				
	4/5	-0.07 <sup>08</sup>	-0.04 <sup>04</sup>	0.1619	-0.01 <sup>02</sup>	-0.08 <sup>08</sup>	-0.04 <sup>03</sup>	-0.11 <sup>08</sup>
	8	0.0808	-0.0404	0.2125	-0.01 <sup>01</sup>	-0.0514		
	11	0.1312						
	15/16	0.3426				0.1614		
	19/22	0.4518	0.2120					
Bark %	6/7	-0.35 <sup>21</sup>	-0.1006	-0.24 <sup>25</sup>	-0.22 <sup>20</sup>	-0.15 <sup>09</sup>	-0.41 <sup>26</sup>	
Pilodyn	6/7	0.2218	0.3921	0.0605	0.0202	0.11110	0.0804	
Deele	12/17				-0.17 <sup>18</sup>	-0.19 <sup>12</sup>		
Basic	/							

detrimental to survival and growth on wetter sites. Within subrace selection for survival across all sites would decrease drought damage, but the picture is less clear for growth.

The contrast in correlations at the subrace and additive levels indicate that there are different mechanisms operating at these two levels.

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## Substitution of K<sup>+</sup> by Na<sup>+</sup> in *Eucalyptus grandis* plantations: consequences on the biological cycle of nutrients

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## Summary

Even though high sodium (Na) contents strongly depress crop yields in saline soils (e.g. Sudmeyer & Simons, 2008), low Na inputs can be beneficial for some crops with the capability to maintain important physiological functions in substitution to K (e.g. Wakeel et al., 2011). Subbarao et al. (2003) proposed to consider Na as a functional element with potential positive effects for the development of numerous plant species in K-depleted soils. A positive response to sodium chloride (NaCl) addition has been demonstrated *in situ* for the first time for trees (to our knowledge) in *Eucalyptus grandis* plantations established on a weathered tropical soil highly depleted in K (Almeida et al., 2010). The commonly admitted hypothesis of little involvement of Na in canopy processes (e.g. Staelens et al., 2008) and more generally in tree growth might thus be invalid in eucalypt plantations established on K-deficient soils. We tested the hypothesis that an improvement in Na availability in a K-deficient soil can to lead to a partial substitution of K by Na in the biological cycle of nutrients, throughout stand development. The main fluxes of nitrogen (N), phosphorus (P), K, calcium (Ca), magnesium (Mg) and Na were quantified in a complete randomized block experiment where applications of 4.5 kmol ha<sup>-1</sup> K (+K), as KCl, and 4.5 kmol ha<sup>-1</sup> Na (+Na), as NaCl, were compared to a control treatment (C) with no K and Na application. Potassium and Na additions were split in 3.0 kmol ha<sup>-1</sup> (116 and 68 kg ha<sup>-1</sup>, respectively) applied the first year after planting and 1.5 kmol ha<sup>-1</sup> applied at age 5 years. A non-limiting fertilization for the other nutrients was applied in all the plots. Aboveground tree components were sampled destructively for 8 trees per treatment at the end of each rainy season and nutrient contents were quantified annually up to age 6 years (harvest age in Brazilian commercial plantations) using

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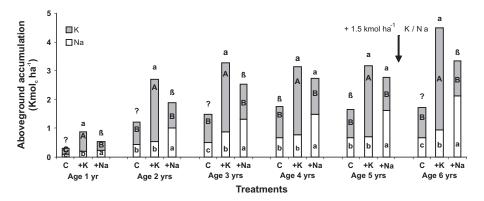
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treatment-specific allometric relationships. Litterfall was collected every 4 weeks over the whole rotation and thoughfall solutions were collected weekly and analysed every 4 weeks in composite samples. Chemical analysis were made to estimate the main fluxes of the biological cycles for N, P, K, Ca, Mg, Na over the whole rotation.

The aboveground biomass at age 6 years amounted to 76, 162 and 107 Mg ha<sup>-1</sup> in the C, +K and +Na treatments, respectively. The amounts of K+Na (expressed in moles of charge per hectare) accumulated in aboveground tree components were significantly higher in +K than in +Na the 3 first years after planting (P < 0.05), but no more at ages 4 and 5 years (Figure 1), before the last fertilization. The accumulation of Na (expressed in number of moles) was higher than that of K in treatment +Na throughout stand development, and the opposite occurred in the +K treatment. The dynamics of K and Na accumulation within aboveground tree components exhibited a similar pattern. From 75 to 88% of the amounts of K accumulated aboveground at age 5 years were already found in the trees at age 2 years, depending on the treatment. The proportions ranged from 62 to 74% for Na. Such dynamics suggest that trees took up early the pools of available K and Na in the soil, and that most of tree demand in K from age 2 years onwards was supplied by the biological cycle. Potassium and Na additions at age 5 years in treatments +K and +Na raised by 43 and 12 kg ha<sup>-1</sup> their accumulation within trees the last year of

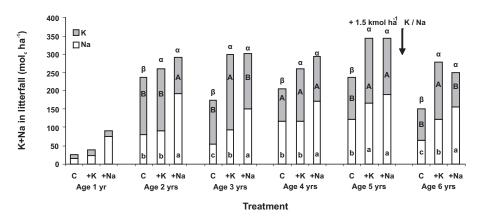


**Figure 1.** Sum of K<sup>+</sup> and Na<sup>+</sup> contents within aboveground tree components (expressed in kmol<sub>c</sub> ha<sup>-1</sup>) estimated at 1, 2, 3, 4, 5, and 6 years of age in the control treatment (C) and in the treatments with 4.5 kmol ha<sup>-1</sup> K (+K) and kmol ha<sup>-1</sup> Na (+Na) additions. Sodium contents are indicated by empty bars and K contents by filled bars. Different lower case, upper case and greek letters between treatments at the same age indicate significant difference in Na, K and Na+K contents (P <0.05), respectively.

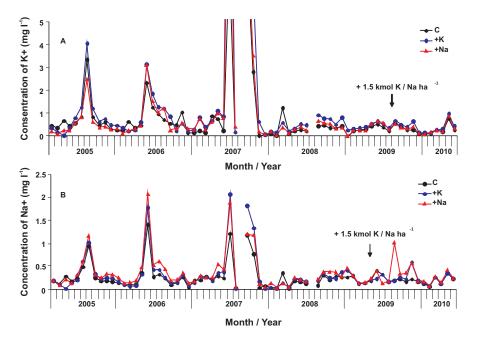
the rotation, respectively. About 30 kg ha<sup>-1</sup> of K was accumulated in stembark the 6<sup>th</sup> year after planting in +K. Half of the amount of K applied at age 5 years was thus likely to be exported off-site with stembark at the harvest.

Potassium and Na additions had the same effects on their return to soil with litterfall. Sodium content in litterfall comprised 40-60% of the amount of K+Na over the rotation in treatments C and +Na. The proportion of K was higher in the +K treatment than in the K-depleted treatments (Figure 2). Resorption proficiencies of K and Na were similarly reduced by the addition of these elements over stand development. Sodium remobilization during leaf senescence and stemwood ageing was much higher in the +Na treatment than in C and +K, suggesting that Na was reused to produce new organs throughout tree development.

Addition of NaCl at the soil surface increase the concentrations of Na<sup>+</sup> in throughfall solutions in comparison with the C treatment, mainly the 3 three first years after planting, when Na foliar concentrations were high (Almeida et al., 2010). While negligible canopy exchange of Na<sup>+</sup> in forest canopies are generally admitted, our results point out the limitations of the canopy budget model for *Eucalyptus* forests established on K-depleted soils. Our study supports thus the hypothesis that NaCl addition enhanced tree growth through a partial substitution of K by Na in *E. grandis* physiology.



**Figure 2.** Sum of K<sup>+</sup> and Na<sup>+</sup> contents within litterfall over stand rotation. Sodium contents are indicated by empty bars and K contents by filled bars. Different lower case, upper case and greek letters between treatments at the same age indicate significant difference in Na, K and Na+K contents (P < 0.05), respectively (from age 2 years onwards).



**Figure 3.** Dynamics of  $K^+$  (A) and Na<sup>+</sup> (B) concentrations in throughfall solutions sampled weekly below the canopy in treatments C, +K and +Na and analyzed every 4 weeks up to harvesting age. One set of 12 funnels installed in each treatment in block 1 in February 2005 was completed by 2 replications in April 2009 in order to sample throughfall solutions in 3 blocks over the last year of stand rotation.

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## Phenology of eucalyptus clones and hybrids growth under contrasting water environments

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Annual phenology at early stages of tree development may be critical to understand how different genotypes interact with environmental constraints such as soil water availability, critical seasonal temperatures and effects of vapor pressure deficit on tree growth(Williams et al. 1997; O'Grady et al. 1999). Success on capturing genetic gain of highly deployed Eucalyptus clonal plantations will require a key understanding in order to match genotypes to site and their phenotipic plasticity under changing climatic conditions (Costa e Silva et al. 2009; Silva et al. 2009).

We evaluated the phenological development of six *Eucalyptus alobulus* clones of high, medium and low productivity, a E. nitens highly improved seed material, and three hybrids (E. nitens x globulus, E. camaldulensis x globulus and E. nitens x E. camaldulensis) under rainfed vs. liquid fertilization irrigated conditions. Fertirrigation was applied during the summer season (November to March) in order to compensate for pan evaporation estimates. Tree height and diameter were carried approximately biweekly during two years since establishment on 3 individual trees for each selected genotype under each irrigation treatment. Climatic data considering daily rainfall, mean, maximum and minimum average temperature were obtained from weather station located side to the study. TDR measurements for 0-30 cm soil available water content were recorded monthly. Annual foliage samples were obtained for nutritional analyses of each genotype at each treatment condition to assess the effect of nutrient additions on irrigation. Daily vapor pressure deficit and evapotranspiration were estimated based on recorded information. Analyses of variance for each measurement period were evaluated for absolute cumulative growth, growth rate and percent seasonal growth rate considering genotype and irrigation treatment effects on a mixed model. Lower soil available water estimates during the first growing season contrasted with higher estimates given a second growing season wet summer.

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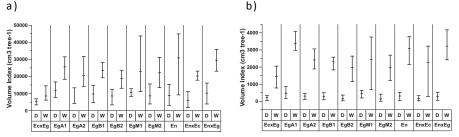
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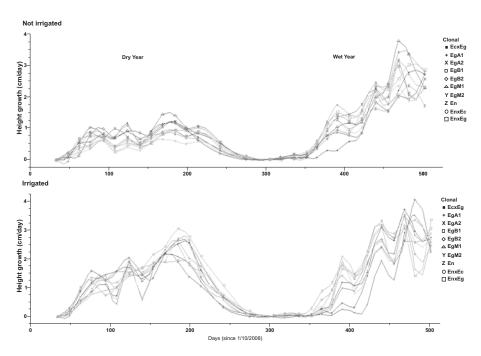
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Average cumulative volume index (height x diameter<sup>2</sup>) growth between rainfeed and fertirrigated genotypes reached a difference of 120% for the first and 200% for the second growing season (Figure 1). Large ranking changes were observed among clones between rainfeed and fertirrigated treatments both seasons (Figure 2).



**Figure 1.** Volume index cumulative growth for a) first and the b) second growing season under rainfed (D) and fertirrigated (W) treatments. Genotypes codes for E. globulus (Eg) are high (A), medium (M) an low (B) productivity, *E. nitens* (En), and hybrids *E.nitens* x globulus (En x Eg), *E. nitens* x camaldulensis (En x Ec) and *E.camaldulensis* x globulus (Ec x Eg).



**Figure 2.** Phenology of height growth since establishment for all evaluated genotypes during the first (dry year) and the second (wet year) growing season under Rainfed and Fertirrigated treatments. Genotypes codes for *E. globulus* (Eg) are high (A), medium (M) an low (B) productivity, *E. nitens* (En), and hybrids *E.nitens* x globulus (En x Eg), *E. nitens* x camaldulensis (En x Ec) and *E.camaldulensis* x globulus (Ec x Eg).

Genotypes differences in volume index accounted for 50% for the first and 220% for the second growing season at the fertirrigated treatment, but accounted for 75% for the first and 160% difference at rainfed treatment. Analysis of *E. globulus* genotypes for the second growing season showed a 215% difference in cumulative volume index between rainfed and fertirrigated treatments, a similar range when comparing all evaluated genotypes. However, rainfed *E. globulus* genotypes differed by 66% compared to a 25% difference for fertirrigated genotypes. This result suggest a large phenotypic plasticity of *E. globulus* genotypes under low resource availability conditions.

Growth rates among genotypes were strongly affected by daily mean temperature and soil available water. However, soil available water affected responses mainly during the first growing season. Large differences in phenology of height and diameter were observed among genotypes between the first (dry) and the second (wet) growing season (Figure 2). *Eucalyptus nitens* and its hybrids showed the longest growing seasonal patterns for height and diameter growth given its almost continuous development along the year and the highest growth rates compared to other genotypes except during the summer season. On the other hand, height growth rates for *E. camaldulensis* hybrids were the highest during the summer season when low soil available water and high atmospheric water demand critically impaired *E. nitens* and somewhat less *E. globulus* genotypes growth rates. Our results suggest that *E. camaldulensis* hybrid genotypes require the highest temperatures for growth initiation and average growth rates compared to other genotypes.

Phenology of E. genotypes may be used as a powerful tool to integrate environmental stresses and understand plasticity of highly developed material for selection of advanced deployed genetic material.

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## Mapping of the Brazil eucalyptus potential productivity

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## Background

The early studies in Brazil trying to identify potential sites for forestry production come from the period of fiscal incentives by highlighting the study of Golfari (1974). After this period is incorporated into the Brazilian forest sector use of Geographic Information Systems. During this time is that it develops the empirical model from the production of timber, where the methods relate the dependent variable with independent variables such as soil properties and climate. However, aiming to understand the processes that govern tree growth, it is necessary to use so-called ecophysiological models, for example, the model 3-PG (Physiological Processes Predicting Growth), published by Landsberg and Waring (1997), which has produced satisfactory results regarding the estimation of forest productivity, with several applications in Brazil (Stape et al., 2004, Almeida et al., 2010, Alvarez 2011). Thus, in a first approximation, the purpose of this study was to determine exploratory level, the potential productivity of Eucalyptus in Brazil using ecophysiological model 3-PG.

## **Materials and Methods**

The 3-PG model was rewritten in computer language of type Visual Basic for Applications (VBA), results consistent with the programs of geographic information systems. For the spatial and interpretation of the model 10 was used ArcGIS (ESRI, 2011). We used the concept of minimum mappable area (IBGE, 2005), in this case was 625 km2, to compose the final maps in the scale of publishing 1:4.000.000. Agrometeorological variables maximum temperature for each month (C), minimum temperature for each month (C), mean monthly temperature (° C), relative humidity for each month (%), monthly rainfall (mm) and daily insolation (h) were rescued from the report climatological normals of the country (Brazil, 1992). The data points were interpolated from weather stations and spatialized obtaining the entry

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of these variables in the model 3-PG. The calculation of solar radiation and net radiation were performed according to Pereira et al. (2002). Data relief (altitude, slope and aspect) were obtained from the model SRTM (Shuttle Radar Topography Mission) (Farr and Kobrick, 2000) in its current version 4 (Jarvis et al., 2008). The soil fertility was based upon the fertility of the soils of Brazil proposed by Camargo (1980). The maximum storage capacity of the soil water parameters and modifiers of the potential availability of water were estimated as the current soil map of Brazil (IBGE, 2001). Finally, we use a comprehensive database design Plots Twin Inventory Program and Brazil Eucalyptus Productivity Potential, the Forestry Science and Research Institute (IPEF) to compare the potential productivity of Eucalyptus observed in the field with the data generated by the model 3-PG.

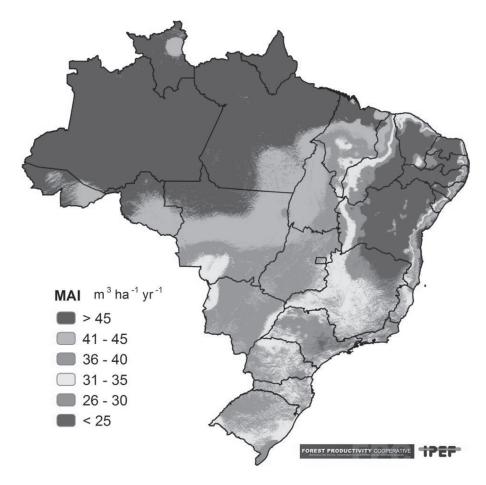


Figura 1. Map of the Brazil eucalyptus potential productivity.

## Results

Productivity was strongly characterized by the pattern of rainfall distribution in Brazil. The Northeast had the lowest productivity, with a mean below 25 m<sup>3</sup>ha<sup>-1</sup>yr<sup>-1</sup>. However, this region is a strong gradient of productivity, with higher mean productivity on the coast, more than 45 m<sup>3</sup>ha<sup>-1</sup>yr<sup>-1</sup>, even the smallest productivity toward the interior. The same effect was observed in the region for Stape et al. (2004). The potential productivity is very high throughout the northern region of Brazil, as the water deficit is very low or zero, with no frost and temperature conditions present in much of the year. In southeast and south region, the increased productivity is replaced by the relief influence, causing different distribution schemes of global radiation. High-average yields were mapped in much of the Midwest. Productivity data were confronted with an extensive network re twin inventory plots (Plots Twin Inventory Program, from PEF) and sites from "Brazil Eucalyptus Productivity Potential" project (IPEF).

## Conclusions

In this first stage of the modeling process, the model 3-PG applied on a national scale showed consistency in determining the magnitude of productive potential. The map showed that the productivity of Eucalyptus in Brazil is strongly controlled by different climatic regions and their monthly variations. The next step will be to identify and understand the sites less accurate estimation of potential productivity.

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## Drought response at age 9 years of 5 *Eucalyptus* species planted at 3 different densities at Highflats plantation, South Africa

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### Background

Long term rainfall data indicated that there is a very high probability that at least one drought event will occur within an eleven year rotation on Highflats plantation, located in the KwaZulu-Natal midlands, South Africa. An assessment of drought conditions during 1999 indicated that geology, age-class and species were all influential in the extent of drought stress. Coarse textured sandstone derived soil with low water holding capacity exhibited the greatest susceptibility to drought. Assessment of the drought tolerance of various species following the 1999 drought event proved difficult because of confounding species-site allocations. Furthermore, the assessment could not provide information regarding the effect of planting density on drought stress. To better understand what species should be planted on these sites and to understand the impact of planting density on productivity and drought damage, a trial containing five tree species planted at three densities was established on a sandstone derived soil that was known to be drought prone. Serious drought symptoms were observed in October 2010 after only 253 mm of rainfall was recorded from April to September of 2010, as opposed to the long term average of 432 mm over the same period.

### **Materials and Method**

A factorial trial with five replications was established to test the response of *Eucalyptus dunnii*, *E. grandis*, *E. paniculata*, *E. sideroxylon* and *E. grandis* x *camaldulensis* clone (G438) to drought. The trees were planted at three densities, namely 816, 1600 and 2066 stems per hectare on 25 x 25 m plots by planting 7 x 7, 10 x 10 and 11 x 11 tree rows per plot at a special arrangement of 3.5 m x 3.5 m, 2.5 m x 2.5 m and 2.2 m x 2.2 m, respectively. The inner 5 x 5, 6 x 6 and 7 x 7 tree rows of the respective planting densities were regularly assessed in terms of diameter and height growth. Crown damage (4 classes – dead, completely defoliated, more than  $\frac{1}{2}$  crown foliage lost and less than  $\frac{1}{2}$  crown foliage loss) was assessed after the

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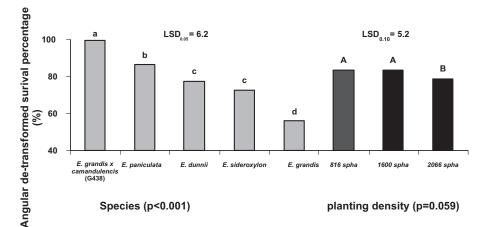
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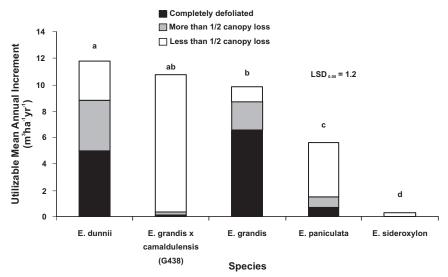
2010 drought event that occurred when the trees were 9 years old. Analysis of variance in association with multiple comparison of means was used to investigate treatment effects.

## **Results and Discussion**

After the 2010 drought there was a significant difference in survival between the various species, with G438 having the highest survival percentage and *E. grandis* the lowest (Figure 1). Planting density had a minimal () effect (p=0.056) on tree survival. The trees planted at 2066 stem per hectare had a lower survival rate than those planted at a lower density (Figure 1). The three commercially planted genotypes, *E. dunnii*, G438 and *E. grandis* outperformed *E. paniculata* and *E. sideroxylon* in terms of mean annual increment (MAI) (Figure 2). The *E. dunnii* had a significantly greater MAI than the *E. grandis*, which was previously planted on these sites. The planting density had no significant effect on volume production and there was no interaction between species and planting density. Ninety percent of the G438 trees retained more than 50% of their canopy during the drought opposed to 13% of *E. dunnii* and 5% of *E. grandis* that retained more than 50% of their foliage. Severe drought symptoms were observed in 63% of the *E dunnii* trees and 42% of *E. grandis* trees. Approximately 31% of the *E. grandis* trees and 13% of the *E. dunnii* died during the drought while none of the G438 died.



**Figure 1.** Angular de-transformed survival percentage across planting densities for the five genotypes at the age of approximately nine years and per planting density across all species. Different letters indicate a significant difference between the species means at the 5% level (lower case) and planting density at the 10% level (upper case).



**Figure 2.** Utilizable mean annual increment of live trees per drought damage class at age nine years recorded for the various genotypes in a trial investigating the effects species and spacing on growth on a site that regularly experience droughts. Different letters indicate a significant difference between the means (total across all drought damage classes) at the 5% level.

## Conclusions

The results from this trial indicate that site-species matching is more important than planting density to optimize yield on drought prone sites. Both *E. dunnii* and the *E. grandis* x *camaldulensis* hybrid (G438) can be considered as suitable genotypes for these drought prone sites as they both had better or similar growth, but showed less symptoms of drought stress than *E. grandis*. We anticipate that G438 will recover faster after the drought than *E. dunnii* as less foliage loss occurred. Unfortunately G438 is more susceptible to *Leptocybe invasa* than *E. dunnii* which will limit further commercial deployment until efficient biological control of this pest is available.

# Clonal variability for water use efficiency and carbon isotope discrimination ( $\Delta^{13}$ C) in selected clones of a few Eucalyptus species

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## Background

Eucalyptus, one of the fast growing tree species extracts water from a depth of up to 15 meters (Peck and Williamson, 1987). As a prolific producer of biomass/ wood (Calder et al., 1997), eucalyptus has been included as commercially important tree species under various afforestation programmes in many parts of the world although there is a controversy regarding the reported reduction of stream flow associated with this species. Therefore in this scenario, it is necessary to make an attempt to use selections with reduced water use or to select and develop high water use efficient clones to cultivate under water limited environments.

## Objectives

The major objective was to assess the eucalyptus clones for variability in WUE and to determine the relationship between WUE and carbon isotope discrimination ( $\ddot{A}^{13}$ C) for large scale screening and selection for high WUE.

## Methodology

Nine clones of *Eucalyptus urophylla*, five clones of *E. pellita* (both procured from Mysore paper mills, Bhadravathi, Karnataka, India) and two hybrids (procured from ITC India Ltd) totaling to 16 entries were subjected to gravimetric measurement to determine WUE. Ten ramets of each clone were planted in cement cisterns containing planting mixture and weighing approximately around 100 kgs were placed under a rain out shelter (ROS). After measuring the initial biomass of a few representative plants (destructive sampling of 3 ramets), pots were weighed everyday and the amount of water lost over a period of 24 hours was replaced to bring the soil to field capacity. This exercise was continued for about 100 days and end of which, the plants were harvested and the final biomass was quantified. Similarly,

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at the end of the experimental period, the cumulative water added (CWA) to each pot from the beginning of the experiment until the end was computed. For evaporative loss correction, empty pots without plants which were weighed everyday were also maintained all through the experimental period. The evaporative loss so obtained was deducted from the cumulative water added to arrive at the cumulative water transpired (CWT). Similarly, the initial biomass of the plants measured at the time of commencement of the gravimetric experiment was deducted from the final

Table 1.	Clonal	variations	in	WUE	across	Eucalyptus clones.
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	WUE (g/kg)
Min	1.44
Max	3.39
Mean	2.28
SD	0.56

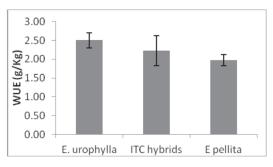
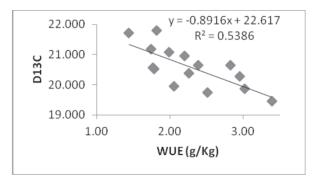


Figure 1. Water use efficiency in different species of Eucalyptus.



**Figure 2.** Relationship between  $\Delta^{13}$ C and WUE in Eucalyptus.

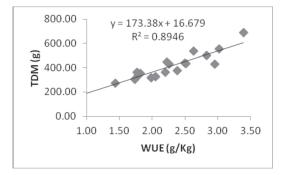


Figure 3. Relationship between WUE and TDM in Eucalyptus.

biomass to arrive at the Delta (differential) biomass. The ratio of D-biomass to CWT therefore forms WUE of the clone.

As gravimetric approach is time consuming, laborious, tedious and cannot be followed for large scale screening, an alternate approach is required to screen and identify high water use efficient clones. In recent times, carbon isotope discrimination approach is emerging as a strong tool to screen for WUE in plants (Farquhar et al., 1989). However, before adopting this approach for large scale screening of germplasm lines/ clones, the relationship between actual WUE and  $\ddot{A}^{13}$ C needs to be established. In this direction, an attempt was also made to determine the relationship between WUE and  $\ddot{A}^{13}$ C in Eucalyptus. Stem wood samples were collected, dried, powdered and carbon isotope fractionation was determined using lsotope ratio mass spectrometer (IRMS) for the  $\ddot{A}^{13}$ C study component.

## **Results and Discussion**

The results of the study indicated a significant clonal variability for WUE in Eucalyptus clones. Accordingly, the WUE measured ranged from 1.44 g/kg to 3.39 g/kg with a mean WUE of 2.28 g/kg (Table 1). This range of WUE is almost similar to that of  $C_3$  crop plants and hence, it may not be appropriate to consider Eucalyptus as water spenders, in terms of biomass production. Of the three different species of Eucalyptus used for assessing the clonal variability, the clones of *E. urophylla* were found to be more water use efficient than hybrids and *E. pellita* (Fig 1). *E. urophylla*, apart from being a good pulp yielder, also appears to be water use efficient and hence, these clones can possibly be popularized under conditions where improved WUE is desired.

A significant negative relationship was observed between actual WUE quantified

gravimetrically and Ä<sup>13</sup>C to indicate that, the clones that are more water use efficient tend to have less discrimination for <sup>13</sup>C carbon (Fig 2). Such a significant negative relationship has been shown earlier both in crop plants (Sheshshaayee et al., 2003) and tree crops (Raju, 2001; Mahadeva Murthy et al, 2006). Establishment of such a relationship will help in large scale screening and identification of WUE clones in Eucalyptus.

A significant positive relationship was also observed between WUE and total dry matter (TDM) in Eucalyptus (Fig 3). This signifies the mesophyll efficiency rather than stomatal regulation for WUE in Eucalyptus clones.

### Conclusion

Clonal variability for WUE does exist in Eucalyptus and therefore, this variability can be exploited for eucalyptus tree improvement. Further, the negative relationship found between WUE and Ä<sup>13</sup>C paves the way for screening large numbers of clones for WUE in the identification of superior clones with high WUE.

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## Estimating potential productivity and identifying the factors affecting growth and growth efficiency in forest plantations in eucalyptus stands in venezuela

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## Background

Increasing forest productivity will permit a more efficient land use, alleviating social conflicts for land which are common nowadays. Forest productivity, which is understood as the amount of stem dry matter produced per unit of area and time (Whitehead et al.,2004), is tightly related to the amount of solar radiation intercepted, light use efficiency (the amount of carbohydrates produced by units of light absorbed) and also to the proportion of carbohydrates allocated to the stem (Vose and Allen, 1988, Battaglia et al., 1998, Bergh et al.,1999; Cannel, 1989, Binkley et al.,2004, Waring and Running, 2007). Other factors like nutrients, water, pest attacks, diseases, wind among others, could affect the amount of leaf area displayed, its duration, and its light use efficiency, because they could affect the photosynthesis rate, stomatal conductance, respiration rates or carbohydrates allocation (Cannel, 1989; Binkley et al.,2010, Landsberg and Sands,2011).

Understanding the factors influencing forest productivity will permit to make correct decisions to select and apply the treatments necessary to reach this goal. But at the same time the potential productivity, reached if not limiting factors exists in the site, is unknown. The gap between the current and potential productivity is useful to recognize where to apply those treatments to maximize the gain attained by the investment.

In this research we tried: 1) to estimate the potential productivity in Eucalyptus plantations in a case study in Venezuela, 2) to determine the factors influencing growth, and 3) to determine the factors influencing growth efficiency.

## Methods

74 pair plots were installed covering all site conditions present in DEFORSA's farms. Every pair consisted of a treated plot which received an intensive silviculture treatment (fertilization + weed control) beside the management regime the firm

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regularly applies to the stands. The control plot consisted on only applying the management regime applied operationally to the stands. The twin plots were selected following a stratified sampling to take into account different site qualities, genetic materials and ages. Each twin plot consisted of 15 X 4 rows of trees (60 trees/plot) in a rectangular shape, with an approximate area of 540 m<sup>2</sup>/plot.

Leaf Area Index measurements and forest inventory were done at the beginning of the study and then one year later at the end of the dry and rainy seasons. Leaf Area Index was estimated using hemispherical photographs, and Hemisfer© to process them. Biomass sampling was performed to fit an allometric relationship between stem biomass and DBH. We measure all tree diameters and 25% of the tree heights, heights of other trees were estimated with a hypsometric regression. Volume was estimated using specific equations for each clone.

A linear model was fitted to test for the significance of variables explaining growth and growth efficiency. Leaf Area Index, site index, stand density, soil texture, were included as independent variables.

## **Results and Discussion**

Significant statistical differences were found for DBH, total height, and biomass increment (Include Figure 1). At some specific sites the difference between treatments reach up to 10 Mg ha<sup>-1</sup>año<sup>-1</sup>, which represents an important increase over the productivity reached with the current stand management. Currently the same management is applied to all the stands, however it is clear that in some

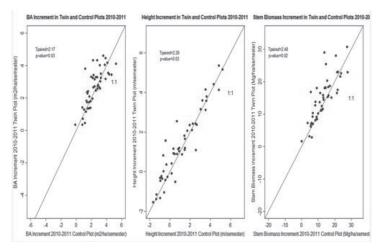


Figure 1. Basal area, height, and stem biomass increment during the period 2010-2011.

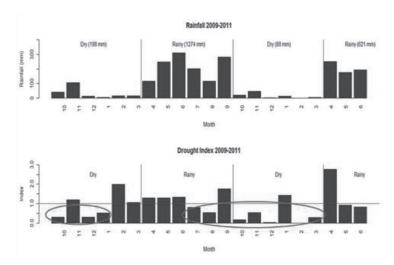


Figure 2. a) Total rain fall by season 2009-2011, b) relative rain fall when compared with historical average red lines indicate months with total rain fall below the average.

stands responses are almost zero, and in others are as high as 10 Mg ha<sup>1</sup>año<sup>1</sup>, which suggest the necessity of a site specific management.

When a linear model was fitted to find the variables that could explain volume growth we found some statistically significant : treatment (p=0.07), LAI (p=0.09), genotype (p<0.0001), age (p=0.0003), and clay content in the first 20 cm (p=0.02). The model had a R<sup>2</sup>=0.71. As expected, growth depends on genotype, declines as the stand ages, and increases with higher LAI. Soils in the area are generally Loam or Silt Loam, and an increase on clay content diminishes the available water for the plants. Soils with higher clay contents are more susceptible to compaction, diminishing the water holding capacity, which could be critical if a prolonged drought occurs. Growth Efficiency is influenced by genotype (p<0.0001) and age (p<0.0001).

This region is characterized by a well defined dry and rainy season, with a large total rainfall variability, which could increase or decrease the productivity in particular years. During the period considered the dry season has been longer and dryer when compared with historical average (Include Figure 2).

## Conclusions

Biomass increment up to 10 Mg ha<sup>-1</sup>año<sup>-1</sup> has been observed in the treated plots in some specific sites after 18 months since treatment application, which suggest the presence of limiting factors in those particular sites. However in most sites the response is not as large, and a possible explanation is the occurrence of dryer and longer dry seasons. Dryer season could cause a stomata closure, a higher litterfall, and in consequence a lower productivity, even when other resources have been supplied. .It is recommended a strict weed control and a better site preparation in order to increase water availability during the dry season.

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## Growth response of eucalyptus grandis in colombia to fertilization with various rates and frequencies of nitrogen

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The Forest Productivity Cooperative established a study evaluating the growth response of Eucalyptus grandis to fertilization with various rates of N applied at different frequencies in Colombia. The study was established at six different locations in southwest Colombia near Cali. Soils were well drained Andisols and Inceptisols derived from volcanic ash and basalt parent material. Site index ranged from 22 to 28 m. The stands were between 11 and 14 months old at the time the study was established. The N fertilizer treatments included applications of 0, 60, 75, 90, 12, 150, 180, and 240 kg ha<sup>-1</sup> applied at 6, 12, 24 or 36 month intervals. At each location, there were 3 or 4 replications of each treatment. Phosphorus and boron were applied to all treatments. Growth was measured at 6 month intervals for 60 months after fertilization. At four of the study sites, there was little response to any of the fertilization treatments. Cumulative volume response over 5 years at these sites was less than 15 m<sup>3</sup> ha<sup>-1</sup>. However, at two of the locations, cumulative volume growth response to fertilization exceeded 60 m<sup>3</sup> ha<sup>-1</sup>. There were substantial increases in stand leaf area in the stands that responded to fertilization indicating that nutrient limitations on leaf area production had been ameliorated by fertilization. The growth response at these two sites was a function of the cumulative amount of N applied, increasing monotonically up to the maximum N applied of 1000 kg ha<sup>-1</sup> over the course of the study. The soil at both of these sites was classified as the Pubenza series, a Hapludand derived from volcanic ash with a with loamy surface. The C/N ratio in these two soils exceeded 15:1 whereas the C:N ratio in the other soils was less than 14:1. Prior to fertilization, foliar nitrogen levels at these two sites were 1.9 and 1.8% at these two responsive sties compared to foliar N levels at the non responsive sites which generally were 2.0 % or greater. These results suggest that nutrient deficiencies can be a major stress factor and limit growth on specific sites in Colombia. Significant increases in productivity are possible following fertilization that eleviates the stresses caused by nutrient deficiencies.

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ORAL SESSION 3. GENETICS

## Genotype x environment interactions for growth and wood traits for eucalyptus hybrids

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## Background

Forest trees are long-lived organisms and the expression of genes in relation to age and competitive environment is likely one of the most important features to consider in tree genetics and tree breeding. If genetic of growth traits is well known in eucalyptus, the understanding of genetic control for wood traits needs some additional experimental data. In this work, based on one experiment including full-sib families of two eucalyptus hybrids planted at two contrasted densities, two specific questions are asked:

(1) Genotype x environment interactions are lower for wood traits than for growth traits.

(2) Heritability of growth and wood traits are affected by the environment effects as tree density

## Methods

Data were obtained from one field trial established in the Republic of the Congo in the experimental area of the "Centre de Recherche et de Developpement des Plantations Industrielles". The climate is tropical humid with a mean annual temperature of 24°C, a mean annual rainfall of 1,200 mm and a dry season from May to October.

The R90-13 experiment used a set of 6 hybrid families created by controlled pollination of different unrelated parent trees : 6 families of *Eucalyptus urophylla* x *E. pellita* and 6 families of *E. urophylla* x *E. grandis* planted in 1991 at two spacing 2 m x 2 m (2,500 trees/ha) and 4 m x 4 m (625 trees/ha).

Within each density, the experiment was a complete block design with a 36-tree square plot in three replicates. To minimise the impact of competition between

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plots, only the 16 inner trees were measured.

Data of circumference at breast height and total height were extracted from data base for growth traits each two years, starting at 12 months to 86 months. Chemistry related traits: Klason lignin, cellulose, S on G ratio, and extractive values were assessed by Near Infrared Spectroscopy (NIRS) predictions based our own calibrations.

Analyses of family by spacing interaction were performed at the individual level, using the following mixed model with ASREML package on R statistical programming language (R Development Core Team 2010):

 $y_{iiklm} = \mu + h_i + d_i + h^*d_{ii} + b(d)_{ki} + f(h)_{ii} + f(h)^*d_{iii} + f(h)^*d^*b_{iikl} + r_{iiklm}$ 

where  $y_{_{ijklm}}$  is the  $m^{th}$  tree in the  $I^{th}$  family in the  $k^{th}$  block in the  $i^{th}$  hybrid in the  $j^{th}$  density

 $\mu$  is the overall mean,

 $h_i$  is the fixed effect of the  $i^{th}$  hybrid,

d, is the fixed effect of the j<sup>th</sup> density

 $h^*d_{_{ii}}$  is the fixed effect of the interaction between the i<sup>th</sup> hybrid and the j<sup>th</sup> density,

b(d)\_{kj} is the random effect of  $k^{th}$  block in the  $j^{th}$  density with variance  $\sigma^{z}$ 

b(d) and mean 0,

f(h), is the random effect of the Ith family in the ith hybrid with variance  $\sigma^2$ 

f(h) and mean 0,

 $f(h)^*d_{_{lij}}$  is the random effect of the interaction between the  $l^{th}$  family in the  $i^{th}$  hybrid and the  $j^{th}$ 

density with variance of  $\sigma^2$ 

f(h)\*d and mean 0,

 $f(h)^*d^*b_{_{ijkl}}$  is the random effect of the interaction between the  $l^{th}$  family in the  $i^{th}$  hybrid and the  $j^{th}$ 

density and the  $k^{th}$  block with variance of  $\sigma^{\scriptscriptstyle 2}$ 

f(h)\*d\*b and mean 0,

 $r_{_{iiklm}}$  is the residual random effect, with variance of  $\sigma^{\scriptscriptstyle 2}$ 

r and mean 0.

Broad-sense family heritabilities were estimated by variance component using the following model:

 $y_{ijkl} = \mu + h_i + b_j + f(h)_{ik} + r_{ijkl}$ 

where  $y_{_{ijkl}}$  is the mth tree in the k<sup>th</sup> family in the j<sup>th</sup> block in the i<sup>th</sup> hybrid  $\mu$  is the overall mean,

h, is the fixed effect of the i<sup>th</sup> hybrid,

b, is the fixed effect of j<sup>th</sup> block,

 $f(h)_{_{ik}}$  is the random effect of the  $k^{th}$  family in the  $i^{th}$  hybrid with variance  $\sigma^2$  f(h) and mean 0,

 $r_{_{iikl}}$  is the residual random effect, with variance of  $\sigma^{\scriptscriptstyle 2}$ 

r and mean 0.

## **Results discussions**

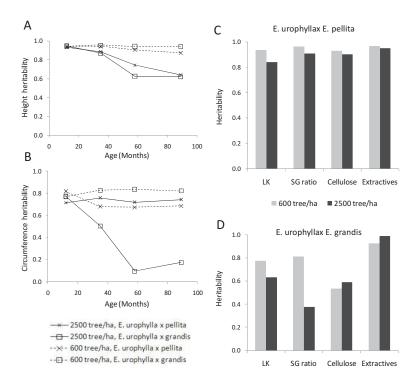
The family\*density interactions were significant for growth traits after 12 and 35 months, respectively for height and circumference (Table1). However for wood traits, there was no family\*density interactions. Wood traits seemed not affected by competition. The variation level for wood traits (generally the coefficients of variation were less than 20-25%) was lower than for growth ones.

Hybrid *E. urophylla* x *E. pellita* showed higher family variances (10 times) for wood traits than for *E. urophylla* x *E. grandis* (Table 1). However this trend was not observed for growth traits. In terms of wood traits E. urophylla was closer to E. grandis than to *E. pellita*. Recombination between *E. urophylla* x *E. pellita* could be one source of higher variability.

The Figure 1 showed the trend of heritability of growth from 12 to 86 months and the heritability of wood traits at 222 months. Heritability of height decreased

Traits	Age (months)	Far Hybrid 1	nily Hybrid 2	Family*densit Hybrid 1	•	Plot	Residual
Height	12	0.251	0.383	0.000	0.016	0.154	0.630
	35	1.039	1.335	0.000	0.431	0.131	3.474
	58	0.569	1.327	0.474	0.972	0.160	7.476
	86	0.016	1.578	1.169	1.441	0.000	10.830
Circ.	12	0.316	0.432	0.000	0.000	1.871	7.142
	35	0.920	1.687	0.629	1.767	0.683	31.120
	58	0.000	1.435	4.599	4.808	0.000	66.660
	86	0.000	2.295	8.780	5.919	0.000	98.944
Lignin		0.161	0.016	0.000	0.000	0.029	0.264
SG ratio		1.144	0.104	0.000	0.000	0.656	2.838
Cellulose		1.230	0.090	0.069	0.015	0.000	2.929
Extractives		2.533	0.867	0.046	0.000	0.067	2.619

**Table 1.** Results of analyses of variances for each hybrid (Hybrid1: E. urophylla x E. pellita, Hybrid 2: E. urophylla x E. grandis) for circumference and height at different ages and for wood traits (in gray significant family x density interactions)



**Figure 1.** Trend of growth trait heritabilities and heritabilities of wood traits - A and B variation of heritability with age for growth traits (A : Height, B : Circumference), C and D heritabilities of wood traits (C: *E. urophylla* x *E. pellita*, D: *E. urophylla* x *E. grandis*)

with age for the high density, whereas low density heritability was stable up to 86 months (Figure 1A). The trend of circumference heritability for high density depended of the hybrid (Figure 1B). For *E. urophylla* x *E. pellita* there was no effect. However for hybrid *E. urophylla* x *E. grandis* heritability decreased dramatically. Heritability of wood traits depended of the hybrid and the density (Figure 1C and 1D). Independently of traits, the values were lower for *E. urophylla* x *E. grandis* than *E. urophylla* x *E. pellita*. The effect of density was higher for this hybrid.

The sensibility to the competition for *E. urophylla* x *E. grandis* hybrid seemed to be higher than the other one. But this sensibility did not appear for height. This was linked to the different functioning under competition of apical meristem and the cambium.

## Conclusions

These results show that planting at very high density can affect the heritability due to a strong increase on environmental effect. The effect is more pronounced for growth trait that for wood chemical traits.

These results confirm also preliminary studies showing that wood chemical properties are less affected by genotype by environment effect than growth traits.

These results present a real interest for selection strategy in eucalyptus breeding when both growth and wood property traits are taken into account.

## Plantation productivity of sixty-five taxa across eight ecosystems in north-eastern Australia

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#### **Background and objectives**

In north-eastern Australia approximately 130,000 ha of hardwood plantations have been established in the last 15 years. As a result of poor taxa selection, approximately 25,000 ha have failed due to drought, pest and disease or extreme weather events (mainly drought and cyclones) (Lee *et al* 2010). To develop a viable hardwood plantation forest industry dedicated to solid wood production, growers need to know that taxa can sustain productivity in the environments of north-eastern Australia. The results presented herein from taxa trials spread throughout the subtropics and tropics of Australia will provide greater certainty for the development of plantations in areas currently considered marginal for the production of economically viable forests.

Particularly for the long rotations associated with the production of solid wood, the predicted impacts of climate change in north-eastern Australia (reduced rainfall, increased temperatures and an increase in extreme weather conditions such as drought, storms and cyclones) makes selection of the right taxa for plantation development even more critical as the taxon planted needs to be able to perform well within existing and future climates.

The paper summarises data from 38 taxa (species, subspecies and hybrids) trials in northeastern Australia. It provides information aimed at improving the understanding of growth rates, pest risks, carbon sequestration rates and adaptability to climate. The data is summarised and presented at a regional level as opposed to individual trial or plot level, to provide broad inferences as to how taxa would perform across a plantation estate.

### Methods

Ten year growth, survival, stem borer and wood property data were collected from 38 replicated taxa trials established with large blocks that had been managed for solid wood production (thinned around age three to 400 - 700 stems per hectare)

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between latitude 16° - 29° south across eight ecosystems in Australia (Figure 1). The plot-level data was collated with climatic and edaphic data for analyses designed to identify those taxa with the best productivity, adaptation to different ecosystems, resistance to stem boring insects, and rates of carbon sequestration.

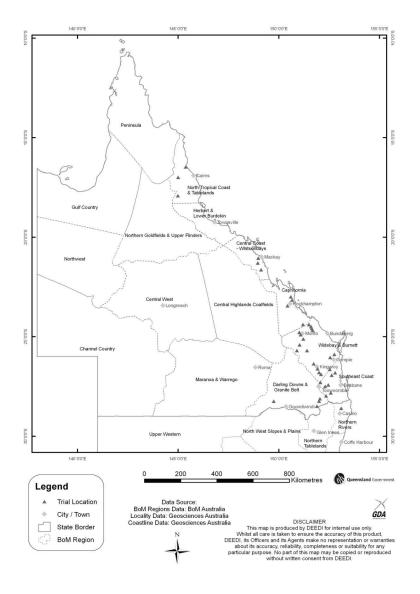


Figure 1. Bureau of Meteorology Forecast Regions (ecosystems) and locations of the 38 taxa trials in north-eastern Australia included in the study.

## **Results and Discussion**

Given that there is some uncertainty about the future climate in north-eastern Australia the taxon chosen for commercial deployment need to be adapted to a large range of soils and climatic conditions. The 38 taxa trials included in this study were generally subject to lowerthan-average rainfall (regional rainfall is presented in Table 1), higher annual average temperatures (0.6 °C higher than the long-term average), insect incursions and many severe weather events (storms, drought and cyclones). The trials were also targeted towards typical soils within the eight ecosystems sampled. Therefore, the taxon performances reported here should be indicative of potential productivity, adaptation and insect resistance under the scenarios predicted for climate change in the region.

Of the 65 taxa included in the study, those that were the most robust and therefore most readily adapted across the region, to the conditions experienced in northeastern Australia (based on volume growth, survival and carbon sequestration rates) were *Corymbia citriodora* subsp. *variegata* (CCV), *Eucalyptus dunnii, E. longirostrata* and *E. argophloia*. All of these taxa appear suitable for solid wood products based on volume growth and carbon sequestration in at least one ecosystem. Of these species only CCV and *E. argophloia* also combined good postthinning survival (survival from thinning to age 10 years) and low stem borer incidence. Lawson (2003) considered levels of over 15% of trees attacked by stem borers to be 'very high', which would preclude the use of a plantation for solid wood products.

Thus only *CCV* and *E. argophloia* appear suitable for solid wood production (Table 1) in north-eastern Australia.

From a plantation development perspective, solid wood production should focus on those ecosystems where the taxa identified above have higher growth rates and risk from adverse weather events (particularly cyclones) is lower. Based on this we propose that solid wood (and carbon sequestration) plantation forestry should focus on the Central Coast Whitsundays, coastal Wide Bay & Burnett and Northern Rivers regions of north-eastern Australia.

#### Conclusions

The taxa with the best growth potential across the diverse ecosystems of northeastern Australia were CCV and *E. argophloia*. These two species also have the lowest borer incidence and have survived an extended drought across this zone, which emulates the climate predicted for the region's future. Based on these trial

Region (trial annual rainfall mm, % of long-term	Taxon⁵	Volume MAI (m³/ha/year)		Post-thin survival <sup>c</sup>		Stem borer incidence		seques	CO2 <sup>d</sup> sequestered (tonnes/ha)	
averagea)		Estimate	StdErr	Est	StdErr	Est	StdErr	Est	StdErr	
<b>Capricornia</b> (863 mm, 77%)	E. longirostrata	8.83	2.49	0.78	0.24	0.47	0.55	108.2	32.70	
	E. argophloia	8.82	2.14	1.00	0.19	0.10	0.65	100.6	25.70	
	CCV	8.49	1.82	0.92	0.15	0.09	0.41	100.6	25.70	
	E. moluccana	7.58	2.46	0.99	0.23	nae	na	90.4	32.00	
Central Coast –	CCC	4.85	2.60	0.90	0.09	0.06	0.32	55.8	34.14	
Whitsunday	E. grandis	10.53	2.07	0.46	0.18	0.93	0.44	159.0	25.45	
(1425 mm, 88%)	E. cloeziana	10.31	1.80	0.48	0.15	0.07	0.33	130.1	19.20	
	E. dunnii	9.97	1.85	0.49	0.16	0.45	0.35	109.2	20.01	
	CCC	9.88	1.85	0.64	0.16	0.00	0.35	128.6	20.08	
Darling Downs	CCV	9.34	2.09	0.53	0.19	0.08	0.42	159.1	24.87	
& Granite Belt	E. longirostrata	4.62	2.23	1.00	0.22	1.00		65.1	23.26	
	E. globulus subsp. maidenii	4.41	1.52	0.67	0.14	0.75	0.60	72.0	31.61	
	E. sideroxylon	4.14	2.23	1.00	0.22	0.00	0.57	44.7	32.12	
	E. grandis	4.09	1.72	0.67	0.16	na	na	65.8	32.97	
	-									
North Tropical	E. dunnii	4.02	1.33	0.64	0.11	1.00		65.1	23.26	
Coast & Tablelands <sup>f</sup> El	E. pellita	29.98 7.92	2.72 2.72	0.56 0.45	0.25 0.25		0.56 0.56	na	na	
(1289 mm, 99%)	laeocarpus grandis CCV	6.69	2.72	0.45	0.23	0.00		na na	na	
(1209 1111, 99%)	EG×EC	5.32	2.55	0.88	0.23	0.05	0.54	na	na na	
Northern Rivers	CCC	5.20	2.04	0.70	0.18	0.11		na	na	
(1594 mm,	E. dunnii	24.39	3.12	0.90	0.26	0.00	0.58	273.6	33.24	
136%)	E. globulus subsp.	12.30	3.12	0.81	0.26	0.00	0.58	138.9	33.24	
	maidenii CCV	10.28	3.05	0.85	0.25	0.01	0.55	135.1	31.91	
	E. grandis	6.08	3.03	0.85	0.25	0.01	0.55	98.6	33.24	
	EG × EU	5.15	3.12	0.64	0.25	1.00	0.55	98.0	55.24	
Southeast	E. longirostrata	5.35	1.37	0.95	0.11	0.90	0.39	79.6	19.08	
Coast	E. dunnii	5.28	1.56	0.77	0.14	1.00		126.0	31.44	
(760 mm, 87%)	E. argophloia	4.94	1.54	1.00	0.14	0.00		43.3	31.31	
	E. sideroxylon	4.20	2.21	1.00	0.22	0.00	0.52	50.5	32.16	
Wide Bay &	C. henryi	3.94	2.27	0.83	0.22	na	na	na	na	
Burnett – coastal (946 mm, 84%)	E. longirostrata	10.59	1.45	0.81	0.14	0.28	0.33	235.2	33.23	
	Pinus caribaea var. hondurensis	9.94	2.21	0.88	0.22	0.29	0.54	na	na	
	CCV	7.91	1.08	0.90	0.09	0.06	0.32	184.5	34.67	
	E. dunnii	7.72	1.19	0.64	0.10	1.00	0.40	na	na	
Wide Bay &	E. grandis	7.60	1.12	0.55	0.09	1.00	0.32	na	na	
Burnett –inland	E. argophloia	4.49	1.09	1.00	0.09	0.29	0.28	55.0	14.51	
(640 mm, 86%)	E. longirostrata	3.56	1.13	0.86	0.10		0.28	43.3	16.19	
, , , , - ,	E. moluccana	3.53	1.25	0.98	0.12		0.31	55.2	18.01	
	CCV	3.50	1.05	0.95	0.09	0.02		48.0	13.22	
	EG×ER	3.27	1.30	0.61	0.12		0.53	68.4	33.64	

 Table 1. Ten year growth, survival, stem borer and carbon sequestration of the best

 five taxa in each ecosystem (BoM Australia region) based on volume MAI.

<sup>a</sup> Trial period annual rainfall in the region and percentage of long term average rainfall.

<sup>b</sup> Taxon abbreviations CCC = *C. citriodora* subsp. *citriodora*, CCV = *C. citriodora* subsp. *variegata*, EG × EU = *E. grandis* × *E. urophylla*, EG × ER = *E. grandis* × *E. resinifera*.

<sup>c</sup> Survival from thinning to age 10 years.

<sup>d</sup> CO<sub>2</sub> equivalents sequestered (stem wood only) - derived as: Volume MAI × average predicted basic density [biomass] × 0.50 [biomass to C] ×10 years × 44/12 [C to CO<sub>3</sub>].

<sup>e</sup> Not Available.

<sup>f</sup> Trials in these regions were not thinned but were damaged by cyclones (hurricanes). Survival at the 10 year assessment was used as an estimate of survival post-thinning.

results, it is recommended that tree improvement activities in north-eastern Australia should focus on these (and related) taxa as they have the potential to produce higher-value products and will be better adapted to the climate of the future.

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## Recurrent Selection of F<sub>2</sub> Genotypes based on GCA and Breeding Values for Improvement of yield in *Eucalyptus tereticornis* Smith Progeny trial

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#### Introduction

Progeny trials are mainly used to test the fidelity of gains achieved in the parent clones and also to create variation for further selection and breeding. Hence, when selection in half-sibs are attempted in a progeny trial, the general combining ability (GCA) and breeding values obtained from best linear unbiased prediction are important criteria for selection of genotypes because GCA reflects interactions between alleles at gene loci and represents an average performance of the progeny of an individual when it is mated to a number of individuals in the field (Zobel and Talbert, 1984; Xie et. al. 2007). It helps in managing inbreeding in the populations through thinning inferior trees and such seed orchards serve as a gene package with improved seed at the end of a generation of breeding. Breeding values were used more recently to select genotypes by recurrent selection to increase the genetic gains marginally compared to forward selection (White and Hodge, 1988). A Eucalyptus tereticornis F2 progeny test was established for evaluation of their performance for fast growth and also for inclusion into F<sub>2</sub> generation breeding program. Based on the performance of progenies, GCA and breeding values of mother trees were estimated and the results are communicated here.

#### **Material and method**

An *E. tereticornis*  $F_2$  progeny test was established during 2002 at Midnapore, West Bengal (latitude 22° 25' 60N, long. Of 87° 19' 60E. and an altitude of 78'). The temperature varies from 6° C to a maximum of 47° C in summer with an average rainfall of 1240 mm with lateritic soil. The trial was established with 49 families collected from two seedling seed production areas and a seedling seed orchard located in Karnataka, Tamilnadu and Pondicherry. These orchards were thinned out based on field performance and the trees are of about 10-12 years old. The trial was laid in RCBD with five replications. Each seed lot has seven plants in each replication assigned randomly within each replication and planted in contiguous

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rows with a spacement of 3 × 2 m. The mortality rate was of 4.77 percent at the end of four years. The growth components *viz*. total height, clear bole height and girth at collar were measured at age of four years and analyzed with SPSS 9.0 package. Unbiased population variability was estimated through Co-efficient of variation (CV) using whole plot data. One-way ANOVA was conducted to find out intra-specific variability based on allocating the families to quartiles through analysis of mean values. GCA and breeding values were estimated as per Zobel and Talbert (1984).

#### Results

Progenies exhibited a good amount of population variability in the form of CV in all growth components. One-way ANOVA revealed highly significant intra-specific variation in all growth components suggesting that the progenies responded positively to the environment and also sufficient diversity is floating within population which can be exploited for further improvement. The progenies exhibited wide range of GCA and breeding values.

#### Discussion

Highly significant variation in the progenies suggested sufficient diversity within the population and such variation is common in species with exploitive and out crossing breeding system growth strategy (Kozlowski and Keller, 1966). Eucalypts have exploitive growth strategy with indeterminate growth which results in lowest rate of bifurcation in the apical meristem. The growth in such species is largely determined by current growing conditions. Disturbances in environment or microsite may create higher buffering actions during development and differentiation stages of growth phases which result in higher amount of intra-specific variation. Another reason may be either due to differences in the genes controlling development of each character or differences in the efficiency of the buffering mechanisms themselves. Further Higher amount of variation may be due to control by a set of pleiotropic genes which control individual component expression in such a way that well balanced genotypes are better able to buffer character development against environmental perturbations (Clarke, 1993). Such phenotypic variations create versatile genotypes for adoptedness and live successfully in a broad range of environments.

All the best performers in terms of GCA and breeding values for all growth traits were from Pudukotai SSPA indicating broad genetic base of the orchard. Like wise, worst performers were from Karnataka SSO and Pondichery SSPA suggesting narrow selection range or family co-ancestry in those parents and they can be culled to

Sl.no	Total height (m)					Clearbole height (m)				Girth at collar (cm)				
	Tree	GCA		BV		Tree	G	iCA	В	V	Tree	GCA	B\	/
	no	Best	Worst	Best	Worst	no	Best	Worst	Best	Worst	no	Best Worst	Best	Worst
1	17	3.4		6.8		17	3.43		6.86		17	6.28	12.56	
2	22	2.19		4.38		6	2.15		4.3		6	5.69	11.38	
3	6	2.12		4.24		22	2.07		4.14		22	5.42	10.84	
4	20	1.84		3.68		20	1.87		3.74		15	3.86	7.72	
5	7	1.71		3.42		7	1.74		3.48		7	3.8	7.6	
6	19	1.67		3.34		19	1.62		3.24		12	3.74	7.48	
7	21	1.5		3		21	1.53		3.06		3	3.62	7.24	
8	11	1.22		2.44		5	1.26		2.52		20	3.54	7.08	
9	10	1.21		2.42		10	1.24		2.48		14	3.49	6.98	
10	5	1.23		2.46		13	1.18		2.36		11	3.38	6.76	
11	35		-4.46		-8.92	35		-4.43		-8.86	8	-7.21	-	14.42
12	44		-3.16		-6.32	44		-3.45		-6.9	43	-6.34	-	12.68
13	43		-2.93		-5.86	43		-3.11		-6.22	44	-5.83	-	11.66
14	8		-2.87		-5.74	8		-2.84		-5.68	49	-4.71		-9.42
15	31		-2.48		-4.96	33		-2.45		-4.9	33	-3.8		-7.6

**Table 1.** Ten best and five worst general combiners and their breeding values for total height and girth at collar (GCA- General combining ability; BV- Breeding value).

avoid dilution of panmixis (Soh, 1994). The parental orchards of these poor performers may also be rejected for further improvement. Further, with the increase in selection intensity, the variations in replicate populations remained significantly large and have a tendency to increase as a function of average response to environment. Hence, more precise selections can be made from these progenies for improvement in growth. This was facilitated by the fact that best performers showed good values for all growth traits indicating correlated growth traits for simultaneous improvement of multiple traits and such strong age-age correlations for both height and mean annual increment were reported in E. grandis (Osorio et. al. 2003). However, due to non-linear nature of multiple alleles, the directions of change in gene frequencies are not constant. During the course of selection, the alleles may change from positive to negative while phenotypic gains improve (Xu et.al. 2004). Hence, there is need to select sufficient number of parents with high breeding values. The breeding values in the present progeny trial favor selection of 10 parents having the highest breeding values for each growth trait to synthesize F<sub>2</sub> generation breeding population (Table 1).

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# Relationship between non-additive and additive genetic variances from full-sib *Eucalyptus globulus* progeny trials: implications for deployment and breeding strategies

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#### Abstract

Variances components were estimated for growth and Pilodyn penetration -an indirect measure of wood density- from twenty-one second-generation control-pollinated *Eucalyptus globulus* Labill. *spp. globulus* progeny trials in South-Central Chile. The progeny tests were established across multiple sites and included 283 full-sib crosses. The parents of the families in the trials had two origins, the local Colcura landrace and Australian seedling selected from trials of the CSIRO natural population collection. The full-sib families were classified into three categories – within landrace (Colc x Colc), between Australian native forest selections (Aus x Aus), and the widest crosses (Colc x Aus).

Each site was analyzed separately in order to obtain single-site genetic parameter estimates, to provide site-specific information for best linear prediction and to examine the importance of both general combining ability (GCA) and specific combining ability (SCA). From this preliminary analysis, unequal error variances among sites were found. Then, a heterogeneous multisite variance model was fitted to account for different error variances among sites and used to evaluate the relative importance of non-additive and additive genetic effects for volume and Pilodyn. The individual site heritabilities averaged 0.11 for volume and 0.57 for Pilodyn. Although the importance of SCA variance relative to GCA variance (SCA/ GCA) varied between sites for each trait, results from combined-sites analyses of twenty-one sites indicated that SCA, GCA, Site x SCA, Site x GCA variances were all significant for both traits. The ratio SCA/GCA was 1.23 for growth indicating very significant non-additive effects on growth. However, the effect was much lower for Pilodyn, where the ratio was 0.13. When combined-sites analysis was performed by each sub-population, the ratio SCA/GCA was 0.40, 0.13 and 0.30 for Aus-Colc, Aus-Aus and Colc-Colc respectively. The ratio of non-additive to additive variance for growth is thus clearly dependent on population structure. The ratio for Pilodyn

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is more stable and with all values close to 0.15.

The results of this study suggest that wide crossing increases the chances of identifying highly heterotic full-sib families for deployment. Breeding Populations need to be structured both to take advantage of gains from recurrent selection for GCA and to retain the ability to generate and test wide crosses for deployment.

### Combining high-throughput sequencing and *in silico* prediction to identify miRNA in *E. globulus* xylem tissues formed upon gravitropic stimulation

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#### Background

The ability to drive wood quality is fundamental for Portuguese economy. Portugal is one of the largest producers of pulp derived from eucalyptus (*E. globulus*), with exports accounting for about 5% of GDP. It is also relevant that *E. globulus* represents about 1/3 of the Portuguese forest and that the use of adequate genotypes will optimize the exploitation of available areas.

Wood is a complex and highly variable tissue, which anatomical, chemical, physical and technological features are ontogenic and environmentally controlled (1). In response to a gravitropic stimulus, Angiosperms differentiate a tension wood showing specific anatomical, chemical and mechanical features (2). Tension wood has been considered an excellent model to study the biosynthesis of xylem cell walls (e.g. cell wall biosynthesis) (3).

The understanding of the molecular mechanisms involved in the biosynthesis of the cell wall is of great importance not only for future production of pulp and paper, but also for the production of bio-fuels and bio-materials. Many of the genes involved in wood formation have been catalogued, but the mechanisms to regulate this process of development are still far from being elucidated (4).

MicroRNAs (miRNA) are small size (21-24nt), endogenous non-coding RNAs (5).

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miRNAs play a crucial role in diverse biological processes such as development, cellular differentiation, and responses to abiotic and biotic stress, including cambium differentiation (6,7). Of the approximately 16,772 miRNA available in the database miRbase v.17 (http://microrna.sanger.ac.uk/), none of the entries relate to *Eucalyptus* species.

Here we present the first results obtained by microEGo project (FCT grant PTDC/ AGR-GPL/098179/2008) on identification of miRNA, combining *E. globulus* smallRNA libraries sequencing and *in silico* miRNA prediction on candidates identified on *E. grandis* genome sequence.

#### **Material and Methods**

#### Plant material

Differentiating xylem (DX) samples were collected on 4-years old *Eucalyptus globulus* certified trees (3 clones) that were bent to induce tension wood formation, along five time points of bending (24h, 1 week, 2 weeks, 3 weeks and 4 weeks). DX samples were collected on upper (tension wood) and opposite side (opposite wood) of the bent tress, and also on vertical trees. Experimental setup was conducted at Quinta do Furadouro, Óbidos (Portugal) on ALTRI Florestal SA. Microscopy and NIR spectrometry were used to study the modification of the anatomy and chemical composition of differentiating xylem during the induction of tension.

#### smallRNA profiling by deep sequencing

Total RNA was extracted using Le Provost et al (2007) protocol, with the following modifications. Instead of LiCL precipitation, RNA were precipitated during one hour at -20°C, after addition of 0.1X (v/v) NaOAc (pH 5.2) 4M and 2.5X (v/v) of absolute ethanol. Total RNA were pool by bending time point and by DX type associated to tension, opposite or vertical wood (control). Ten micrograms of each pool were sent to FASTERIS (Genève, CH) for smallRNA libraries preparation and sequencing. The TruSeq ™ SBSv5 sequencing kit (Illumina) was used for sequencing, using the Illumina Hi-Seq 2000 instrument, on a multiplex run with 1x50+7(index) cycles.

Sequence tags ranging between 18 and 30bp in length were selected for further analysis. Sequence tags holding homopolymers (larger than half the sequence length) and unknown bases were discarded. Sequences were trimmed when base quality score was <15 using in house developed PERL scripts. Remaining sequence tags ranging 18 and 30bp in length were aligned to the *E. grandis* genome (version

1.0) allowing up to two mismatches using Bowtie 0.12.7. Uniquely aligned sequence tags which were annotated by positional comparison as *E. grandis* coding regions (version 1.0) were excluded using BEDtools. Remaining sequence tags were searched against currently known miRNAs of *A. thaliana*, *V. vinifera* and *P. trichocarpa* (miRBase version 17), *A. thaliana* miRNAs database (http://bioinformatics.cau.edu.cn/PMRD/), *A. thaliana* non coding RNAs (available from Rfam v10.1) and *A. thaliana* repeats (Repbase v) and classified as homlogs. Finally, non-homologue sequence tags were clustered using IRanges package of Bioconductor 2.8 in order to identify potential novel miRNAs.

#### In silico smallRNA prediction

The computational search for miRNAs in *Eucalyptus grandis/Eucalyptus globulus* was made using the framework CRAVELA (http://www.cravela.org) [8] previously developed for metazoans that was adapted to the search for plant miRNAs. The approach we are using is based in a three-pillar evaluation of candidates extracted from a single-genome. The first pillar refers to the combination of four stability and robustness measures which have been proved to segregate pre-miRNAs from the majority of spurious genomic stem-loops. The second pillar consists in the analysis of the secondary structure of the candidates by comparing a large number of structures against a set of known precursors, and the third pillar refers to the assessment of the transcriptional potential of each candidate.

Then datasets of both approaches were combined to retrieve potential *E. globulus* miRNA linked to tension wood formation.

#### Results

A set of 26 miRNA homologs were identified by enumerating the BLASTn two-way best matches between known pre-miRNAs in *A. thaliana*, *V. vinifera* and *P. trichocarpa* and pre-miRNA candidates extracted from the *E. grandis* genome, restricted to those which show perfect conservation of the mature sequence of the miRNA precursor. A set of new 22 miRNA precursors are proposed by identifying the top-scoring candidates enumerated by the CRAVELA platform which show evidence of transcription in a deep-sequencing assay on *E. globulus*. These candidates exhibit highly favorable robustness and stability properties, are structurally similar to the 26 homologs previously identified and include a presumably mature sequence in one of the stem arms (with a length between 19 and 26 nt) obtained from the sequenced small RNA libraries of *E. globulus* transcripts.

Northern analysis are being used to confirm the expression of conserved and non-conserved miRNA on *E. globulus* developing xylem.

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# Identification, phylogeny and tissue-specific expression of some members of multigene families involved the lignin biosynthesis in *Eucalyptus*

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#### Background

Lignin is only exceeded by cellulose as the most abundant organic polymer on Earth. Given their abundance, chemical and physical properties, this complex, highly heterogeneous biomolecule has an inherent significant biologicaland ecological function, determining an increasing economic importance for several industrial areas and technological applications.

A restricted number of eucalyptus species are among the fastest growingplanted trees and have been gaining a growing economic importance as wood providers since the traditional interests of pulp and paper production have been extended to the emergent areas of bio-fuels and bio-materials. The conversion efficiency of ligno-cellulosic biomass to ethanol is determined largely by lignin nature and content. Considerable differences in lignin concentration and composition exist among species, genotypes within species, tissues and organs. Lignification is under close genetic control so that plants, depending on internal and external factors, coordinate its levels, timing and spatial occurrence. Therefore, we are interested in comprehensively identify and characterize the genes involved in lignin biosynthesis to contribute for the future definition of educated strategies towards wood quality improvement.

This work aimed three tasks: 1) to identify and list members fromtenmultigene families (MGF) involved in *Eucalyptus*lignin biosynthesis pathway; 2) to analyze protein phylogeny (in terms of predicted proteins phylogeny and grouping based on sequence identity) in each of these MGF; 3) to unravel gene expression patterns of some of those genes (selection based on the phylogenies, our group's accumulated knowledge and bibliographic references) in several *Eucalyptus*species, tissues and conditions.

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#### **Material and Methods**

#### Identification of putative MGF members and predicted proteins

For gene identification, database searches were conducted over the 44,974 *Eucalyptusgrandis*(Egr) gene models predicted and annotated by the *JGI/CIG-Phytozome* (annotation JGI\_v1.0). We used both *Phytozome* (http:// www.phytozome.net/) and *BOGAS* (http://bioinformatics.psb.ugent.be/webtools/ bogas/) resources while performing keyword searches over sequence annotations and BLASTp homologies searches using as queries, orthologous protein sequences from *Arabidopsis thaliana* (Ath) and *Populustrichocarpa*(Ptr).A comprehensive list of predicted proteins was assembled from the gene models found to be the best candidates based on annotation and stringent cutoff values established over the BLASTp results. Sequence comparisons and homologies with known proteins from landmark, *bone fide* genes (previously published, proven experimentally as involved in lignin biosynthesis) and occasional manual curation, resulted either on the correction of some predicted proteins or in the elimination of incorrectly truncated protein predictions.

#### Protein phylogenetic relationships

Phylogenetic relationships were revealed for the selected predicted proteins of each MGF, after sequencesalignment using *MAAFT* and tree computing and assemblage using several clustering algorithms and substitution models from *MEGA5*. In these trees, we included orthologs identified in *Phytozome*parallel searches over the Ptr (JGI\_v2.2), and Ath (TAIR\_10) genomes. Additional landmark genes from other species like: *E.gunnii,E. globulus,Nicotianatabacum, Pinuspinaster, Pinustaeda*; were included in the phylogenies.

#### Gene expression studies

Based on the phylogenetic clustering results and giving priority to genes closely associated with landmark genes, we selected 41 to unravel their specific-transcript abundance patterns over a wide range of tissue collection. A set of primary and secondary eucalyptus tissues was used, featuring contrasting biological conditions in which lignin formation and content is expected to be variable: apical meristem; fruit capsule; floral buds; roots; leaves; stem; cambium; phloem; xylem. Moreover, the tissues were sampled under differential conditions of formation: leaves (age; cold stress); stem (primary/secondary); roots (cold stress); cambium (age); phloem; xylem (age; gravitropic stimulus; nitrogen supplementation), comprising a total of 26 conditions (including controls) and involving 4 species: Egr, Egl and an *E. gunnii* x *dalrympleana*hybrid. Three biological replicates were used. Theassay was conducted using the qRT-PCR system from *FluidigmBioMark*'shigh-throughput platform. The normalized data (using 3 reference genes) were transformed and inputted for hierarchical clustering in *Expander5.2*.

#### Results

Overall, the database searches and filtering criteria leads us to retrieve an early selection of 116 full-gene models containing ORFs encoding: 9 PAL, 2 C4H, 6 4CL, 5 HCT, 5 C3H, 29 CCoAOMT, 2 CCR, 2 CAld5H, 37 COMT and 19 CAD.

In the larger families chromosomal clustering of an important number of members has been observed. Interestingly, this genomic architecture is reflected in many phylogenetic associations which corroborate the possibility of a link between transcriptional regulation and chromosomal gene order, an interesting element while determining gene function and interactive behaviors in such complex processes of tissues development like wood formation. The phylogenetic clusters allowed us to identify Egrorthologs for the landmark *bone fide* genes, which facilitated the selection for the gene expression studies. This selection included 9 PAL genes, 2 C4H, 2 4CL, 5 HCT, 4 C3H, 3 CCOAOMT, 2 F5H, 2 CCR, 7 COMT and 5 CAD.

The gene expression results indicated the existence of two main groups of tissues, generally matching their primary or secondary nature. These results support the presumption of a preferential expression of many of the selected genes in secondary, wood-related tissues, in which lignin content is higher. This sub-set of genes includes at least one member of each gene family and seems to configure the core of participants from the traditional version of the lignin biosynthesis pathway. Special situations regarding the gene expression patterns particularly from several MGF members are identifiable, requiring a deeper analysis involving comparison of samples formed under contrasting situations and eventually further work which might reveal a specialized role of these genes in particular tissues, organs and/or conditions.

#### Acknowledgments

The authors would like to thank all those involved in the Egr sequencing effort for the data and resources which were pivotal for this work.

ORAL SESSION 4. ECOPHYSIOLOGY AND HYDROLOGY

# Estimating potential productivity of cold-tolerant *Eucalyptus* benthamii in the Southestern US

Jose S. Alvarez<sup>1</sup>, Jose L. Stape<sup>1</sup>, Thomas Fox<sup>2</sup>, Rafael Rubilar<sup>3</sup>, Timothy Albaugh<sup>1</sup>

#### Background

There is an enormous interest in new sources of energy for the US. Among these possibilities, the biomass production from forestry species has been taken a relevant position (http://energy.gov/biomass), and the *Eucalyptus* genera are among the considered species due to its fast growth, wood quality and coppice ability. Several previous experiences using species of *Eucalyptus* had little success in growth and frost tolerance in the SE US, especially because the temperatures can drop from 20° C to subfreezing temperatures in one day (Hunt and Zobel, 1978). However, with the availability of new genetic materials, new propagation techniques and tested silvicultural technologies, new efforts have been initiated in the SE US in the search for the adequate species and management. Species such as *E.grandis, E.tereticornis, E.amplifolia* and *E.camaldulenis* had survived the conditions in parts of the SE US but with marginal development due to the low tolerance to frosts and freezes. A new relevant project, supported by several forest companies, is being developed by the Forest Productivity Cooperative since 2009, establishing a network of 6 field trials in order to evaluate the performance of a range of *Eucalyptus* species and entries. The trees have been assessing for frost tolerance after 12 months, after a significant cold winter, which allowed a strong screening among species and entries. Among the few promising species, *E.benthamii*was the best species regarding both growth and cold tolerance, for the 6 studied sites in North Carolina, Alabama, South Carolina and Florida. Others experimental and commercial *E.benthamii* plantations across the SE US also showed the species potential to become biomass option for the region. In order to have a regional overview of the productivity of *E. benthamil*across the SE US, a productivity map was generated using the 3-PG process-based model and contrasting it with the available, although limited, growth information of the species in the region.

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#### Meth.ods

We obtained climate and soil data for the states of Alabama, Arkansas, Florida, Louisiana, Mississippi, North Carolina, South Carolina, Oklahoma, West Virginia, Tennessee and Texas. Data of solar radiation, maximum and minimum temperature, frost days, and precipitation come from public sources. Soil data were obtained from NRCS (Natural Resources Conservation Service). The 3-PG (Physiological Processes Production Growth) from Landsberg and Waring(1997) model was used for estimations of stand growth. The spatial resolution was 1000 [m] x 1000 [m] (100 ha). We followed same spatial approach as Coops *et al.* (1998) and Almeida *et al.* (2004), doing the integration of climate surface maps and some soil characteristics.

The 3-PG model was run for the SE US considering a 10 year rotation cycle. Soil water content (< 150 cm soil depth) was between 0 and 400 mm. Parameters were obtained from previous calibrations with *Eucalyptus nitens* (Rodriguez et al., 2009) and *Eucalyptus grandis* (Stape *et al.*, 2004).Climatic requirements for *E. benthamii* were obtained from Jovanovic and Booth (2002).

#### **Results and discussion**

3-PG predicted stand volume across the entire area of the Southeast, excepting some locations with very low soil water content (Figure 1). Simulated volumes at 10 years old varied from less than 50 to more than 250  $[m^3 ha^{-1}]$  The best sites were

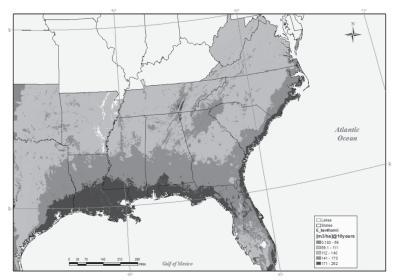


Figure 1. Potential productivity E. benthamii in the Southeastern US using 3-PG model.

the ones with mild temperatures, like near the Gulf of Mexico (Louisiana and Mississippi) and the Atlantic Coast (Georgia and north of Florida). The water supply was not a limiting factor in most of the SE US (rainfall above 1200 [mm yr<sup>-1</sup>], although poor sandy soil affect the productivity in central and south of Florida.

Although the lack of commercial plantations data for validation, a trial located in Summerville, SC was measured to verify the model prediction. The stand had 123 [m<sup>3</sup> ha<sup>-1</sup>] at 5 years old while the 3-PG prediction showed a range of 70–100 [m<sup>3</sup> ha<sup>-1</sup>] at the same age, slightly underestimating the productivity

The results should be taken as a general view of the expected spatial variation of *E.benthamii* productivity in the SE US. More commercial and experimental trials should be available and measured for a full validation of the model.

#### Conclusions

We were able to provide the first approach for *Eucalyptus benthamii* productivity in the US, in a regional scale. Our results could indicate some insights for the installation of new experiments or pilot plantations. Further work is required for validation.

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### Aboveground biomass in *Eucalyptus grandis* plantations fertilized with different sewage sludges

Alexandre de Vicente Ferraz<sup>1</sup>, Paulo Henrique Müller da Silva<sup>2</sup>, Fábio Poggiani<sup>3</sup>

#### Introduction

In the last decades, soil fertilization in eucalypts stands has become a common practice worldwide. In Brazil, this practice is very important to maintain the sustainability in forestation, given the high weathered soils in Brazil. In turn, the mineral fertilizers used in the forestry come from non-renewable mineral reserves.

Sewage sludge is a residue produced in sewage treatment plants (SWP) and its disposal occurs mainly in landfills, harming the environment. Alternatively, the use of sewage sludge as fertilizer and soil conditioner in forest plantations has gained relevance in view of the benefits attributed to the recycling of nutrients (VEGA et al., 2004), in addition to wood production without affecting the human food chain (MAGESAN and WANG, 2003).

The aim of this work was evaluate the aboveground biomass production in *Eucalyptus grandis* plantations fertilized with different sewage sludges or with mineral fertilizer.

#### Materials and methods

The study was carried out in the Experimental Station of Forestry Sciences (ESFS) of ESALQ/USP in Itatinga, São Paulo-Brazil, located at 23° 10′ S and 48° 40′ W, 860 m altitude. The climate in the Köppen classification is Cwa - mesothermal humid with dry winter. The average annual precipitation is 1,300 mm and temperature is 19.4 °C. The soil is Ferralsol, with medium texture and low fertility.

The experimental design was in blocks, with four replications and randomized plots.

The treatments comprised: 1) Control (C), without fertilization; 2) Mineral fertilization (MF), normally used by forestry companies in Brazil; 3) 15 t ha<sup>-1</sup> (dry basis) of sewage sludge from *Barueri* STP (SB); 4) 15 t ha<sup>-1</sup> (dry basis) of sewage sludge from *São Miguel* STP (SS) and 5) 15 tha<sup>-1</sup> (dry basis) of sewage sludge from

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*Parque Novo Mundo* STP (SP). The plot size was 20 m x 30 m, totaling 600 m<sup>2</sup> per plot. Tree spacing was 2 m x 3 m resulting in 100 trees per plot and 36 useful trees.

The sewage sludge was applied on the soil surface, on the planting rows. Since the sewage sludge lacks mineral potassium, KCl was equally added to all the treatments. In the STP, sludge from the municipalities of *Barueri* and *São Miguel* was conditioned with polyelectrolyte, but sludge from the region of *Parque N. Mundo* was conditioned with CaOH, and FeCl<sub>3</sub> instead.

After eucalyptus trees reached five years old, the production of aboveground biomass was quantified as follows: 1) Inventory of the trees; 2) Twelve trees per treatment (distributed in twelve basal area classes defined by the inventory) were cut down, and the major components were isolated: stemwood and stembark were gathered to diameters of 4 cm, along with top trunk (the trunk with diameters less than 4 cm), living branches and leaves.

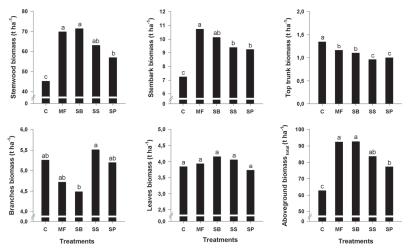
The fresh weight of components of the trees was measured in the field and samples were taken to measure moisture and nutrient concentration in the laboratory. The estimation of aboveground biomass was calculated through linear regressions using inventory and sampling biomass field data. Differences between treatments and blocks were tested with SAS using one-way ANOVA and the means were compared by Tukey test (P>0.05).

#### **Results and discussion**

The stemwood biomass produced in MF and SB was nearly 50% (23 t ha<sup>-1</sup>) higher than the control (T) and 20% (11.3 t ha-1) than in the SP treatment (Figure 1). Moreover, the stemwood biomass produced in SP was similar to that of SS and both were 25% (11.7 t ha<sup>-1</sup>) higher than the control.

There were no differences among sewage sludges treatments in terms of stembark biomass accumulate in trees. However, the stembark biomass produced in SB, SS and SP was approximately 30% (2.3 t ha<sup>-1</sup>) higher than in the control. This result can be very important considering the minimum cultivation practice, because the recycling of the stembark in the field reduces nutrient and organic matter losses; contributing to maintaining soil fertility in the long term (GONÇALVES et al., 2004).

Biomass of leaves and branches were similar among all treatments, with average production of 4.0 and 5.0 t ha<sup>-1</sup>, for each. In case of the top trunk, the biomass produced in the control was 20% (200 kg ha-1) higher than in MF and SB and 40% (400 kg ha-1) than in SS and SP. This can be explained by the smaller size of trees in the control which consequently caused a larger portion of the trunk with diameter



**Figure 1.** Aboveground biomass in *Eucalyptus grandis* plantations according to treatments: Control (C), Mineral fertilization (MF), Sewage Sludge from *Barueri* (SB), Sewage Sludge from *São Miguel* (SS), Sewage Sludge from *Parque N. Mundo* (SP). For each tree component, means followed by same letter do not differ in the Tukey test (P> 0.05).

inferior to 4 cm (commercial threshold diameter for pulpwood).

The total biomass production was similar to the stemwood production pattern, which is expected due the considerable contribution of stemwood (70 to 80%) in the total biomass of the trees.

#### Conclusion

The fertilization of *Eucalyptus grandis* plantations with sewage sludge, regardless of the STP, increased biomass production of stemwood and stembark as well as the total aboveground biomass in relation to the control treatment.

The sewage sludge from *Barueri* and *São Miguel* provided a aboveground biomass production similar to that by mineral fertilization.

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### Ecophysiological processes and wood anatomy related to growth and drought resistance in genotypes of *Eucalyptus grandis*

Tesón, N.<sup>1</sup>, Monteoliva, S.<sup>2</sup>, Licata, J.A.<sup>1</sup> and Fernández, M.E.<sup>3</sup>

#### Introduction

*Eucalyptus grandis* has proven outstandingly successful in subtropical latitudes of the Mesopotamia Argentina where is the preferred species for sawn timber (Marcó, M. 1995). However the species is highly intolerant of adverse conditions such as water and temperature stress.

Growth and stress-resistance processes are interrelated and in general there are tradeoffs between them. In addition, wood density, which is a key variable for wood quality, could also influence water transport processes and consequently it plays a role in drought stress resistance. Understanding the interrelations among these factors and the underlying processes would allow the selection of better adapted genotypes.

The main objective of this study is to identify ecophysiological processes and wood functional characteristics of four different clones of *Eucalyptus grandis* that would explain their growth differences under normal and water stress situations. This abstract examines preliminary results and outline our in progress research projects.

#### Methods

The study was performed on a 14 year-old *E.grandis* clonal productivity trial located in Concordia, Entre Ríos, Argentina (Lat: 31°22' S, Long: 58° 07' W Alt: 43 m) in a deep sandy soil. The trial design was RCB, with 4 replication of 9 trees per plots and the spacement was 3 x 3 m. The climate of the area is humid, with a mean annual precipitation of 1345 mm, without dry season. During the study an unusual drought occurred between October 2008 and February 2009 (rain was only 161 mm when the normal mean for this period is 563 mm).

Four clones named: 2, 4, B and K were selected from the Tree Breeding Program of INTA (Instituto Nacional de Tecnología Agropecuaria) with a variable range of growth rates and contrasting values in wood density: high and low.

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In one replication total height, diameter breast height, sap flow density, leaf water potential (predawn and midday), specific leaf area, osmotic adjustment, and weekly measurements of soil water content were measured. In addition, wood anatomy was characterized through morphometric measurements of sapwood samples from nine trees per clone (3 per rep) at 1.3 m height.

Analysis of variance using Statistica 7.1 software (StatSoft, Inc.) were conducted for all variables.

#### **Results and discussion**

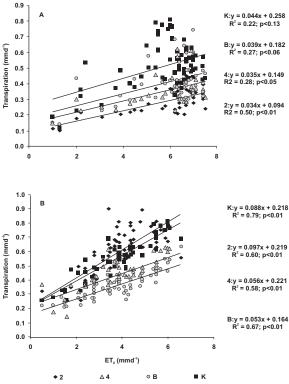
The clones evaluated showed different response patterns to drought stress that could be explained by the measured physiological and anatomical variables. Clone 2 growth, one of the high wood density clones, was the most negatively affected (both in absolute growth rate and relative to previous mean growth rate) during the severe drought period. However, the other high wood density clone (clone K) was the one with the best performance during the drought period. This differential growth rate was related to water use during the drought period (Fig. 1), where the clone K showed the highest and clone 2 the lowest transpiration rates. The better performance by the clone K could also be explained by the significantly higher osmotic adjustment capacity than the other clones.

During the period after drought, all the clones increased their water use and growth rates (Table 1). However, during the post drought period transpiration rates differed among clones in correspondence with their wood density, suggesting that the high density clones (K and 2) recovered from the drought stress faster than the low density clones (B and 4). The transpiration response sensitivity to evaporative demand (slope of regressions in Fig. 1), also showed a higher sensitivity of high density than low density clones. This could imply a loss of either leaf area or hydraulic conductivity in the low density clones, which would require a longer period to recover to a previous drought condition. High wood density clones showed a greater fiber walls thickness than low density clones.

Clone B, the clone with the highest mean growth rate and poor performance after drought had the thinnest fiber walls, the highest percentage of vasicentric tracheids, the vessels with the largest lumen diameter, and the lowest midday leaf water potential during the drought period (-3.3 +/- 0.23 MPa vs. -2.9 +/- 0.3 MPa in other clones). This suggests that a lower stomatal control of water potential maybe leading to cavitation under severe drought. When calculated the theoretical wood specific hydraulic conductivity (*ks*) of wood, clone B had the highest *ks* (Table 1).

Therefore, there could be a tradeoff between conductivity and resistance to cavitation in this clone, implying a high growth rate when resources are available, but a fast shutdown when they are not, plus a slower recovery than non cavitated trees.

On the other hand, this tradeoff does not appear to occur for clone K, which presented a small reduction in growth rate during the drought period, and at the same time a high growth rate after the drought. It is possible that wood and leaf anatomy and physiology could explain this behavior. Clone K had high density wood, but also high *ks* due to large vessel diameters. Additionally, its leaves showed a high osmotic adjustment, and low specific leaf area. Specific leaf area was also lower in both clones with higher wood density (SLA: 80 and 124 mm<sup>2</sup> g<sup>-1</sup>), suggesting



**Figure 1.** Relation ships between transpiration (estimated from sap flow density and sapwood area, mm day<sup>-1</sup>) and potential evapotranspiration (mm day<sup>-1</sup>) observed in the four clones of *Eucalyptus grandis* during soil water deficit (A) and subsequently to it, after recharge by rainfall (B). Regression slopes represent the sensivity of each clone to atmospheric demand, being higher when water constraints are lower.

#### 208 - Oral Session

**Table 1.** Mean values (+/- standard deviation) of growth, wood density, some key wood anatomical variables and estimated specific hydraulic conductivity (theoretical estimation based on Hagen-Poiseulle law, ks) of four *Eucalyptus grandis* clones differing in growth rate and wood desity. Note high variance in theoretical ks values within each clone, which was explained by their high plasticity (significant block effect on all clones). Different letters within each column mean statistical differences between clones.

Clone	Wood Desnsity	Annual mean growth	Mean growth during	Mean growth post-dry dry season	Mean vessel diameter season	Vasicentric tracheids	Fibre waal thickness	Theoretical specific hudraulic
	(kg m⁻³)	(kg DM m <sup>-2</sup> y <sup>-1</sup> )	(kg DM m <sup>-2</sup> y <sup>-1</sup> )	(kg DM m <sup>-2</sup> y <sup>-1</sup> )	(mm)	(%)	(mm) (	conductivity kg m <sup>-1</sup> MPa <sup>-1</sup> s <sup>-1</sup> )
2	444.2 a	1.29 ac	0.18 bc	2.06 a	81.9 a	3 a	3.4 b	25.5 a
-	(+/-23.5)		(+/-0.13)	(+/-0.74)	(+/-30.6)	(+/-1)	(+/-0.2)	(+/-10.9)
4	401.1 b	1.14 bc	0.66 a	1.60 a	89.3 b	3 a	3.3 ab	46.2 ab
	(+/-1.47)	(+/- 0.45)	(+/-0.47)	(+/-1.20)	(+/-43.1)	(+/-1)	(+/-0.1)	(+/-35.5)
К	439.5 a	1.42 ac	0.73 a	2.78 a	107.5 c	3 a	3.4 b	64.3 b
	(+/-10.5)	(+/-0.26)	(+/-0.44)	(+/-1.23)	(+/-47.0)	(+/-1)	(+/-0.2)	(+/-44.5)
В	397.2 b	1.72 a	0.52 ac	1.83 a	104.7 c	6 b	2.9 a	72.2 b
	(+/-13.2)	(+/- 0.53)	(+/-0.32)	(+/-1.41)	(+/-47.2)	(+/-2)	(+/-0.1)	(+/-47.5)

a functional relationship between wood and foliar anatomy which deserves to be more explored.

Some of the wood anatomical variables, such as vessel diameter and frequency, showed a significant block effect, implying a high plasticity of these features that determine the wood's capacity to conduct water. More measurements are needed to understand which specific conditions of the sites created this response.

#### **Final Remarks**

The results presented above are still preliminary. However, given the response differences found among clones, we expect that anatomical and ecophysiological measurements will help to understand the differential responses to environmental conditions of *Eucalyptus grandis* and will consequently become useful tools to aid in the selection of breeding material.

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# Soil water retention characteristics explain growth variability within *Eucalyptus* plantations: a modelling analysis

Claire Marsden<sup>a,b</sup>, Yann Nouvellon<sup>a,c</sup>, Jose-Luiz Stape<sup>d</sup>, José-Leonardo de Moraes Gonçalves<sup>f</sup>, Jean-Paul Laclau<sup>a,g</sup>, Guerric le Maire<sup>a</sup>

#### **Background and objectives**

Monitoring and predicting stand productivity is of crucial importance for forest plantation managers, yet its difficulty is increased by the observation that growth is often highly variable both in time and in space. Temporal variability can now be addressed by process-based growth models, which take into account the effect of measured meteorological variables on plantation biophysical functioning. Spatial variability has typically been dealt with by using an empirical site fertility parameter such as the site index, which is determined for each stand of a large plantation on the basis of previous inventory data. Attaining a mechanistic understanding of the determinants of site fertility is an exciting challenge for process-based modellers, as it would allow the simulation of inter-stand growth variability with less reliance on inventory data.

The present modelling study examines the causes of inter-stand variability in productivity in Brazilian commercial *Eucalyptus* plantations, as part of a research effort to model plantation carbon, water and nutrient budgets at different temporal and spatial scales. A modified version of the G'Day model (Corbeels *et al.*, 2005) of ecosystem C, N and  $H_2O$  fluxes was applied to a selection of sixteen commercial stands of contrasted productivity all located within one climatic region, and its ability to reproduce observed growth differences was tested. We considered that the climatic contribution to sub-plantation scale spatial variability would be insignificant. We also expected that the company's stand-specific fertilization schemes, designed to provide adequate nutrition on all stands, would strongly reduce the contribution of nutrient availability constraints to growth variability. We therefore hypothesized that soil water retention characteristics would explain a large part of the variability of tree growth rates and leaf area index on the 16

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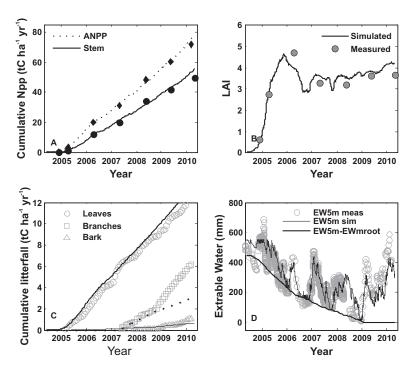
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stands. In view of this hypothesis, specific attention was paid to the modelling of the response of short-rotation *Eucalyptus* stands to soil water availability. In particular, the progressive and rapid exploration of deep soil layers by roots was modelled in a simple way.

#### Methods

The G'Day model (Corbeels *et al.*, 2005) simulates carbon, water and nitrogen fluxes between the atmosphere and a number of soil and biomass pools. The main ecophysiological processes are represented: C assimilation, water and nitrogen extraction, plant respiration, allocation of C and N to the growth of different plant compartments, litter fall, soil organic matter decomposition and immobilisation, and evapotranspiration. The daily time-step version used was that presented in



**Figure 1.** Simulated and measured variables on the parameterization stand. Simulated and measured cumulative above-ground net primary productivity (ANPP, dotted line) and stem biomass (black line) (A); simulated and measured leaf area index (B); cumulative leaf, branch and bark litter fall (C); measured extractable water in the top 5m of soil (grey circles), simulated equivalent (thin black line) and difference between maximum extractable water at 5m (EW5m) and in the rooting zone (EWm<sub>root</sub>) (thick black line) (D).

Corbeels *et al.* (2005), but some changes were made in the plant carbon assimilation and water balance submodels, in order to follow a more mechanistic framework using parameters obtained from measurements. The C allocation submodel was also slightly modified on the basis of features observed on our study sites. A significant change was made to the water balance submodel, to take into account the progressive increase of maximum plant available water with root system growth. Soil water availability affected plant growth by modulating stomatal conductance, which in turn limited C assimilation.

The model was parameterized using detailed experimental measurements made throughout the 6-year rotation of a *Eucalyptus grandis* stand located at the Itatinga Experimental Station (University of São Paulo) in São Paulo State, south-eastern Brazil (described in Laclau *et al.*, 2010). After parameterization, the model was applied to simulate 1 to 6 years of growth of 16 stands of different ages and productivity levels. A first set of simulations was run with identical parameterization for all stands, while in a second set, stand-specific soil water retention capacity parameters were used. Models were compared on the basis of their capacity to simulate wood biomass and production during 1 to 3 intervals between inventory dates.

#### **Results and Discussion**

Simulations by the modified model on the parameterization stand showed a good correspondence with measurements (Figure 1). With identical parameterization on all of the 16 application stands, model performance was high, with a coefficient of determination of the regression between measured and simulated stem biomass at inventory dates,  $r^2$  of more than 0.8. Model performance increased significantly when spatial differences in soil water retention capacity were taken into account, attaining more than 95% of explained variability in stem biomass and more than 80% of the variability of growth rates. The temporal variation of leaf area index was well simulated, as was shown by comparison with time series retrieved from satellite data (le Maire *et al.*, 2011). Results showed the importance of taking into account water extraction by roots to a depth of 5m.

#### Conclusions

Progress has been made in the modelling of the effect of water availability on stand productivity, and therefore of the effect of intra-plantation variability of soil water retention characteristics, which were highlighted as a spatially critical model

parameter strongly related with site fertility. In addition, leaf area index dynamics simulated by the model compared well with estimations obtained from satellite image time series. Such data are now freely available at a temporal and spatial resolution that is compatible with commercial *Eucalyptus* plantations. This study's results suggest the possibility of estimating soil water retention characteristics on large zones by using satellite data with model inversion or data assimilation techniques, therefore potentially significantly increasing the capacity of process-based models to simulate the spatial variability of plantation productivity.

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# Quantifying water use and biomass production by forest and pasture at plot- to catchment-scales

Auro Almeida<sup>1,3</sup>, Anders Siggins<sup>2,3</sup>. Craig Baillie<sup>1,3</sup>, Philip Smethurst<sup>1,3</sup>

#### Background and Objectives

Water scarcity demands a better understanding of the spatial and temporal patterns of water use by different vegetation types, and a synthesis of this knowledge in models that simulate water use, biomass production, ground water levels, and runoff and stream flows.

Quantifying water use by vegetation in catchments is normally limited by a scarcity of data at the required spatial and temporal scales. An alternative approach is to use hillslope and catchment hydrological models which provide relevant outputs for understanding water dynamics in the landscape and explore scenarios that trade-off alternative uses for the water e.g. wood production, pasture or environmental flows. Measures of water-use efficiency of alternative vegetation types may enhance decision-making in catchment management.

The objectives of this study are to measure biomass production and water use in a large, diverse catchment, and to simulate biomass production and stream flow using a catchment-scale, hydrological adaption of the 3-PG spatial model (Almeida et al., 2010a). The experimental catchment is located in north-west Tasmania, Australia, and contains native forest, eucalypt and pine plantations, and pasture. The catchment has 43 years of streamflow measurements, and land-use conversion to plantations has occurred during the past 20 years (Almeida et al., 2010b). Stream flow measured at the lowest part of the catchment prior to this land-use change enabled pre-treatment calibration.

#### Methods

#### Study area and measurements

The study catchment called Flowerdale is located between latitudes 40.97°S to 41.06°S and longitudes 145.62°E to 145.58°E. The catchment has an area of 152 km<sup>2</sup>; altitude is 28 to 570 m above sea level, and mean annual rainfall varies from

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1600 mm in the south to 1100 mm in the north. Maximum and minimum mean annual temperature also shows a gradient from south to north ranging from 20 to 22 °C and 10 to 13 °C, respectively.

Using historical Landsat images, we classified land-use change from 1980 to 2009. The Flowerdale catchment is now 33% occupied by forest plantations (predominantly *Eucalyptus nitens*), 49 % is native forest, and 18% is pasture and minor areas with annual crops. The plantations replaced native forest. Pasture and cropping are concentrated in the northern half of the catchment.

In order to estimate water use and water-use efficiency, four sub-catchments, each with a different land use, were delineated. One sub-catchment of 421 ha contains *Eucalyptus nitens* and *Pinus radiata* plantations and one sub-catchment is



Figure 1. Flowerdale catchment showing the four sub-catchments: pasture (P), native forest (N), *Eucalyptus nitens* (E1) and *Eucalyptus nitens and Pinus radiata* (E2).

*Eucalyptus nitens* plantation (220 ha), one sub-catchment is native forest (179 ha) and the fourth is pasture (96 ha). Instruments in each sub-catchment measure rainfall and streamflow (from which ratios of stream flow:rainfall were calculated), soil water on hillslope transects, biomass production and leaf area index.

#### Modelling

The modified 3-PG model was used to estimate the growth, water use, and water-use efficiency of the three vegetation types using a catchment approach, and to quantify water storage and movement across the landscape. The model simulates growth and water-use efficiency at a monthly time step, and water use, available soil water, drainage and runoff at a daily time step, for nominated spatial configurations of land use in the catchment, and then integrates these values for the entire catchment to predict streamflow. The model is being tested and validated for the total catchment and four sub-catchments. The sub-catchments are being used to separate the treatment effect of vegetation from other sources of variation that affect flow including landscape position, soils and climate (mainly temperature and rainfall). The model requires spatial surfaces of climatic variables, digital elevation, soil depth, soil texture, wilting point, field capacity, saturation, and hydraulic conductivity.

#### **Results and Discussion**

Analysis of historical annual streamflow and rainfall data for the entire catchment shows a trend of reduction in the ratio of runoff:rainfall (Fig. 2a); annual values are 40%-70%. Potential causes of this variability are rainfall or other climatic patterns, irrigation practices, or vegetation change that affects

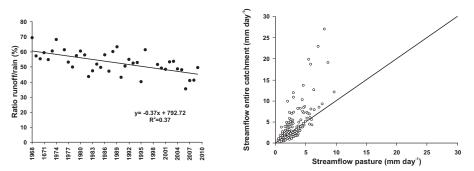


Figure 2. (a) Ratio of annual runoff:rainfall from 1968 to 2010 for the entire catchment. (b) Measured daily streamflow in the entire Flowerdale catchment and pasture sub-catchment.

evapotranspiration. Stream flows June 2010 to June 2011 indicated higher runoff for the entire catchment than for the pasture sub-catchment. Rainfall for the entire catchment (spatially interpolated) was 1,717 mm with a discharge of 985 mm (runoff:rainfall 55%). The pasture sub-catchment received 1,502 mm of rainfall and produced a discharge of 703 mm (runoff:rainfall 47%). These results are inconsistent with the expectation that runoff will be higher from pasture than from forest (Zhang et al. 2001); in this case the opposite is evident. Modelling so far indicates a strong correlation between observed and predicted plantation growth across the plantation sub-catchments ( $R^2 = 0.8$ ). Observed and predicted hillslope variations in growth are minor. Further modelling of wood production and stream flows will be presented, and hydrological analysis will evaluate the importance of spatial and temporal patterns in soil water holding capacity, hydraulic conductivity, rainfall patterns and evapotranspiration.

#### Acknowledgment

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### Predicting long term growing stock and carbon sequestration in Portuguese eucalyptus stands under different wood demand and climate scenarios

Margarida Tomé, Susana Barreiro, Josep Crous, Luís Fontes, João Palma, Sónia Palma, José Tomé

Interest in net primary productivity (NPP) modelling and carbon budget estimation increased following the signing of the Kyoto protocol as countries began to develop methodologies to quantify the carbon balance and terrestrial ecosystem carbon sequestration. In Portugal NPP modelling is particularly important for the production forest, namely for eucalyptus plantations. The use of process-based models that simulate forest ecosystem dynamics for this purpose gained therefore relevance. Such models, integrating the main physical, biogeochemical and physiological processes involved on forest growth and development, give a mechanistic description of the interactions between the living plants and their environment and are able to assess the energy balance and the cycling of water, carbon and nutrients within a given ecosystem. In Portugal the 3PG model, a simple process-based stand model requiring few parameter values and only readily available data as inputs has been selected as appropriate for simulation of NPP in eucalyptus plantations. The 3PG model includes modules for biomass production, biomass allocation, stem mortality, soil water balance and information for managers (stand information). Some modifications have been made in 3PG in the equations used to predict stand volume and basal area. Several equations/modules were added in order to obtain a detail in output similar to the one obtained with a growth and yield model that is operationally used in the country. The resulting model, designated by 3PG-out<sup>+</sup>, has been implemented in a regional simulator – SIMPLOT – that uses data from the Portuguese National Inventory to make longterm projections of the eucalyptus forest – area and growing stock per each one year age class - taking into account different scenarios of wood demand, biomass demand, annual rate of afforestation and deforestation, annual area of new energy plantations and annual area of burned stands. The substitution of the traditional model GLOBULUS by the 3PG-out<sup>+</sup> model allows taking also into account different climate scenarios.

This presentation describes the implementation of 3PG-out<sup>+</sup> into the regional simulatorSIMPLOT and presents its application to analyse the evolution of the

Portuguese eucalyptus plantations under different scenarios. Most of the scenarios show the consequences of a serious overharvesting that is occurring in the country. The simulator can be used to estimate the needs to the annual import of wood or to estimate the effort that will be needed in new plantations in order to be able to sustain the wood and biomass demands that are expected.

# Using the remote sense technique to monitor Eucalyptus forest plantation in Brazil

Cristiane Lemos<sup>1</sup>, Rodrigo Hakamada<sup>1</sup>, Thiago Freitas<sup>2</sup>, Jose Luiz Stape<sup>3</sup>, Kevin Hall<sup>4</sup>, Amanda Vergani<sup>5</sup>, Israel Lima<sup>5</sup>, Stephen Kinane<sup>4</sup>

#### Background

Brazil is the fifth largest country in territorial extension of the world; however less than 1% of its area is designated for forest plantation, 6.51 million hectare, and 4.75 million ha of which is composed of *Eucalyptus* plantation. The *Eucalyptus* plantation forest is characterized to have the highest level of productivity and uniformity in the world. The homogeneity of that kind of forest benefit immensely uses the remote sensing technique to monitor the quality of plantation, mainly through the analysis of the canopy pattern. The amount of leaf can be estimated using the leaf area index (LAI) concepts, which is direct connected by important ecological processes, such as evapotranspiration and nutrient cycling (Flores et al, 2006).

The reflectance spectrum of canopy is captured by satellites and it has its own standard reflectance. For green vegetation, the reflectance spectrum is characterized by low reflectance in the red region (0.6 - 0.7 im), associated to chlorophyll absorption, and strong near infrared reflectance (0.7 - 1.2 im), related to internal leaf structure. Based on this approach it is possible to create a vegetation index, which can be empirically related to LAI (Flores, 2006). Simple ratio (SR) is a kind of vegetation index, calculated by dividing surface reflection in near infrared and red spectral bands. SR has been applied for different types of forest to estimate the LAI, adopting a linear relationship (Running et. al 1986; Law and Waring, 1994; White et al. 1997, Flores 2003) that is not affected by site, stand structure, or time of the year (Flores, 2003). Monitor forest using the remote sense technique is cheaper than field measure and can be applied in less time for wide area extension.

Our object was to develop and apply the remote sense technique to monitor the quality of *Eucalyptus* plantation.

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#### **Material and Methods**

We chose the Landsat 5 satellite images because it is available every 15 days for free and its resolution (30 m x 30m) is large enough to monitory *Eucalyptus* forest plantation. The image was process at Erdas software and we did the atmosphere and geometric correction.

First of all, we did field's campaigns to determinate the real LAI (destructive sample and indirect measure using LAI manual made for this specific forest plantation) and after that we could fit the equation: SR function of LAI.

To monitor forest was necessary to determinate the LAI trend for forest plantation all over the rotation time, for so we could be able to infer the forest quality and health. To do that we selected seventy inventory plots for one type of Eucalyptus clone, planted along 1998 to 2001. All rotation (7 years) had the LAI estimation for two month: March (end of wet season, the upper LAI) and September (end of the dry season, the lower LAI) and then we could analyze the LAI trend during the Eucalyptus growth, planting at different seasons.

After that we evaluate the potential to use of remote sense technique to monitor forest, applying it at different scenarios of canopy damage: storm and defoliation occurred due to bug pest attack.

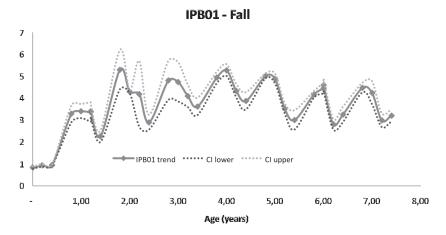
#### **Results and Discussion**

Fitting the curves of LAI versus forest age, we identified the necessity to share the LAI trend by planting season, quarterly: summer, fall, winter and spring, because when we analyzed it every six month, the details of natural oscillation of LAI was attenuated. An example of LAI trend planted in fall time is represented below (Figure 1).

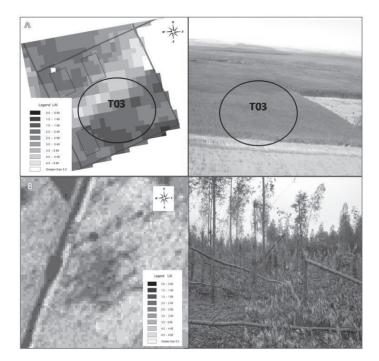
The LAI trend is applied to evaluate the forest quality in each age as a systematic way for a wide commercial forest plantation area. An option that we found was include the LAI trend intro a software system, which aware the forest manager to pay attention in a specific site when the LAI estimated is under of the lower confidence interval.

To evaluate the potential use of this technique, we applied it for two canopy damage situation: 1) to determine the amount of area damaged by the storm (Figure 2a); 2) to monitor the forest area defoliation by pest occurrence (Figure 2b). Remote sense technique was able to capture any canopy damage: such as defoliation (case 1) or dead trees and broken trees (case 2).

Moreover, this technique has potential to aid silvicultural decision making and



**Figure 1.** Example of LAI trend of Eucalyptus along the age planted in fall season (continues line is the trend and dotted lines are upper and lower confidence intervals).



**Figure 2.** Example of using the LAI to determine: a) the amount of Eucalyptus plantation area damaged by the storm on July 2011 (Map of LAI in the right and the damage in the left) b ) the defoliation area of Eucalyptus plantation caused by bug pest occurred on September 2010 (Map of LAI in the right and aerial photography in the left).

avoidance silviculture cost, for example, using this tool we could be able to avoid 80% of labor cost during the estimation the amount of area damaged by the storms.

This study is some steps towards the development of commercial tools for monitoring forests and associates the LAI estimation as an input for ecophysiological models to predict the *Eucalyptus* growth rate all over the years.

#### Conclusion

This technique has been applying as a commercial tool in monitoring the forest plantation, providing financial and time gains.

Remote sensing has the potential to become a very useful tool in order to monitor *Eucalyptus* forest plantation in Brazil.

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ORAL SESSION 6. BREEDING

# Cooperative eucalypt breeding program of nucleus population – PCPN/IPEF – Brazil

Edson Seizo Mori<sup>1</sup>, Paulo Henrique Muller da Silva<sup>2</sup>, Mario Luiz Teixeira de Moraes<sup>3</sup>

# Introduction

The eucalypt culture is the main forest commercial activity thoughout Brazil. The most planted commercial clones have *Eucalyptus grandis* and *E. urophylla* as the base species, which have mainly been studied in different private companies and governmental institutions. In 2008, after a IPEF meeting among IPEF and several associated companies, was created a Cooperative Eucalypt Breeding Program of Nucleus Population (PCPN), modified on Cotterill et al. (1989), with the goal to join the best genetic materials from the companies and experimental stations of Brazil and overseas, having as objective the amplification of potential genetic base of companies (Amapá Florestal e Celulose S.A. – Amcel; Arborgen Tecnologia Florestal Ltda; ArcelorMittal BioEnergia Ltda; Celulose Nipo-Brasileira S/A – CENIBRA; Consórcio Paulista de Papel e Celulose (Suzano); Duratex S/A; Eucatex S/A Indústria e Comércio; Fibria Celulose S/A; Forestal Oriental; Jari Celulose, Papel e Embalagens S.A; Lwarcel Celulose Ltda; Masisa do Brasil Ltda; Palmasola S.A; Stora Enso Florestal RS Ltda; Suzano Papel e Celulose S.A.), as can be seen in Figure 1, and cooperative members of institutions (São Paulo State University – UNESP-Brazil, University of São Paulo- USP - Brazil, and INTA - Argentina). The recombination of superior genotypes should establish a germplasm source for different breeding programs. Other important factor is setting up an experimental network to obtain data on ecological zones of both main commercial eucalypt species.

# Objectives

The Cooperative Program objectives are: (1) To install populations of broad gene pool came from several breeding programs of companies and experimental stations; (2) To make available the genetic materials for cooperative participants to amplify their gene banks; (3) To set up experiments into the companies to obtain data on ecological zones by the analysis of stability and adaptability of progenies/ provenances through the studied regions; and (4) To generate scientific works with ndergraduation and graduation students.

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Figure 1. Locals of *Eucalyptus grandis* and *E. urophylla* experimental network of progeny/ provenance trails through Brazil.

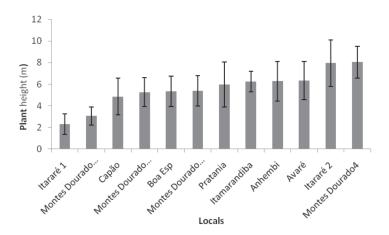


Figure 2. Plant height of Eucalyptus grandis in different locals in Brazil.

## **Experimental Network**

The Cooperative Program was created in 2008, with selected progenies seeds from advanced generations of participant members. In 2009 the Instituto Nacional de Tecnologia Agropecuária (INTA) of Argentina became a cooperative member make available their several *E. grandis* progenies, which will be distributed in 2011.

During the period from 2008 to 2010 we set up 18 experiments for both species, and installed 155 progenies of nine *E. grandis* provenances and 165 *E. urophylla* progenies of 6 provenances. To amply the genetic base of *E. urophylla* genetic material we have made an agreement with Forestaciones Operativas company of México, opening the possibity to introduce new progenies of different provenances.

The objective of experimental network is to establish ecological zones based on the analysis of stability and adaptability of progenies and provenances for different regions by Brazil and Argentina. The companies will have an opportunity to select the best progenies within their main population which will be established their own seed orchards by the 2011.

# **Preliminary Results**

The progênies of both species are presenting good development and Figure 1 shows *E. grandis* plant height by the locals throughout Brazil.

Montes Dourados 4 is presenting the best plant height by the one year old (about 8 m height) and Itataré 1 the lowest average height (about 2 m height). However, Itararé has presented the larger genetic variation than all of them (Figure 3).

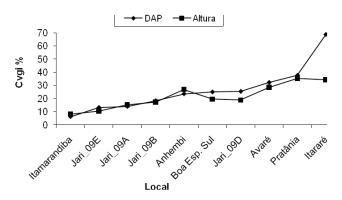


Figure 3. Coefficient of genetic variation in *Eucalyptus grandis* progenies by 01 year old.

# **Final Considerations**

The trails are just in an earlier age, but showing great potential of development and genetic variation that can be used into the genetic breeding programs in all of companies. Many progenies are pointing as stable for different environmental conditions, opening possibilities for cooperative elite trees into the joining programs.

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# The role of seed orchard factors in genetic gain and plantation yield of *Eucalyptus nitens* in South Africa

Tammy Swain<sup>1</sup>, Steve Verryn<sup>2</sup>

# Background

*Eucalyptus nitens* is an important forestry species grown for pulp and paper production in the temperate, summer rainfall regions of South Africa, particularly in environments where the growth of other eucalypt species is limited due to low Mean Annual Temperature and frequent snowfall occurrences. An *E. nitens* tree improvement programme to develop robust genotypes that are able to grow over a range of temperate sites has been ongoing at the Institute for Commercial Forestry Research for two decades. However, genetic improvement in the species has been slow as a result of delayed and infrequent flowering and subsequent seed production in the species.

With the aim of firstly, quantifying the gain that has been made in the 1<sup>st</sup> generation of improvement and secondly, determining whether the amount of flowering in the seed orchard, seed orchard origin and composition of seed orchard seed bulks influence performance of the progeny, an *E. nitens* trial series was established over diverse sites in the summer rainfall region.

# Methods

Three trials were established on temperate sites in KwaZulu-Natal and Mpumalanga in South Africa in 2001. Diameter at breast height and height measurements were completed in the trials between 87 and 97 months after establishment, and timber volumes calculated. In addition to comparing improved with unimproved material, as indicators of genetic gain, treatments included seed orchard bulks comprising a mix of the same mother families originating from different seed orchards, i.e. half sibs, to determine whether seed orchard origin plays a role in progeny performance. All bulks from a specific seed orchard were also combined in another comparison, irrespective of flowering percentage, to further examine the relationship between seed orchard origin and gain. The seed orchards differed in terms of both site and climatic variables. Treatments were

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included that comprised bulks of the same families, but which were collected in different years to represent different percentages of trees flowering simultaneously in an orchard. Flowering assessments were made in these orchards over several years to acquire the necessary flowering figures, which fell in two groups of between 15 and 20% or 40 and 47%, for this study.

## **Results and Discussion**

Improved seed orchard bulks performed significantly better (p < 0.05) than the unimproved controls (Table 1). Gains ranging from 0.2 to 21.4% increase in diameter at breast height and 9.3 to 94.4% in total volume per hectare were observed when comparing improved seed orchard bulks over unimproved commercial seed. Improvement in survival and stocking of the advanced generation material played an important role in the gains achieved. Significant differences (p < 0.05) were also found between flowering percentage for progeny growth, with seed collected from seed orchards that had more than 40% flowering producing significantly larger progeny than seed that was collected from those with 15-20% flowering (Table 1). Composition of seed orchard bulks is not critical to progeny performance in these breeding populations, as total volume did not differ significantly (p > 0.05) for this comparison (Table 1).

Despite there being significant differences in progeny growth as a result of flowering levels, there were no significant differences (p > 0.05) based on seed

		DBH (cm)	Height (m)	Individual treevolume(m <sup>3</sup> )	Total volume(m <sup>3</sup> ha <sup>-1</sup> )
Level of	Improved	15.25 a	20.67 a	0.179 a	208.05 a
improvement	Unimproved	13.78 b	18.23 b	0.130 b	128.46 b
Flowering	40 - 47%	15.48 a	20.78 a	0.182 a	226.75 a
percentage	15 - 20%	14.81 b	20.33 a	0.167 a	202.44 b
Year of seed	1999	15.44 a	21.04 a	0.185 a	230.79 a
collection	2000	15.39 a	20.65 a	0.182 a	224.30 a
	1998	15.06 a	20.58 b	0.174 a	191.33 b
Seed orchard	Amsterdam	15.82 a	20.68 a	0.194 ab	228.23 a
origin	Helvetia	15.49 a	20.79 a	0.185 a	231.67 a
	Jaglust	15.27 a	20.81 a	0.180 a	199.77 a
	Jessievale	15.04 a	20.57 a	b0.173 bc	213.85 a
Composition of	С	15.82 a	20.68 a	0.194 a	228.23 a
seed orchard bulk	E	15.79 a	20.23 a	0.193 a	241.42 a
	А	15.22 ab	20.74 a	0.177 ab	234.41 a
	В	15.17 ab	20.77 a	0.175 ab	207.33 a
	D	14.66 b	20.23 a	0.160 b	198.67 a

**Table 1.** Comparison of growth within treatment groups in *Eucalyptus nitens* genetic gain trials across all sites. (p > 0.05).

orchard origin (Table 1). Thus, seed orchard climatic variables did not appear to have influenced progeny performance. One of the possibilities for the seed orchard origin negating the effect of flowering levels in a seed orchard may be that the inbreeding depression associated with selfing is not expressed in the progeny as a result of very few of the selfs developing into viable seedlings due to low seed numbers per capsule. Should any selfs have developed into viable seedlings, it is also possible that they may have been selected against in the nursery.

# Conclusions

Significant improvements have been made over the first generation of selection in the ICFR

*E. nitens* breeding population. Improvement in survival and stocking of the advanced generation material plays a significant role in the gains achieved. Indications are that levels of flowering have an impact on progeny growth. Higher flowering percentages resulted in better progeny growth and survival, suggesting that seed should only be collected from seed orchards where more than 40% flowering was observed in the previous year.

However, seed orchard origin appears to have had no effect on progeny growth in this trial series, irrespective of flowering levels and climatic differences. This suggests that seed collected from any of the four seed orchards tested in the trial series will produce trees with significant improvement in growth.

# Evaluation of fertility and progeny performance in seedling and clonal seed orchards of *Eucalyptus camaldulensis* in southern India

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# Background

Seed orchards are production populations that serve as a means of packaging genes and generate improved seed at the culmination of a generation of breeding. Clonal seed orchard (CSO) can give greater gain than a seedling seed orchard (Borralho, 1997) when the best progeny tested clones are used in a high input breeding strategy. The gain however depends on the degree of outcrossing among clones and adaptability of offspring to the deployed site. A seedling seed orchard (SSO) gives comparatively lower gain but maintains higher diversity and hence is considered a conservative but safe strategy. Clonal forestry is a very attractive option for obtaining quick gain but diversity of the deployed crop is quite low. Majority of the eucalypt planting in India is of the local land race though it is one of the largest eucalypt planting countries in the world. Seed collected from good plantations is often used by the farming community to meet the huge demand for planting stock. Several clones are available but only a few clones are often planted over large areas, greatly reducing the genetic diversity of the planting stock.

A breeding programme for *E. camaldulensis* was implemented in 1996 in southern India. Improved seed was supplied to farmers from several unnpedigreed and pedigreed seedling seed orchards developed using natural seedlots (Kamalakannan *et al.* 2007) from Australia. Orchard seed was estimated to give a gain of 17 % in tree height, when evaluated in genetic gain trials, over the normally planted local land race (Varghese *et al.* 2009). Now several such plantations of orchard seed are used as a source of open pollinated seed by farmers. A study was therefore taken up to evaluate the performance of seed collected from orchard origin plantations (referred as SSO) in comparison to commercial clones and seed from plantations of commercial clones (referred as CSO).

#### **Material and Methods**

Seeds were collected from 10 individual trees each, in two unthinned genetic gain trials (established initially to compare bulk seedlots from five seedling seed

orchards in 49 tree plots) and one thinned plantation (established using seed from one seedling seed orchard) at five years of age. Seeds were also collected from 10 trees each in two unthinned clone evaluation trials (22 commercial clones) at five years. Fecundity was recorded (Varghese *et al.* 2003) in the five plantations (hereafter referred as SSO 1-3 and CSO 1&2) to estimate fertility variation ( $\psi$ ) and effective population size (*Ns*). Fifty families (30 from SSOs 1-3, and 20 from CSOs 1&2) were in all evaluated in progeny trials at three locations along with forty natural *E. camaldulensis* families (Kennedy river provenance from Australia) and 10 commercial clones as control. Twelve seedlings per family were evaluated in six replications using two trees plots. Growth and survival were evaluated in the progeny trials at one year. Fertility data of the orchards are given in Table 1 and field performance of families and clones is summarized in Table 2.

# **Results and Discussion**

Fertility was low in the SSOs with less than 30% fertile trees where as almost 80% of the clones were fertile in the CSOs. The genetic gain trials were not thinned, hence with a stocking of more than 1200 trees, fertility variation (as indicated by the sibling coefficient) was very high ( $\psi$ =15) in SSOs 1&2. The third seedling orchard (SSO 3) was thinned but the sibling coefficient was still quite high ( $\psi$ =9.4) indicating high genetic drift, since 70% of the trees had not flowered. The two clone trials located at two contrasting sites, differed considerably in fecundity. Two clones in CSO 2 had unusually high fecundity contributing almost 80% of the flowers and fruits in the plantation. Though 20 clones (out of 22) were common to both sites, CSO 1 had low but balanced fecundity compared to CSO 2 and hence had a very low sibling coefficient of 1.95 indicating close to a panmictic situation. Four of the orchards (except CSO 2) had more than 30% trees contributing 80 % fruits in the orchard.

It was surprising to see that progeny from the orchards (despite not being ideal

Orchard	No of F trees/Clones	ertile trees/ Clones (%)	Fruits/ tree	Ψ	Ns	Trees/Clones contributing 80% fertility (%)
SSO 1	1518	21.9	638	14.89	102	31.6
SSO 2	1272	21.4	984	15.18	83.82	30.9
SSO 3	369	27	136	9.40	41.38	36
CSO 1	22	77.3	1173	1.95	10.8	36
CSO 2	21	81	11600	10.59	1.98	9.5

 Table 1. Fertility and effective population size (Ns) in clonal and seedling seed orchards of *E. camaldulensis*.

Orchard	Ht 12 (m)	Dbh 12 (cm)	Survival (%)
Site I (Inland)			
Kennedy River	3.24	2.34	87.4
SSO 1	3.39	2.57	94.2
SSO 2	3.45	2.51	85.8
SSO 3	3.14	2.41	90.2
CSO 1	3.59	2.63	91.7
CSO 2	3.50	2.59	81.2
Clones	3.52	2.36	70.8
Site mean	3.35	2.44	86.4
SE	0.079	0.073	3.83
Site 2 (Coast)			
Kennedy River	5.09	4.29	80.3
SSO 1	5.28	4.77	88.0
SSO2	5.26	4.52	91.0
SSO 3	5.14	4.53	75.6
CSO 1	4.95	3.96	74.3
CSO 2	4.59	3.76	78.3
Clones	4.63	3.86	80.0
Site mean	5.04	4.28	81.3
SE	0.164	0.190	5.46
Site 3 (Inland)			
Kennedy River	2.89	2.03	73.2
SSO 1	2.99	2.27	81.7
SSO2	2.88	2.18	85.0
SSO 3	2.78	2.03	75.0
CSO 1	3.09	2.30	72.2
CSO 2	3.00	2.48	75.0
Clones	2.79	1.84	54.2
Site mean	2.91	2.12	73.5
SE	0.089	0.100	5.61

 Table 2. Field performance of seed orchard progeny of *E. camaldulensis* at three sites.

orchards), had done on par or better than the commercial clones. Even where the performance was on par, the survival of the clones was quite low as seen in site 3 where the progeny of SSOs 1 &2 had 50% higher stocking than the commercial clones and 12% more survival than the natural Australian families. There was a clear difference in adaptability of the commercial clones showing better performance in the inland locations (Site 1 followed by Site 3). The SSOs and the native Australian families did well in the coastal site (Site 2) indicating the need to select families and domesticate them to the coastal site.

## Conclusion

It is quite clear from the study that the commercial clones (which have not originated from any systematic breeding programme) show variation in adaptability

to different sites. In such a situation, to meet the planting stock demand, it is a safe strategy to develop advanced generation unpedigreed orchards by mixing seeds from different seed orchards (Kamalakannan *et al.* 2008) to maintain diversity and promote production of outcrossed progeny. With careful planning many such plantations can be used to identify genotypes for deployment as clones (in the same region) as well as for seed production in community orchards.

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# Simultaneous Selection for Productivity, Stability and Adaptability in progenies of *Eucalyptus grandis*

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Experiments conducted in different edaphoclimatic conditions help identifying genotypes with adaptability and stability in different environments (Maia et al. 2009). The use of mixed models is an alternative for stable genotype selection through locations, accomplished through the harmonic mean method of the relative performance of predicted genetic values (MHPRVG), recommended by Resende (2004).

This study aims to evaluate the selection of stability and adaptability and estimate genetic parameters of DBH (diameter at breast height) character in progenies open pollination of *Eucalyptus grandis*, at three sites with different edaphoclimatic conditions.

#### **Material and Methods**

The progeny tests were conducted at three sites in the towns of Anhembi and Itararé in the State of São Paulo and Itamarandiba, in the State of Minas Gerais (Table 1).

The soil in the experimental areas was classified according to the Brazilian Soil Classification of EMBRAPA as Quartz-Sand Neosol (Neossolo Quartzarênico) in Anhembi/SP, typical Dystrophic Red Latossols (Latossolo Vermelho Distrófico típico) in Itararé/SP and Yellow Red Latossols (Latossolo Vermelho Amarelo) in Itamarandiba/MG.

The experimental design used was the random blocks with treatment range **Table 1.** Characterization of sites in the study of *Eucalyptus grandis* progenies.

Site	Number of progenies	Latitude (S)	Longitude (W)	Altitude (m)	TMA (C°)	PP (mm)	<b>Climate</b> <sup>1</sup>
Anhembi	153	22º 47'	48º 07'	472	21.8	1300	Cwa
Itararé	160	24º 06'	49º 19'	740	17.0	1549	Cfa
Itamarandib	a 160	17º 45'	42º 46'	1097	22.5	1083	Cwa

<sup>1</sup>Cwa: s humid temperate climate, with dry winter and hot summer; Cfa: - humid temperate climate, with hot summer; TMA: average annual average temperature; PP: pluviometric precipitation.

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from 153 to 160 (progenies), linear plots of six plants, the spacing used was the 3 x 2 m, and, in order to reduce external border effects, double line external borders were used. The experiment was assessed at 12 months of age, for DBH (diameter at breast height).

# **Statistical Analysis**

The estimates of variance components and genetic parameters were obtained by the REML/BLUP method, using the genetic program-statistic SELEGEN-REML/BLUP (Resende 2007).

For the analysis of stability and adaptability, the harmonic mean method of the relative performance of preset genetic values (MHPRVG) was used, predicted by BLUP (Resende 2004; Oliveira et al. 2005). Thus, the statistic model used was:

y = Xr + Zg + Wc + e

where: y, r, g, c, e = vectors of fixed effects data (average of blocks), random (genetic effect, of the genotype x environment interaction and error), respectively. X, Z and W = incidence matrixes of r, g and c, of the respective effects.

# **Results and Discussion**

The average joint analysis of the environments for DBH was 4.04cm, the genetic variation coefficient was 9.09%, considered high (Sebbenn et al. 1998), and the experimental variation coefficient was low (15.82%).

There was low individual heritability free from location interaction was observed, medium heritability of progenies presented high magnitude (0.64), indicating excellent selection possibilities. The accuracy was high (0.80), confirming precision and control of causes for environmental variation. The genotypic

	MHVG			PRVG			MHPRVG	
Order (	Genotype	MHVG	Genotype	PRVG	PRVG*MG	Genotype	MHPRVG	MHPRVG*MG
1	13	4.41	13	132	5.36	13	1.27	5.14
2	14	4.41	14	1.32	5.34	14	1.27	5.14
3	163	4.26	163	1.28	5.17	15	1.24	5.03
4	15	4.21	15	1.27	5.15	163	1.23	5.00
5	30	4.17	30	1.25	5.07	30	1.21	4.91
6	22	4.16	22	1.24	5.04	22	1.21	4.89
7	29	4.02	29	1.22	4.94	29	1.20	4.86
8	131	4.00	131	1.21	4.91	131	1.19	4.82
9	104	3.95	104	1.20	4.87	104	1.18	4.80
10	107	3.95	107	1.20	4.85	107	1.18	4.77

**Table 2.** Best selected *Eucalyptus grandis* progenies based on stability (MHVG), adaptability (PRVG) and simultaneously for productivity, stability and adaptability for DBH character.

correlation between the progenies performance in those environments was 0.53, showing that the best progeny in an environment isn't necessarily the best in another location, justifying the need for considering the adaptability and stability for selection purposes. Although the genetic correlation was considered average, progenies were stable over the three sites.

The simultaneous selection, considering the DBH factor and the stability and adaptability parameters, highlights the ten best progenies, with better performance for these parameters, and the ten best progenies coincide in the joint analysis. The small variation observed indicates that the most productive progenies are also stable and great adaptability.

The selection of the best progenies in all sites provides high genetic gain. The progenies show great stability in the assessed environment. The selected progenies show adaptability and stability in the three sites simultaneously (Table 2).

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# Breeding *E.tereticornis x E.grandis* and *E.tereticornis x E.alba* dihybrids: From Seed Production to Macropropagation

B.Nagarajan, V.K.W.Bachpai, K.S.Venkatramanan, and M.Ganesan

# Background

Until the late 1980s the average yield in the Indian land race ('Mysore gum') was low as 5 -10 m<sup>3</sup>/ha <sup>-1</sup>/year –1 (Kulkarni, 2002). The major reason for the low levels of productivity was admittedly the failure of infusing fresh populations over a long period. Industries in India has raised the yield of Eucalyptus under rainfed condition from 7m<sup>3</sup>/ha/yr (untested seedling origin) to 20m<sup>3</sup>/ha /yr (from clonal selections) even though the latter are of poor pedigree (Davidson, 1988).

Realizing the need of improved yield and domestication requirements within the country the Institute of Forest Genetics and Tree Breeding, Coimbatore with the technical support of Australia Tree Seed Center (ATSC), CSIRO, Australia initiated a genetic improvement program in *E.tereticornis* and *E.camaldulensis* to widen the genetic base of the during 1996 (Varghese *et al.*, 2000). Based on extensive provenance testing five sublines (based on altitude) consisting 49 provenances was recommended for Indian conditions (Davidson, 1988). Priming on the said knowledge a *E.tereticornis* seedling seed orchard cum progeny trial (SSO) with 37 open pollinated families belonging to thirteen different provenances was established.

Though India initiated hybridization studies in red gums during the mid sixties (Venkatesh and Sharma, 1976) availability of pedigreed material was an impedance for further progress. Globally most hybrids tested or deployed are  $F_{1s}$  or composites derived from spontaneous hybridization (Camphinos and Ikemor, 1977). These are from sections *Maidenaria* (e.g.*E.globulus*), *Exsertaria* (e.g.*E.camaldulensis E.tereticornis*) and *Transversaria* (e.g.*E.pellita*) of the subgenus *Symphomyrtus* (Eldridge et al 1993). In the past it has been understood that hybrid superiority has been understood to arise through heterosis, epistasis or trait complimentarity (Nikles and Griffin, 1992). Hybrids selections have resulted in higher productivity (Verryn 2000) than either parents or sometimes lower (Potts and Dungey 2004). Apart from growth, key traits which have been identified for improvement by hybridization include clonal propagation, coppicing, frost, drought and salt resistance, resistance to

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pests, wood density and pulp yield (Dale 2000). In this study we attempted to develop dihybrids subline specific *Eucalyptus teretcornis* resource as a seed parent with the following objectives:

• To develop viable inter-sectional dihybrid combinations *E. tereticornis* x *E. grandis* and *E. tereticornis* x *E. alba* and understand its suitability to growth under coastal and inland conditions.

• To quantify the said combinations for their amenability to macro-propagation and industrial requirements.

#### Methods

#### **Eucalyptus Improvement**

The Institute of Forest Genetics and Tree Breeding, Coimbatore with the technical support of Australia Tree Seed Center (ATSC), CSIRO, Australia initiated a genetic improvement program in *E.tereticornis* and *E.camaldulensis* to widen the genetic base of the during 1996 (Varghese *et al.*, 2000). In *E.tereticornis* a seedling seed orchard cum progeny trial (SSO) with 37 open pollinated families belonging to thirteen different provenances (OP familes: 28 from Queensland, New South Wales and Northern Territory, Australia and 9 from Papua and New Guinea) and about 5 sub lines (TRT1-North Queensland, Helenvale-Laura Subline, TRT2-North Queensland, Palmer River Subline, TRT3-North Queensland>500m Subline, TRT4-<500m Subline and TRT5-Papua New Guinea Subline). In the present study we tried control pollination in TRT 1, TRT3 and TRT5 sublines of *E.tereticornis*.

#### **Pollen parents**

An *E.grandis* provenance resource stand of 13017 Lorne, New South Wales (31° 37' S 152°43' E) provenance established during 1988 to an extent of 10 hectare was assessed for growth performance. The entire area was divided into three blocks. Block mean with respect to girth was estimated for every block. The entire plantation was surveyed and identified about 60 candidate trees based on the baseline survey. Further, these trees were assessed for height growth and identified about 39 CPTs. Ten trees were been selected on the basis of volume and stem form. The *E.alba* trial consisting 220 trees at a coastal location was used to make two selections with the highest biomass and were cloned through cleft grafting. Pollen from the said clones were used for control pollination.

# Seed Set and Survival

From a modest seed set of  $4\pm 2$  seeds/capsule from 2001 we have improved the dihybrid seed set to over  $20\pm 3$ /capsule that results 95-100% of germination. Though 100% fruit set was observed initially fruit abortions at 2 weeks were noted in *E.tereticornis x E.alba* (10-15%) in *E.tereticornis x E.grandis* (10%) combinations.

Morphological Expressions and Yield

The dihybrids *E.tereticornis x E.alba* and *E.tereticornis x E.grandis* showed 100% survival under coastal and inland rain fed conditions. However, outbreeding depressed (5-7%) phenotypes exhibiting lanky stems and highly reduced leaf areas were noted in both. Morphologically both dihybrids were akin to the pollen parents in terms of foliage and stem color. Flowers were of perfect intermediary nature. At 60 months fresh weight of individual trees varied from  $120\pm10$  kg (*E.tereticornis x E.alba*) to  $150\pm10$  kg (*E.tereticornis x E.grandis*) inclusive of bark. The number of coppice shoot emergence per individual ortets of *E.tereticornis x E.grandis* families were significantly higher compared to *E.tereticornis x E.alba*. However, lead coppice shoots that established as a crop in *E.tereticornis x E.alba* ( $4\pm1$ ) was higher compared to *E.tereticornis x E.alba* ( $4\pm1$ ) was higher compared

# Hybridity confirmation

A simple study using RAPD dominant marker confirmed the introgression of both *E.tereticornis* x *E.alba* and *E.tereticornis* x *E.grandis* combinations.

# Macropropagation

Rooting experiments carried out in poly-tunnels using150cc capacity root trainers with agricultural grade-4 vermiculite supplemented with 4000 ppm IBA resulted in 24 – 48% rooting in *E.tereticornis* x *E.alba* crosses and a range of 27-71% in *E.tereticornis* x *E.grandis* crosses. Both the dihybrid clones showed 100% survival and establishment after field transfer. At the state of coppicing and during nursery phase the dihybrid *E.tereticornis* x *E.alba* was resilent to leaf gall insect pest *Leptocybe invasa* LaSalle while a few clonal selections *E.tereticornis* x *E.grandis* succumbed to the infection

# Conclusion

We find in terms of growth and pulp quality the *E.tereticornis* x *E.grandis* hybrids to be superior compared to the seed parent. We also infer the qualitative and

quantitative influence of the pollen parent in the dihybrid progeny. Both the hybrid combinations were found suitable for industrial requirements such as paper pulping, veneering and structural timber. The implication of dihybrids in overall productivity improvement in Red Gum breeding needs to be upscaled. Further, development of mapping populations for screening QTLs that are relevant to industrially important traits are the immediate foreseen outputs.

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# Evaluation of mini-cuttings as a propagation system for *Eucalyptus* hybrids

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# Background

Clonal asexual propagation by cuttings is an efficient technique for capturing genetic gain in forestry. However, selected clones (selected for growth, wood properties and stem form) often prove to be difficult to root, thereby limiting the rate of deployment for further field testing and subsequent commercialisation. This constraint will also delay the time taken for new clones to be identified. It is thus imperative that a propagation system runs efficiently and economically to realise genetic gain.

Three factors are crucial to rooting success of *Eucalyptus* species, (i) the condition of the mother plant, colloquially referred to as a hedge (i.e. adequate nutrition, no water-stress and free from debilitating pests and diseases), (ii) conditions in the rooting environment, and (iii) genetic disposition. The first aspect is difficult to control in field hedges as achieving and sustaining optimal nutritional balance is often influenced by edaphic factors (Alpoim, 2004, Proceedings of the IUFRO Conference on *Eucalyptus* in a Changing World. 1-15 Oct. 2004: 493-499). Irrigation can limit water stress in field hedges but this is not efficient as water is lost to the surrounding soil resulting in an excess being applied. The second factor, conditions in the rooting environment, is a complicated balance of adequate light intensity, relative humidity (that governs vapour pressure deficit), temperature (air and growing medium) and hygiene. The last aspect, genetic disposition, cannot be manipulated in the Nursery unless rooting potential is a prerequisite for selection and can thus only be addressed by using clones with the desired properties.

# Methods

Using *E. grandis* hybrid clones, two propagation systems were compared in this study. The traditional macro-cutting system involves growing macro-hedges in the ground, from which coppice is harvested for the generation of macro-cuttings while the nascent mini-cutting system involves growing mini-hedges in soil-less media,

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under plastic cover, resulting in well-nourished hedges that produce juvenile cuttings (Stape, 2001, *New Forests* 22: 19-41; De Assis, 2004, *Plantation Forest Biotechnology for the 21<sup>st</sup> century*: 303-333).

The main aims of this study were to: (i) develop appropriate regimes for the setup and management of *Eucalyptus* clonal mini-hedges, (ii) monitor productivity and (iii) record rooting obtained from the mini-hedges. The ancillary aim was to record plant quality and gauge field survival after planting.

#### **Results and Discussion**

Results indicated that the quality and preparation of the sand, prior to bed establishment, is imperative to provide a medium with suitable characteristics for hedge maintenance. This, in combination with adequate fertilisation (via fertigation and foliar feeding), should ensure vigorous, well-nourished hedges that provide juvenile, herbaceous cuttings for production. It is essential that these mini-hedges are not over-harvested as this will result in hedge mortality. Monitoring of EC, pH and foliar testing is important to understand the dynamics of a soil-less, hydroponic-type system.

Mini-hedge productivity (number of cuttings set per month) was superior, on a per hedge basis, for widely-spaced hedges compared to more closely-spaced hedges (6.7 vs. 4.2 cuttings per hedge per month). However, on a per unit area basis, the more closely-spaced hedges yielded better productivity. Rooting was greater for mini-cuttings compared to the conventional macro-cutting approach with internodal cuttings (cuttings taken along the length of the coppice) outperforming apical cuttings. The rooting of cuttings from mini-hedges was initially comparable to macro-cuttings but the former propagation system increased in rooting efficiency over time and the mini-hedges remain viable after three years in production. Minicuttings had superior new shoot growth relative to macro-cuttings indicating that if the mini-cutting approach is adopted, production turn around time could be reduced to allow more batches to be produced per season. This, coupled with improved rooting speed, root system quality and increased rooting percentage, could significantly improve the efficiency of a production nursery resulting in large economic gains if the nursery uses mini-cuttings exclusively. In addition, field results showed similar survival performance after six months since planting for an *E. grandis* x *E. urophylla* (GU) clone (Figure 1).

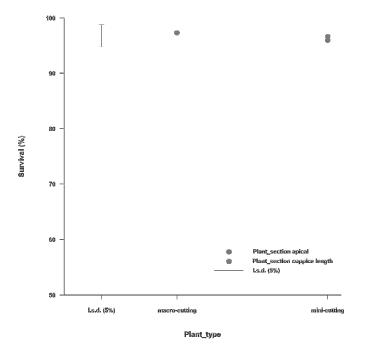


Figure 1. Six month field survival percentage of a GU clone comparing macro- versus mini-cutting and apical versus coppice length (inter-nodal) cuttings.

# Conclusion

It is widely hypothesised that rooting ability of clones is under genetic control. Although true for some clones, this study showed that the sand bed mini-hedge system resulted in improved rooting percentages through rejuvenation, better nutrition and improved climatic control of hedges. Additional benefits of this system included a more robust root system, faster growth and improved plant quality of mini-cuttings, which are favourable traits to reduce transplant stress when planted in-field.

Further work has been identified and is in progress (hedge shaping, cutting morphology and origin of hedge). This work will (i) investigate means of ameliorating the shortfalls and gaps discovered in this study and (ii) contribute to increasing our understanding on the root system and field performance of mini-cuttings. Keywords: mini-cuttings, *Eucalyptus*, propagation, rooting

# Influence of stockplants management on cutting production and rooting efficiency of cold-tolerant *Eucalyptus grandis x nitens* hybrids in KwaZulu-Natal

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## **Background and objectives**

Clones of the *E. grandis* x *E. nitens* (GN) hybrids were produced and selected through the CSIR's breeding programmes for colder plantation sites in South Africa. Some GN clones consistently exhibit high and superior pulp properties, which makes them valuable for commercial plantations in South Africa. In nurseries, stockplants are usually seven cm in length and maintained at of 5 x 5 cm planting density. However, rooting frequency varies with season and little is known on the impact of position of cuttings on overall rooting frequency of a clone. The aim of this study was to investigate the effect of size and planting density of stockplants in mini-hedges, and the position of cuttings on GN clones (Clone 1: unimproved and Clone 2: improved) on the yield and subsequent rooting of cuttings.

#### Methods

Stockplants (10 cm vs. 20 cm) were established at high (100/1.5 m<sup>-2</sup>) and at low (25/ 1.5 m<sup>-2</sup>) densities for Clone 1 and Clone 2 under commercial nursery conditions in a polyethylene tunnel. Cuttings were harvested every two to three weeks in September, October (spring), December 2010, January 2011 (summer), April and May 2011 (autumn). The harvested material was 5 – 7 cm in length and the light intensity received by individual shoots at the two planting density levels was recorded. Harvested cuttings from the three positions (apical, middle and basal shoots) were used for: (i) rooting experiments under nursery conditions and (ii) bio-stimulant analysis using the mung bean bioassay and (iii) soluble sugars analysis.

# Results

Between September and October 2010, Clones 1 and 2 established at low density yielded a similar number of cuttings, but differences in the rooting frequencies were significant (Fig. 1). Similar observations were made at high density in terms of production of cuttings, but the significant differences in the rooting observations were reversed between the clones. For both clones, short stockplants produced

fewer cuttings but had a higher rooting frequency than cuttings from tall stockplants, with a high rooting frequency recorded from basal cuttings (Table 3; 4). Similar results were observed in the mung bean bioassay which showed high rooting frequency Clone 2 cuttings of stockplants maintained at high planting density (Table 2). A high and low rooting frequency was also observed in autumn although the two clones responded differently to fungal infestations (Fig. 1). Sucrose concentration was highest sugar present in stockplants grown under low planting density. Position, size and genotype had a significant impact on type and concentration of sugar (i.e. sucrose, glucose and fructose), particularly in Clone 2 (Table 1).

## Discussion

High carbohydrate (i.e. soluble sugar) contents and auxin concentration increased production and subsequent rooting of cuttings across both clones,

	Sucrose	High density Glucose	Fructose	Sucrose	Low density Glucose	Fructose
Genotype						
Clone 1	$4.45 \pm 0.61$	2.68 ± 0.47	2.45 ± 0.38	$8.10 \pm 0.44^{a}$	6.37 ± 0.39	5.05 ± 0.52
Clone 2	$5.38 \pm 0.61$	$2.59 \pm 0.47$	$2.03 \pm 0.38$	$9.75 \pm 0.44^{a}$	$7.01 \pm 0.39$	6.19 ± 0.52
Position						
Apical	7.98 ± 0.74 <sup>a</sup>	3.45 ± 0.57 <sup>a</sup>	$2.72 \pm 0.47^{a}$	$11.23 \pm 0.54^{\circ}$	$7.18 \pm 0.48$	5.93 ± 0.63
Middle	5.15 ± 0.74 <sup>a</sup>	3.02 ± 0.57 <sup>a</sup>	$3.02 \pm 0.47^{a}$	7.5 ± 0.54ª	6.72 ± 0.48	5.47 ± 0.63
Basal	$1.62 \pm 0.74^{a}$	$1.45 \pm 0.57^{a}$	$0.99 \pm 0.47^{\circ}$	$8.04 \pm 0.54^{\circ}$	$6.18 \pm 0.48$	5.45 ± 0.63
Size						
Small	$5.53 \pm 0.61$	2.40 ± 0.47	2.42 ± 0.38	$10.82 \pm 0.44^{\circ}$	8.75 ± 0.39 <sup>a</sup>	7.52 ± 0.52 <sup>a</sup>
Tall	$4.31 \pm 0.61$	2.88 ± 0.47	2.07 ± 0.38	$7.02 \pm 0.44^{a}$	$4.63 \pm 0.39^{a}$	3.71 ± 0.52 <sup>a</sup>

**Table 1.** Effect of spacing density on soluble sugars concentration (mg  $g^{-1}$  DW) on genotype, position of cuttings and size of stock plants during spring.

Values are means (± s.e.)

Values followed by <sup>a</sup> are significantly different at P = 0.05.

**Table 2.** Effect of spacing density on number of roots per mung bean cuttings betweentall and small stock plants of GN 018B and PP 2107 from sample collected in springand summer.

Genotype	High d	ensity	Low density			
	Small	Tall	Small	Tall		
Spring						
Clone 1	20.67 ± 1.76 <sup>a</sup>	20.00 ± 1.76 <sup>a</sup>	20.92 ± 1.76 <sup>a</sup>	20.33 ± 1.76 <sup>a</sup>		
Clone 2	53.58 ± 1.76 <sup>a</sup>	26.50 ± 1.76°	$45.00 \pm 1.76^{a}$	$23.92 \pm 1.76^{a}$		
Summer						
Clone 1	23.08 ± 1.13 <sup>a</sup>	21.00 ± 1.13 <sup>a</sup>	27.67 ± 1.68ª	28.75 ± 1.68°		
Clone 2	39.83 ± 1.13°	$27.67 \pm 1.13^{a}$	$32.92 \pm 1.68^{a}$	$23.92 \pm 1.68^{a}$		

Month/year	Positions of cuttings on stockplants						
	Ар	Apical Middle			Bas	al	
	HD	LD	HD	LD	HD	LD	
Sept. 2010	42.3 ± 3.8 <sup>a</sup>	26.0 ± 2.9 <sup>a</sup>	45.8 ± 3.8 <sup>a</sup>	46.4 ± 2.9 <sup>a</sup>	47.9 ± 3.8 <sup>a</sup>	80.7 ± 2.9 <sup>a</sup>	
Oct. 2010	36.4 ± 3.3ª	18.8 ± 3.6ª	33.5 ± 3.3ª	39.1 ± 3.6ª	55.6 ± 3.3°	74.5 ± 3.6ª	
Dec. 2010	28.6 ± 3.9 <sup>b</sup>	16.7 ± 4.5 <sup>b</sup>	$31.8 \pm 3.9^{b}$	5.2 ± 4.5 <sup>b</sup>	30.2 ± 3.9 <sup>b</sup>	16.1 ± 4.5 <sup>b</sup>	
Jan. 2011	4.2 ± 2.5ª	15.6 ± 5.6ª	17.1 ± 2.5ª	35.9 ± 5.6ª	17.7 ± 2.5ª	40.1 ± 5.6 <sup>a</sup>	
Apr. 2011	4.7 ± 4.7 <sup>b</sup>	14.1 ± 2.7ª	13.5 ± 4.7 <sup>b</sup>	31.2 ± 2.7 <sup>a</sup>	14.6 ± 4.7 <sup>b</sup>	36.5± 2.7°	
May 2011	8.9 ± 2.3 <sup>b</sup>	$24.0 \pm 3.8^{\circ}$	9.9 ± 2.3 <sup>b</sup>	$27.1 \pm 3.8^{a}$	$8.3 \pm 2.3^{b}$	$30.7 \pm 3.8^{\circ}$	

Table 3. The effect of position of cuttings on stockplants and planting density on GN clones on rooting rates over different seasons (HD: High density, LD: Low density).

The values within column are (means  $\pm$  S.E) <sup>a</sup> = variables which are significantly different at P = 0.05 and <sup>b</sup> = variables which are not significantly different.

 Table 4. Effect of season, size and planting density on overall rooting rates of GN clones (HD: High density, LD: Low density).

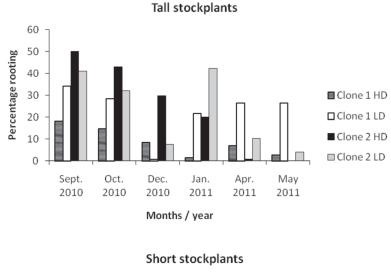
Months	Tall stock	plants	Short stockplants		
	HD	LD	HD	LD	
Sept. 2010	33.2 ± 3.1 <sup>a</sup>	37.5 ± 2.4ª	57.5 ± 3.1ª	64.6 ± 2.4 <sup>a</sup>	
Oct. 2010	28.9 ± 2.7 <sup>a</sup>	30.2 ± 2.9 <sup>a</sup>	54.9 ± 2.7 <sup>a</sup>	58.0 ± 2.9 <sup>a</sup>	
Dec. 2010	19.1 ± 3.2 <sup>a</sup>	4.2 ± 3.6 <sup>a</sup>	41.3 ± 3.2 <sup>a</sup>	21.2 ± 3.6 <sup>a</sup>	
Jan. 2011	12.9 ± 2.1 <sup>b</sup>	35.5 ± 4.6 <sup>b</sup>	13.2 ± 2.1 <sup>b</sup>	25.7 ± 4.6 <sup>b</sup>	
Apr. 2011	3.8 ± 3.8 <sup>a</sup>	18.4 ± 2.2 <sup>a</sup>	18.1 ± 3.8ª	36.1 ± 2.2 <sup>a</sup>	
May 2011	$1.4 \pm 1.9^{a}$	15.3 ± 3.1ª	$16.7 \pm 1.9^{a}$	$39.2 \pm 3.1^{a}$	

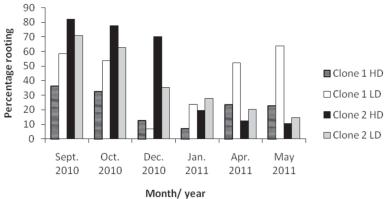
The values within column are (means  $\pm$  S.E) <sup>a</sup> = variables which are significantly different at P = 0.05 and <sup>b</sup> = variables which are not significantly different.

particularly in spring. Furthermore, rooting was enhanced by relatively higher light intensity intercepted by individual stockplants. Light intensity in the low and high planting densities caused variation in the rooting frequencies of the two clones. Light intensity and fertiliser application levels at tall and short stockplants impact on endogenous hormone levels thereby increasing or decreasing rooting. High sugar levels in Clone 2 increased its susceptibility to fungal infection thereby decreasing its rooting frequency in April and May.

#### Conclusion

It is concluded that Clone 2 has high rooting frequency than Clone 1, in particular at high planting density and if stockplants are not infected by fungal diseases. High sugar levels correlated with high growth vigour of Clone 2 stockplants. Season, planting density and size of stockplants affect the rooting frequency of GN clone. Thus, short stockplants maintained at low and high planting densities are recommended of Clone 1 and Clone 2 respectively.





**Figure 1.** Overall rooting frequency of tall and short *E. grandis* x *nitens* (GN) clones evaluated at high and low planting density over season.

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# Provenance Variation of *Eucalyptus urophylla*forPhysical and Chemical Wood Properties in Genetic Trials in FiveDifferent Countries

G. R. Hodge<sup>1</sup>, W. S Dvorak<sup>1</sup>, J. A. Espinoza<sup>1</sup>, W. Woodbridge<sup>1</sup>

*Eucalyptus urophylla* is native to seven islands in Indonesia. From 1996 to 2003, Camcore, NC State University, and PT Sumalindo Lestari Jaya made seed collections from 1104 mother trees in 62 provenances across all the islands (Figure 1). Visual assessments were made in all the natural populations to quantify their conservation status. Many of the populations had never been tested before. With the seeds collected, more than 100 provenance/progeny trials were established in Latin America and southern Africa. Preliminary results from these trials indicated that *E. urophylla* exhibits high levels of variation for both growth and wood properties.

In this paper, we use a subset of 30 Camcore trials of *E. urophylla* grownin Argentina, Colombia, Mexico, Venezuela and South Africa to examine provenance variation for wood density and wood chemical properties of this species. A total of 2140 wood samples from 56 provenances were taken and included representativesfrom all seven islands.

We developed models for analyzing the data using Near Infrared Spectroscopy (NIRS) and X-ray densitometryto determinecellulose content and wood density, respectively.Breeding values obtained from best linear unbiased prediction (BLUP) were used to rank the provenances across sites for the different wood properties. Because the majority of the provenances were tested on at least three different sites, this information will give us precise results on the genetic variation in wood properties by island of collection and across the different planting locations. At the same time, it will allow us to evaluate GxEfor wood properties across geographic regions. We will use the results to broaden our breeding base of *E. urophylla* to include both productivity and wood properties important to the pulp and paper industry while at the same time conserving genes in special conservation plantings across countries.

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# NIR spectral heritability: a promising tool for wood breeders

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# Introduction

As a fast-growing and short-rotation source of wood, *Eucalyptus* plantations are the basis for several industries, such as paper and steel producers. Forest sectors demand phenotypes exploitable for a wide range of processing needs according for different end-uses, allowing effective response to the rapidly changing market, technological, and natural environments (Verryn 2008). Hence, the determination of the genetic factors contributing to quantitative trait variation of wood properties is essential for tree breeders. This study focuses on verifying in which extent the Near Infrared (NIR) spectra variations recorded on woods from *Eucalyptus* are under genetic and environmental controls. NIR spectroscopy is a fast, non-destructive technique based on vibrational spectroscopy which expresses the interaction between radiation and the material.

# Material and methods

One hundred fifty (150) discs at 25% of tree height of 6-year old *Eucalyptus grandis* x *E. urophylla* hybrids coming from clonal tests established in Brazil on three nearest sites (19°17′ S, 42°23′ W, alt 230-500 m) were used in this study. NIR spectra were recorded using a spectrophotometer (model Vector 22/N, Bruker Optik GmbH, Ettlingen, Germany) along radius (outer, intermediate and outer) of radial face of the wood discs. The samples were stabilized at 12% of moisture content beforehand. Second derivatives (25-point filter and a third order polynomial) were applied on the spectra to enhance the quality of the information. NIR spectra were recorded from 9,000 to 3,500 cm<sup>-1</sup> totalizing 1,400 absorption values. Each NIR spectrum was reduced along its wavenumber by a reduction factor of 5 producing a NIR spectrum containing 280 absorption values. Each wavelength was analyzed

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independently (univariate analysis) to estimate the variance components by using an individual mixed linear model as  $y = \mu + \text{Clone} + \text{Site} + \text{ClonexSite} + \varepsilon$  where i is the mean value, Clone is the random effect, Site is the fixed effect, Clone×Site is random interaction effect and  $\varepsilon$  is the residual. The variances associated to random and fixed effects were estimated by restricted maximum likelihood (REML) analysis by using computer routines written in the R statistical programming language (R Development Core Team 2008). As the variances are assumed to be independent, the total phenotypic variance was calculated as  $\sigma_p^2 = \sigma_g^2 + \sigma_{gxe}^2 + \sigma_e^2$ , where  $\sigma_p^2$  is the phenotypic variance,  $\sigma_g^2$  is the clonal variance,  $\sigma_{gxe}^2$  is the clone by site interaction variance and  $\sigma_e^2$  is the environmental (error) variance. The broad-sense heritabilities were estimated as  $H^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{gxe}^2 + \sigma_e^2)$ . As the NIR spectrum consisted of 280 wavenumbers, the same number of broad-sense NIR spectral heritabilities was performed.

#### **Results and discussion**

NIR spectra contain a lot of information about wood, this complex threedimensional biopolymer material composed of an interconnected network of cellulose, hemicelluloses, lignins and extractives. Considering a NIR spectrum as a source of information concerning many wood traits, genetic parameters were calculated from them.

The broad-sense NIR spectral heritabilities and the radial variation are

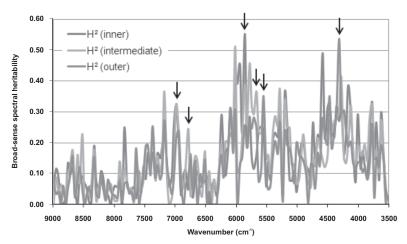


Figure 1. Radial variation of NIR spectral broad-sense heritability estimates.

presented by colors (Figure 1). The analysis of the NIR spectra heritabilities by wavenumber plot is useful to investigate the underlying relationships that have made the estimation of genetic parameters possible by NIR spectroscopy. The assignments of absorption bands are useful to identify which wood components present higher broad-sense heritabilities from NIR spectroscopic data. It helps to understand how NIR spectroscopy can evaluate genetic control over wood traits.

Some ranges of the NIR spectra presented heritabilities greater than 50%. This means that NIR spectra are able to capture the potential genetic of some chemical components and wood traits indirectly. The bands normally associated with cellulosic or lignin type biomolecules may be observed in the NIR spectra of wood. For comparative purpose, the heritability of the wood density estimated by NIR-based models at the same points linearly increased from 0.24 to 0.49 towards the cambium.

The indicated bands yielded high NIR spectra heritabilities. These bands are normally associated to cellulose and lignin contents. According to Workman and Weyer (2007) the band around 6,831cm<sup>-1</sup> is associated to O-H polymeric (2íO-H) and is assigned to cellulose type I. The NIR spectral heritabilities also were high around 5,669 cm<sup>-1</sup> and this band is associated to functional grouping CH<sub>2</sub>: stretching (2í) being related to cellulose content. The bands around 5,553 cm<sup>-1</sup> and at 4,313 cm<sup>-1</sup> are also related to the cellulose content of the wood. Lignin is representative of aromatic natural product compounds. The band around 7,180 cm<sup>-1</sup> is associated to C-H (2íCH<sub>2</sub> and äCH<sub>2</sub>) and is interrelated to lignin content. The band around 5,940 cm<sup>-1</sup> belongs to functional grouping C-H (2í), ArcC-H: C-H aromatic associated to C-H, also related to lignin content. The NIR spectral heritability estimates also were high around 4,585 cm<sup>-1</sup> and this band is associated to C-H stretching and C=O combination related to lignin content of wood.

# **Concluding remarks**

In moisture content controlled situation, the variations in NIR spectra are related to variation of chemical traits as lignin, and cellulose, and hemicelluloses, and extractives contents. It appears that variations in the indicated wavenumbers (which are related to chemical components of wood) can be attributed to genetic effects.

As NIR spectra is a countless source of information concerning wood traits, the analysis of genetic parameters from NIR spectra of wood appears to be an efficient and promising way to indirectly evaluate the genetic control over many wood traits at once and the efficiency of the design.

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ORAL SESSION 7. MIXED PLANTATIONS AND AGROFORESTRY

# Belowground carbon allocation in mixed *Eucalyptus - Acacia* plantations: a comparison between Brazil and Congo

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# **Background and objective**

Large nitrogen output associated with wood exportation in short rotation eucalypt plantations impact the low term productivity after several rotations (Gonçalves et al. 2008). Owing environmental and economical issues of fertilizers, the introduction of nitrogen fixing species such as *Acacia mangium* in mixture with *Eucalyptus* is an attractive alternative to fertilisation to improve soil fertility (Bouillet et al. 2008). Nitrogen fertilisation is known to enhance aboveground net primary productivity both by an increase in carbon uptake and by a change in carbon allocation towards aboveground organs, but the response of mixed-species plantations have led to contrasted results that might reflect interactions with other environmental factors. A better understanding of environmental control on carbon allocation is therefore required for accurately predicted tree yield in mixed-species plantations.

Trees transfer large amounts of carbon belowground to support the growth, the turnover and the respiration of both roots and associated microorganisms (Litton et al. 2007). Belowground carbon allocation compete with above ground growth, and therefore wood production, but is essential for the acquisition of belowground resources (nutrients and water) that are often limiting forest stand productivity. In addition, belowground carbon allocation plays a key role in carbon sequestration in the soil.

Our objectives were to compare belowground carbon flux (TBCF) in pure *Eucalyptus* and *Acacia* stands and in mixed-species stands in southern Brazil and coastal Congo at the end of the rotation. We hypothesized that TBCF is lower in mixed-species stands compared to pure *Eucalyptus* stands because of the positive effect of N<sub>2</sub> fixing species on soil fertility and higher in the mixed-species stands

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compared to the pure *Acacia* stands because of the energetic cost of nitrogen fixation, but that the magnitude of these changes depend on local pedoclimatic conditions that differ between the two sites.

#### Methods

Complete randomized block design including monocultures of *Eucalyptus grandis* in Brazil or *Eucalyplys urophylla x grandis* in Congo (100%E) and *Acacia mangium* (100%A), and mixtures of these species (50%E:50%A) were set up at the Itatinga experimental station (SP, Brazil, 23°02′ S and 48°38′ W, 850m elevation) in May 2003 and at Kissoko experimental station (Congo, 4°47′ S, 11°59′ E, 100-m elevation) in May 2004. The deep soils are classified as Ferrasols at Itatinga and Ferralic Arenosols at Kissoko. Mean annual air temperatures are respectively 19°C and 25°C at Itatinga and Kissoko and annual rainfall averaged 1400 mm in both places.

TBCF was estimated using a mass-balance approach as cumulative soil CO, efflux (FCUM) minus the carbon input from aboveground litter plus the changes in the C stored in roots, in the forest floor, and in the soil (Giardina and Ryan 2002). Soil CO, efflux was measured every two weeks during the last year of the rotation with a dynamic closed-path Li8100 system equipped with a 20 cm diameter Li8100-103 respiration chamber (LiCor Inc., Lincoln, NE, USA). Soil volumetric water content in the 0–6 cm soil layer was measured at the same time with a soil moisture probe. FCUM was estimated using linear interpolations between each measurement date. Litter on the forest floor was collected at the ages each year in 4-6 quadrats (50 cm x 50 cm) in each plot and sorted into leaves, twigs, bark, and miscellaneous, ovendried at 65°C weighed, and corrected for soil contamination using ash content. Aboveground litter fall was collected every month in litter traps, separated into leaves, bark and dead branches, then oven-dried at 65°C and weighed. The annual increment of coarse and medium root mass was calculated using allometric relationships. We assumed no change in fine root biomass and necromass at this age, no change in soil organic matter after several decades of afforestation with Eucalyptus, and no exportation of carbon off site.

#### Results

At Itatinga, FCUM was higher in mixed-species plots compared to *Eucalyptus* plots (intermediate) and to *Acacia* plots (the lowest). In contrast, there was no difference in FCUM at Kissoko between pure *Eucalyptus* plots and mixed-species plots while pure acacia plots displayed lower FCUM values.

Because aboveground litter fall were lower in mixed-species plots compared to pure *Eucalyptus* plots at Itatinga, TBCF was higher in the mixed-species plots compared to the pure *Eucalyptus* or Acacia stands. In contrast, higher aboveground litter fall in the mixed-species plots in Kissoko accounted for a lower TBCF compared to *Eucalyptus* plots.

# Conclusions

These results suggest that the enhancement of biomass production by introducing nitrogen fixing species in *Eucalyptus* plantation is driven by changes in belowground carbon allocation that depends on local pedoclimatic conditions. No significant difference in TBCF was reported between pure and mixed-species plots of *E. globulus* and *A. mearnsii* in southern Australia (Forrester et al. 2006). This is similar to the response of *Eucalyptus* to fertilization indicating that fertilization increases aboveground growth without affecting the amount of carbon allocated belowground (Giardina et al. 2003). In our study, the introduction of *A. mangium* decrease TBCF at the stand level on low fertile soil in coastal Congo while it failed to decrease TBCF in southern Brazil at the limits of the climatic range of *A. mangium*. The individual response of each species in the mixed-species plants is still unknown and further experimentations are required to better understand the effect of interspecific competition on belowground carbon allocation in mixed-species ecosystems.

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# Dynamics of soil N mineralization in mixed-species plantations of *Eucalyptus grandis* and *Acacia mangium* in southern Brazil

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#### Introduction

*Eucalyptus* is the genus most planted in tropical regions and covers about 20 million hectares (Iglesias-Trabado and Wilstermann, 2008), of which 4.5 million are in Brazil (ABRAF, 2011). *Acacia mangium* Wild is an NFT species largely used to recover degraded tropical lands (Macedo et al. 2008; Wang et al. 2010). These fast-growing trees have been planted on about 2 million ha in Southeast Asia, mainly for pulpwood production (Yamashita et al. 2008). Mixed-species plantations combining *Eucalyptus grandis* W. Hill ex Maiden and *Acacia mangium* trees might be planted on a large scale to supply pulpwood and firewood in the Tropics if a clear benefit over mono-specific plantations on soil fertility and/or biomass production were to be demonstrated.

Introducing fast-growing legume trees in *Eucalyptus* plantations might be an attractive option to sustain high yields, combining ecological processes of facilitation between  $N_2$ -fixing tree species (NFT) and non- $N_2$ -fixing trees (non-NFT) with large N inputs resulting from biological fixation of atmospheric  $N_2$  (Binkley et al. 2003; Kelty, 2006). Multi-species forests with annual or perennial  $N_2$ -fixing species may generate greater productivity than monocultures (Binkley et al. 2003; Forrester et al. 2006), but some studies have shown no impact, or a depressive effect of mixtures on the overall stand production (Forrester et al. 2006; Firn et al. 2007). The objective of our study was to assess the potential of *Acacia mangium* plantations to enhance nitrogen availability in soils where highly productive *Eucalyptus* plantations have been managed for several decades.

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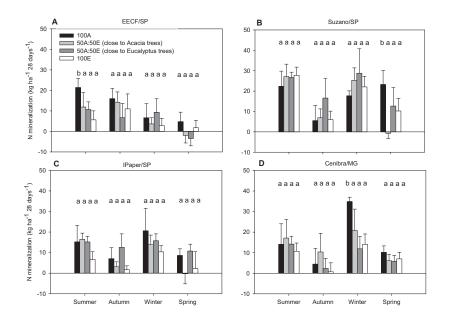
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#### **Materials and Methods**

The study was carried out at four sites located in contrasted environmental conditions (EECF, Suzano, and IPaper in the São Paulo state and Cenibra, in the Minas Gerais state). Soils were Ferralsols (FAO), mean annual temperatures ranged from 19°C (EECF and Suzano) to 25°C (Cenibra) and annual rainfall ranged from 1200 to 1360 mm, with a dry season length ranging from 2 months (EECF and Suzano) to 6 months (Cenibra). The same experimental design had been set up in 2003 (EECF) or 2005 (other sites). The study was carried out in 3 of the original 7 treatments planted without N fertilization: 100A (mono-specific A. mangium), 100E (mono-specific *E. grandis*), 50A:50E (mixture in a proportion of 1:1 between *E. grandis*) and A. mangium). Monthly nitrogen (N) mineralization in the 0-20 cm soil layer was estimated 4 times over one year at each site (one incubation per season) using the *in situ* coring technique (Raison et al.1987). Soil incubations were conducted in twelve plots (three treatments x four blocks) per site. At the onset of each sampling season, three pairs of cores (70 mm in diameter) in 100A and 100E and six pairs of cores in 50A:50E were driven 20 cm into the soil with a hammer. The pairs of soil cores were located 35, 105 and 175 cm from the nearest tree. One soil core from



**Figure 1.** Seasonal dynamics of net nitrogen mineralization in the upper 0-20 cm of soil at four sites in southern Brazil. Standard errors are indicated by vertical bars (n = 4). Different letters indicate significant differences between treatments (P < 0.05).

each pair was withdrawn immediately. In each of the twelve plots at the four sites, three soil cores (covered with plastic caps to eliminate mineral N leaching) were incubated for 4 weeks under field conditions. Samples were transported in cooled insulated containers and extractions were initiated on the same day for one composite sample in each plot. Net ammonification and nitrification were estimated by the difference between post-and pré-incubation concentrations of NH<sup>+</sup><sub>4</sub>-N and NO<sup>2</sup>-N, respectively. Before extraction, soil samples were homogeneized manually and roots were removed (no gravel in this soil). A subsample was collected for determining the water content (at 105°C). Mineral N was extracted by shaking 10g of soil with 50 ml of 1 M KCl and extracts were analysed for NH<sup>+</sup><sub>4</sub>-N and NO<sup>-</sup><sub>3</sub>-N by an automated flow injection system (Ruzicka and Hansen, 1981). Net N mineralization was obtained for each sampling season by summing net ammonification and net nitrification. Annual mineralization was roughly estimated multiplying by 3 the net mineralization rates measured one month per season. The probability level used to determine significance was P<0.05. When significant differences between treatment levels were detected, the Student-Newman-Keuls multiple range test was used to compare treatment means.

#### **Results and discussion**

Net N mineralization rates were about twice higher in 100A than in 100E at all the sites but Suzano, with intermediary mineral N production in the mixed-species stands (Figure 1A, C, D). Monthly net productions of mineral N ranged from -3.5 kg to 34.9 kg ha<sup>-1</sup> 28 days<sup>-1</sup> across these 3 sites and annual net N mineralization rates were on average 162 kg ha<sup>-1</sup> yr<sup>-1</sup> in 100A, 114 kg ha<sup>-1</sup> yr<sup>-1</sup> in 50A:50E (close to Acacia trees), 111 kg ha<sup>-1</sup> yr<sup>-1</sup> in 50A:50E (close to Eucalyptus trees), and 74 kg ha<sup>-1</sup> yr<sup>-1</sup> in 100E. Most of the differences between treatments were not significant due to large inter-block variability, whatever the season. The highest mineral N production was found at the Suzano site (Figure 1B), with 207 kg ha<sup>-1</sup> yr<sup>-1</sup> in 100A, 176 kg ha<sup>-1</sup> yr<sup>-1</sup> in 50A:50E (close to *Acacia* trees), 254 kg ha<sup>-1</sup> yr<sup>-1</sup> in 50A:50E (close to *Eucalyptus* trees), and 198 kg ha<sup>-1</sup> yr<sup>-1</sup> in 100E.

Within only 6-8 years after introducing *A. mangium* trees in soil that have been cultivated for several decades with *Eucalyptus* plantations, the production of mineral N was multiplied by 2 under the N-fixing trees at 3 sites. A similar pattern has been reported under other N-fixing plantations established on tropical soils (Siddique et al., 2008, Voigtlaender et al., 2011). The C and N stocks in the upper soil layer were not significantly influenced by *A. mangium* trees at these 3 sites (data not

shown), suggesting that the rise in net N mineralization was a result of a fast N recycling in *A. mangium* plantations. However, the overall pattern of increase in net N mineralization rates under N-fixing trees was not valid at the Suzano site, where the production of mineral N was very high (Figure 1B). The soil type was similar at the EECF and Suzano sites and the contrasted productions of mineral N at these 2 sites might be a result of different fertilization regimes over the last decades. Whilst N fertilizer additions were very low at the EECF site, large N fertilizer additions (between 100 and 200 kg ha<sup>-1</sup> rotation<sup>-1</sup>) applied at the Suzano site might have greatly increased soil N availability at this site. The beneficial effect of *A. mangium* trees on mineral N production might thus be reduced in N-rich soils. Our results suggest that the introduction of N-fixing species in highly productive *Eucalyptus* plantations might be a promising way to reduce the long-term N fertilizer requirements.

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ORAL SESSION 8. WOOD TECHNOLOGY

# Improving the pulp productivity of a eucalypt suited to challenging environments - *Corymbia citriodora*

J.T. Brawner<sup>1&2</sup>, Roger Meder<sup>1</sup>, D.J. Lee<sup>3&4</sup>

#### Introduction

*Corymbia citriodora* ssp *variegata* (CCV) is a species of interest in Australia (Lee et al. 2010) and South Africa (Gardner et al 2007) due to its adaptability, tolerance of endemic pests and diseases, and its inherently high wood density and pulp yield. Nevertheless, the taxon is relatively undomesticated for pulp production objectives. Compared to volume production, wood quality traits are more difficult to quantify and their genetic control is typically poorly understood in tree improvement programs. Near infrared (NIR) spectroscopy has been utilised in a number of species to provide a rapid and inexpensive means of assessing wood quality compared to standard laboratory techniques (Downes et al. 2009). This study combines the results of productivity and wood property assessments to evaluate selection strategies so that the limited resources available for CCV breeding can be focused on the traits that will lead to the largest impact in pulp productivity (PP).

# Methods

Progeny trials utilised for this study were established with the intent of converting trials into seedling seed orchards; a description of the trial's early performance has been provided by Brawner et al (2011). Trials were measured for total height and diameter to estimate total tree volume. A pair of growth assessments was selected to represent volume production before and after thinning to remove biases in genetic parameter estimates and breeding value predictions. Five provenances from the Gympie region, which were being used as part of an association genetics study, were sampled for wood properties with an average of 6.7 stems from 211 families sampled and scanned with a near infrared (NIR) spectrometer. Pre-existing NIR calibration models for wood traits were utilised to provide predictions of pulp yield and wood density.

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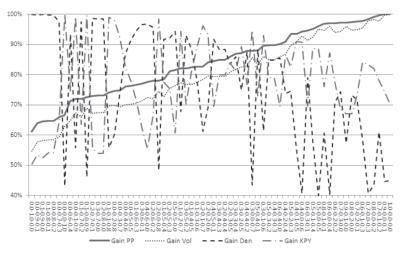
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Two multivariate mixed models were used for the estimation of genetic parameters: one model considered different environments as separate traits while the other differentiated the assessment traits and pooled data across the three trials. Genetic parameters were estimated using standard formulae for heritability and genetic correlations (Brawner et al 2011). Breeding value predictions for each parent were generated from the later analysis and scaled so that predictions reflected growth and wood quality in the largest trial (451D) at 72-months of age. Pulp productivity was then calculated for each parent as: Later-age Volume × Density × Pulp Yield. A deterministic simulation was used to estimate genetic gain for various combinations of selection index trait weightings. Index weights were varied in 10% increments so that the relative importance for each trait varied from 0 to 100% with the sum of the three traits driving pulp productivity equal to 100%. Genetic gain for each selection index was estimated as the ratio of the average of the top 10% of the parent's breeding values (37 of 374) to the population average assuming selections would interbreed at random in an isolated clonal seed orchard.

#### Results

Differences between provenances were significant for all traits with coefficients of variation for volume much higher relative to wood properties: 1.01, 0.83, 0.05



**Figure 1.** Percent of maximum gain achievable for pulp productivity (PP), individual tree volume (Vol), basic density (Den) and Kraft pulp yield (KPY) from selection of the top 10% of the *Corymbia citriodora* population using an index with weights incremented by 10% for each selection trait and restricted to sum to 100%. Indices on X axis indicate percent weighting applied to the selection traits Vol, Den and KPY and are sorted by maximum gain achievable in pulp productivity.

and 0.04 for early volume, late volume, density and KPY, respectively. Genetic parameters showed that genetic control was greater at the family than at the provenance level and across-site stability was high at both levels for volume production and pulp yield; some between-site interaction was noted for wood density (Table 1). When all trials and traits were assessed simultaneously, heritability estimates for volume were lower and wood quality estimates were higher than the average individual site estimates and again, the proportion of provenance variance estimates were consistently lower than estimates of

**Table 1.** Performance statistics (trial average with standard deviation in parentheses) for each trait assessed in each trial (451C, D & G) and genetic parameter estimates from model A) separate across-site analyses undertaken for each trait assessed within the three *Corymbia citriodora* progeny trials and model B) one multivariate analysis for all traits pooled across sites. Family parameter estimates include narrow-sense heritability on the diagonal (bold) with between-site type-B additive genetic correlations for model A and between-trait genetic correlations for model B below the diagonal. Provenance parameter estimates include proportion of phenotypic variance on the diagonal (bold) and among provenance correlations below the diagonal. Standard errors of genetic parameter estimates are provided in parenthesis.

	Tree volume Early (m³)		Tre	Tree volume Late (m³)			Density (kg/m³)			Kraft Pulp Yield (%)		
	451C	451D	451G	451C		451G	451C	451D	451G	451C	451D	451G
				Tr	ait Perf	ormance						
	0.02	0.01	0.03	0.07	0.05	0.15	749	755	764	55.16	55.1	55.6
	(0.02)	(0.01)	(0.03)	(0.06)	(0.05)	(0.10)	(34)	(37)	(37)	(2.2)	(2.4)	(2.4)
			Model A)	Across site	analys	es of each	assessme	nt trait				
Family												
451C	0.33 (0.07)			0.43 (0.11)			0.12 (0.28)			0.15 (0.30)		
451D	0.84 (0.11)	0.25 (0.03)		1.00 (0.13)	0.23 (0.03)		0.95 (1.19)	0.46 (0.10)		0.87 (0.92)	0.38 (0.09)	
451G	0.96 (0.26)	0.66 0(0.19)	0.20 (0.04)	0.92 (0.34)	0.95 (0.18)	0.25 (0.07)	0.84 (1.15)	0.94 (0.19)	0.79 (0.20)	0.74 (1.38)	0.97 (0.33)	0.33 (0.18)
Provenance	()	- ( /		( /	()		( - )	()		( )	()	
451C	0.17 (0.08)			0.21 (0.09)			0.13 (0.15)			0.13 (0.16)		
451D	0.94	0.02		1.00	0.07		-0.13	0.02		0.99	0.06	
	(0.16)	(0.01)		(0.03)	(0.03)		(1.06)	(0.03)		(0.30)	(0.07)	
451G	0.78	0.94	0.04	0.94	0.92	0.15	0.48	0.79	0.07	0.99	1.00	0.13
	(0.29)	(0.22)	(0.03)	(0.11)	(0.12)	(0.08)	(0.80)	(1.00)	(0.11)	(0.28)	(0.20)	(0.12)
	-		lel B) Acros				s all assess					
	Tree volume Early (m³)		Tree volume Late (m³)			Density (kg/m³)			Kraft Pulp Yield (%)			
Family		()			( )			(			()=)	
Vol. Early	(	0.25 (0.03	)									
Vol. Late		0.98 (0.01)		0.	22 (0.02	2)						
Density		0.42 (0.10)		0.	.39 (0.10	))	(	0.51 (0.08	3)			
Pulp Yield		0.45 (0.11)		0.	.48 (0.11	.)	(	0.26 (0.14	L)	0	.32 (0.0	7)
Provenance												
Vol. Early	(	0.07 (0.03	)									
Vol. Late		0.98 (0.01)			10 (0.04							
Density		0.09 (0.88)		-0	.10 (0.89	<del>)</del> )	(	0.01 (0.03	3)			
Pulp Yield		0.82 (0.21)		0.	.92 (0.14	4)	-	0.47 (0.90	))	0	.11 (0.0	7)

heritability. Age-age correlations between the two growth assessments were very high and correlations between pulp yield and growth were also strongly positive; however density was not clearly correlated with other traits.

Estimates of genetic gain indicated significant improvements in the objective trait of PP could be realised following selection on indices designed identify the top 10% of the parents within this base population of CCV. Maximum genetic gains achievable using were 59.8, 54.2, 3.8 and 2.5 percent for PP, late volume, density and KPY, respectively. It is evident in Figure 1 that selection for volume is the principal driver of PP and this is primarily associated with high coefficient of variation for this trait as both heritability and proportion of provenance variation estimates were smaller for volume relative to either wood trait.

#### **Discussion and Conclusions**

Significant population effects for growth and the strong association of this trait with overall improvement of pulp productivity highlight the importance of directing effort towards the evaluation of a range of populations in initial stages of an improvement program. However, this should be balanced with the finding that variation among families is much greater than among populations. Genetic improvement programs may realize significant genetic gain for a pulp productivity objective using the existing CCV populations.

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# Using NIR to assess of Kraft pulp yield in breeding trials: In-field sampling considerations

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#### Introduction

Tree breeders would like comprehensive data on every individual tree in a breeding trial. For some properties this is a simple matter, however for properties such as stiffness or Kraft pulp yield (KPY) this traditionally requires destructive sampling followed by costly analysis (KPY costs ~US\$1,000/tree). Near infrared (NIR) spectroscopy is now regularly used to determine KPY to minimise the cost of analysis, which until recently required the development of a calibration matched to the site and species under consideration by destructive sampling of a subset of trees from the population being assessed (Meder *et al.* 1994, Michell 1995, Michell *et al.*, 1998, Downes *et al.* 2009, Ramadevi *et al.*, 2010). Most recently, non-destructive sampling protocols have been developed to determine whole-tree KPY value. This paper reviews recent developments and highlights some of the protocols to enable non-destructive sampling and in-field determination of KPY.

#### Methods

Across a number of studies, individual trees (*Eucalyptus, Corymbia* and *Acacia* spp.) were sampled directly in the field or by non-destructive sampling of wood frass. Screened KPY was predicted from spectra acquired using either a handheld or laboratory NIR spectrometer.

#### NIR Spectroscopy – Breast Height Cores

From each tree a breast-height increment core (10-mm diameter, bark-to-bark) was obtained, air-dried and ground to woodmeal. NIR spectra of the woodmeal were acquired using a Bruker MPA (Bruker, Germany, www.brukeroptics.com) laboratory NIR instrument (4,000-10,000 cm<sup>-1</sup> (1,000-2,500 nm), 8 cm<sup>-1</sup> resolution). Spectra were also acquired using a Polychromix Phazir (ThermoScientific, USA, www.thermoscientific.com) handheld NIR (950–1,800 nm, 8 nm resolution).

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#### NIR Spectroscopy – Outerwood Drill Frass

From each tree, drill frass was obtained from a 20-mm diameter hole, ~40 mm deep into the wood, using a spade bit. Frass was air-dried and ground to woodmeal. NIR spectra were acquired using a Bruker MPA as above.

# NIR Spectroscopy – Bark-free Windows

Bark windows were prepared by removing the bark surface at breast height. NIR spectra were obtained by averaging five spectra to provide a single spectrum representing each tree using a Polychromix Phazir (as above) to acquire spectra from the exposed cambial surface.

#### **Kraft Pulping**

Pulping was performed on whole-tree chips using a Haato 12 autoclave air pulping digester. Pulping conditions were: wood charge 300 g OD equivalent; liquor ratio 3.5:1; cook temperature 170 °C; nominal time to/at temperature 90/90 min; sulphidity 25%; H-factor 2865; activation energy 147.5 kJ mol<sup>-1</sup> with %NaOH charge varied to give a target Kappa value of 18.

#### **KPY Calibration**

Partial least squares (PLS) calibration models for KPY were made using *The Unscrambler* (v9.8, Camo A/S, Norway, www.camo.com). A common sample preparation was used for comparison, namely woodmeal generated from the whole-tree wood chip sample. Each calibration model was optimised for each instrument. Generally spectra were transformed using a Savitzky-Golay derivative (1<sup>st</sup> or 2<sup>nd</sup>) with 15-point windows for the MPA and 3-point windows for the Phazir.

#### Results

The quality of NIR calibration models for the prediction of Kraft pulp yield were similar for the laboratory and handheld NIR systems, even taking in to account the larger spectral range and higher resolution of the laboratory NIR (Table 1). This trial was performed using woodmeal from breast height cores (calibrated against whole-tree KPY). Transferability of calibrations between four MPA laboratory units (Meder et al. 2009) or between two Phazir units provided similar calibration and prediction statistics.

The logical use of the Phazir is in-forest acquisition of NIR spectra via a bark window to expose a fresh wood surface. Early results indicate that the season at which the spectra are acquired plays a role in the quality of the NIR calibrations due to intra-annual variation of pulp yield in the cambial zone (Downes *et al.* 2010), and the fact that NIR obtains spectra from only the surface 2-4 mm of wood. It appears that late summer and autumn (fall) are the best seasons to undertake inforest sampling for NIR prediction of whole-tree KPY (Meder *et al.* 2010).

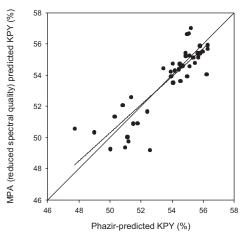
Previous trials have shown the average KPY value decreases between ages 3 and 5 years based on actual pulping data, but these trials suffer from having only few representatives of each age class and the results are not statistically significant. It must also be remembered that density increases with age and hence the pulp productivity (KPY x density) also increases irrespective of any apparent decrease in KPY. More recent and extensive trials using *Acacia mangium* sampled at 2 and 3 years of age show an increase of 0.5% in mean KPY (predicted from NIR of cores) across >700 individuals.

To quantify the benefit of NIR in a breeding programme, Figure 1 shows the economic value of genetic gain that can be theoretically expected when NIR is used

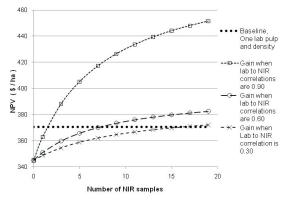
	R <sup>2</sup> (calib. / valid.)	PCs required	RMSEP
MPA	0.939 / 0.83	5	0.95
Phazir	0.913 / 0.81	5	0.99
MPA – reduced*	0.87 / 0.78	5	1.1

**Table 1.** Summary statistics for NIR calibration of KPY for the MPA and Phazir. Woodmealfrom increment core samples.

\* Spectral range and resolution reduced to be similar to the Phazir.



**Figure 1.** Correlation between KPY predicted from the laboratory NIR (using reduced range and resolution) and the handheld NIR.



**Figure 2.** Economic value of genetic gain from increasing NIR sampling with phenotypic and genetic correlations between laboratory estimates and NIR predictions having calibrations with regression coefficients varying from r = 0.30 to 0.90.

to provide predicted values of KPY on individual trees rather than using one laboratory pulp value per family and density on every tree to provide familial variation. Assuming a trial design of 40 progeny assessed for DBH, a NIR calibration model for KPY with r = 0.90, indicates that NIR spectra from a minimum of two trees are needed to obtain a greater genetic gain than that which would be achieved with one laboratory-pulped tree.

#### **Discussion and Conclusions**

Near infrared spectroscopy provides a powerful tool to rapidly and nondestructively determine KPY. While there are still some precautions in applying the technology in terms of sampling and sample presentation, it allows every individual tree in a breeding population to be assessed, thereby improving the calculation of genetic gain parameters. Handheld NIR systems enable data to be collected inforest on standing trees, provided due consideration is given to seasonal variation.

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# Variation of wood stiffness and genetic control within eucalyptus hybrid stems from clonal plantations in Brazil

Hein PRG<sup>1</sup>, CHAIX G<sup>2</sup>, ROSADO AM<sup>3</sup>, TRUGILHO PF<sup>4</sup> and LIMA JT<sup>5</sup>

The aim of this study was to explore the spatial variations of wood stiffness and its genetic control in *Eucalyptus* stems. The elastic modulus strongly increased from pith to bark. The variations in modulus of elasticity along the stem are less consistent than those in the radial direction. These findings are interesting for sawn timber breeders because it is stiffness that is desirable rather than its constituent traits or density. The genetic control of modulus of elasticity varied up to 0.4 depending of the region of the stem. We found greater genetic control over wood stiffness in two specific zones of the stem of *Eucalyptus*.

**Keywords:** modulus of elasticity, NIR spectroscopy, clonal test, heritability, *Eucalyptus* 

# Introduction

A number of wood traits were identified as worthy of attention for *Eucalyptus* tree improvement, including wood density, stiffness and microfibril angle (Raymond, 2002). While most research on Eucalyptus genetics were focused on growth traits and wood density (Greaves et al., 1997, Hamilton and Potts 2008) relatively few studies have investigated the genetic aspects of variation in wood stiffness in *Eucalyptus*. The aim of this study was to explore the variations of the genetic control of wood stiffness in *Eucalyptus* stems.

# **Material and Methods**

Wood discs removed at 0, 25, 50, 75, and 100% of the tree height from 150 *Eucalyptus grandis* x *E. urophylla* hybrids (6-years-old) coming from three nearest but contrasted clonal tests (CT) established in Brazil (19°17′ S, 42°23′ W, alt 230-500 m) were used. The main difference between sites was their declivity level.

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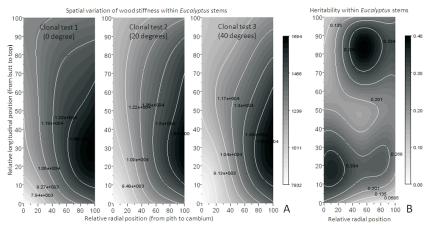
Clonal test 1 is planted on a flat terrain, the slope of CT 2 and CT 3 is 20 degrees and 40 degrees respectively. Previous calibrations based on near infrared (NIR) spectroscopic data (Hein 2011) were used for estimating modulus of elasticity (E) on radial positions of 750 wood discsm and broad-sense heritability of elastic modulus. The NIR-estimated E values were analyzed independently (univariate analysis) to estimate the variance components by using an individual mixed linear model as  $y = \mu$  + Clone + Site + ClonexSite + å where i is the mean value, Clone is the random effect, Site is the fixed effect, Clone×Site is random interaction effect and å is the residual. The variances associated to random and fixed effects were estimated by restricted maximum likelihood (REML) analysis by using computer routines written in the R statistical programming language (R Development Core Team 2008). As the variances were assumed to be independent, the total phenotypic variance was calculated as  $\sigma_{p}^{2} = \sigma_{g}^{2} + \sigma_{gxe}^{2} + \sigma_{e}^{2}$ , where  $\sigma_{p}^{2}$  is the phenotypic variance,  $\sigma_{g}^{2}$  is the clonal variance,  $\sigma_{_{gxe}}^{^{2}}$  is the clone by site interaction variance and  $\sigma_{_{\rho}}^{^{2}}$  is the environmental (error) variance. The broad-sense heritabilities were estimated as  $H^{2} = \sigma_{g}^{2} / (\sigma_{g}^{2} + \sigma_{gxe}^{2} + \sigma_{e}^{2}).$ 

#### **Results and Discussion**

The spatial variation of modulus of elasticity along the *Eucalyptus* trees at three contrasting sites are presented in Figure 1A. The variations in modulus of elasticity along the stem are less consistent than those in the radial direction, especially in the bottom. The E strongly increased from pith to bark at the base (~6,300 MPa), at 25% of height (~6,900 MPa), at 50% of height (~5,600 MPa) and at 75% of height (~3,900 MPa). The stiffness at the base and at 25% of height increased in the same magnitude (~6,500 MPa) towards the bark, but the absolute E values at 25% of height were, on average, 2,500 MPa higher. These findings are interesting for sawn timber breeders because stiffness is more desirable rather than its constituent traits or density.

The pith to cambium variations in wood stiffness was lower in trees from the clonal test 1 (terrain slope 0°) and higher in trees from clonal test 2 (terrain slope 20°). The higher pith to bark variation at the base was found in trees from the clonal test 1, where there was no inclination.

Broad-sense heritability estimates for modulus of elasticity strongly varied within the stem (Figure 1B). Null heritability was found in the sapwood of the base while H<sup>2</sup> of 0.36 was estimated in the outer heartwood at 75% of height. Figure 1 shows that genetic control over stiffness is larger in two regions of the stem: (i) in



**Figure 1.** Spatial variation of modulus of elasticity (MPa) in clonal tests 1, 2 and 3 (A) and of heritability estimates within in *Eucalyptus urophylla* x *Eucalyptus grandis* clones (B).

the inner heartwood region (0-25% of relative radial position) of the lower zones of the tree (10-30% of the relative longitudinal position) (dark and round spot at the bottom of the chart) and (ii) at the top of the tree, a zone localized at 40-80% of relative radial position and at 70-90% of the relative longitudinal position (dark and round spot at the top of the chart). No clear patterns of radial variation were established for H<sup>2</sup> estimates of modulus of elasticity along the stems of *Eucalyptus*.

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# Wood Quality studies of ITC Bhadrachalam Clones of Eucalyptus

H D Kulkarni<sup>1</sup>

A study is undertaken to investigate selected wood properties of basic density and fiber morphology in relation to clone, age, spacing, soil type and cultural practices amongst the species and "Bhadrachalam clones" of eucalyptus to identify the suitable clones for raising large scale commercial plantations under farm forestry for meeting quality raw material for production of pulp and paperboard.

The species to species comparison revealed that wood of *Eucalyptus camaldulensis* clones were significantly denser than *E. tereticornis, E. torelliana* and *E. urophylla*. The longest fibers were found in *E. torelliana* and *E. urophylla*.

Amongst 98 clones of eucalyptus studied, clone 4 showed denser wood (613.9 kg/m<sup>3</sup>) and tendency of having shorter fibers (0.81 mm). Clone 3 & 7 are the most desirable ones for pulping in view of their relatively long fibers (0.88 & 0.84 mm) and basic density (576 to 581 kg/m<sup>3</sup>).

Age seems to be the most crucial factor influencing the pulpwood quality. Basic density, heartwood per cent and fiber morphology (length, diameter, wall thickness) improved with age 1 to 9 years. However, tissue proportion and vessel diameter did not vary significantly. Age studies indicated that there is no advantage of extending the rotation age from 3 to 5 years in terms of wood quality improvement of these clones.

On the influence of tree position, the basic wood density initially increased from the base up to 25% of tree height before start decreasing towards the top while the increase in fiber length continued up to 50% before declining towards the tree crown.

Clones grown in red and black soils indicated that black soil promoted higher girth with higher moisture and heartwood content with wider fiber lumen and thinner fiber walls compared to red soil suggesting that black soil is more favourable for better tree growth. Irrigation and fertilization response in wood properties showed higher vessel percentage with larger vessels and greater moisture content indicating better conducting efficiency.

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#### Introduction

ITC started Pulpwood Tree Improvement Programme in the year 1989 and developed promising eucalyptus clones (Kulkarni, 2004 & 2008). A collaborative work between ITC and Kerala Forest Research Institute (KFRI) was carried out to characterize 98 "Bhadrachalam clones" of eucalyptus in terms of pulpwood qualities particularly wood basic density and fiber morphology. The purpose of this study was to identify and short list the most potential clones of superior pulp and paper qualities for recommending commercial cultivation in clonal plantation Programme of ITC's paper division.

Wood basic density and fiber dimensions have long been accepted as the most crucial quality indicators of pulpwood. Low density and longer fiber woods are mostly preferred for pulping process for eucalyptus. The basic density is generally an indicative of pulp yield of any species for raw material production as it is a measure of the mass of wood on oven dry basis. Fiber length is known to determine the tearing strength of paper. If low density aids impregnation due to a more open wood structure, longer fibers promote higher tear index (Banham, *et al.*, 1995). The studies suggest that in eucalyptus, the optimum basic density is around 480 to 520 kg/m<sup>3</sup> and above the upper limit of 600 kg/m<sup>3</sup> (Dean,1995). The other crucial parameter of fiber morphology that favours the pulping properties is the ratio of double wall thickness of fiber/lumen width of fiber and the value of upper limit is 1; the desired Runkel ratio of 2 x fiber wall thickness/lumen diameter is < 1. The pulpwood demand and quality assessment studies are reported by Singh and Naithani (1994). Further, a detailed account of eucalyptus for pulp and paper making are also given by Sharma and Bhandari (1983) and Tewari (1992).

Obviously, the investigation of these wood proprieties of newly developed clonal eucalyptus is a pre-requisite to evaluate the wood qualities most desirable for pulp and paper and to give preference for wood of optimum basic density and fiber morphology for mechanistic outcome of the raw material from future planting stock.

The goal of this study is to evaluate the wood properties of "Bhadrachalam clones" of eucalyptus for identification and short listing the best clones for mass multiplication and commercial planting. In order to achieve this objective, it is desirable to investigate the influence of various silvicultural factors such as early harvest (tree age, site) soil type, (black, red, alkaline, saline), cultural practices (irrigated *vs.* rain-fed, espacement etc.,) on prime wood quality attributes of most potential clones. The specific objectives are to investigate the selected wood

proprieties *viz.*, basic density and fiber morphology (fiber length, lumen width and the wall thickness) in relation to clone, age, spacing, soil type and cultural practices among the most potential "Bhadrachalam clones" of eucalyptus for identifying the most suitable clones to prepare plantation management strategies for production of quality wood.

#### **Materials and Methods**

#### **Field sampling**

The material consisted of 98 clones, with 2 ramets, and seeding crops of two different ages. Clones have been sampled from six localities representing the ages ranging from 1 to 9. After determining the most potential clones with desired wood properties, selected clones (clone 3 and 7) were sampled from ITC paper mill campus, Sarapaka fields, Jangareddygudem (red soil), Santhravur (Black soil), Ravipad and Eleru (poor sites) to study the effect of age, site/soil types, irrigation, and espacement etc. A total of 217 trees were sampled for wood quality investigations. Cross sectional discs of 3 cm thickness were removed from each tree at breast height (1.37 m from the ground level). From clone 3, additional discs were removed from 25%, 50% and 73% of tree heights to study the longitudinal pattern of variations in wood properties from tree base to top.

The list of 98 clones with 4 Seedling Control (SC) is as follows:

1, 3, 4, 6, 7, 10, 27, 52, 71, 72, 99, 105, 115, 116, 122, 124, 128, 130, 147, 158, 222, 223, 265, 266, 269, 271, 272, 273, 274, 277, 279, 283, 284, 285, 286, 288, 290, 291, 292, 315, 316, 317, 318, 319, 320, 330, 405, 407, 409, 411, 412, 413, 417, 433, 436, 437, 439, 455, 456, 470, 471, 499, 501, 514, 518, 526, 532, 540, 541, 2011, 2014, 2018, 2021, 2022, 2023, 2040, 2045, 2046, 2048, 2051, 2057, 2059, 2069, 2070, 2120, 2121, 2125, 2135, 2253, 2254, 2259, 2261, 2264, 2267, 2268, 2282, 2286, 2287, Seedling control 1, 2, 3 and 4.

#### Laboratory investigations

For laboratory investigations, 5 cm thick cross sectional discs were collected from the breast height level of standing trees. They were transversely cut into two halves of 2.5 cm thick discs. One half (entire disc) was used for measuring the unextracted basic density (oven dry weight to green volume basis) by determination of green volume using water displacement method after removing the bark (Bhat, *et. al.*, 1987 a,b). Bark percentage was estimated on oven dry weight basis by noting

the weight with and without bark of the second disc.

For wood anatomical examination under microscope, 1cm<sup>3</sup> block representing the radial position near the bark was sectioned on a sliding microtome in order to obtain about 15 im thick transverse sections. They were double stained with Chrysoidin red and Astra blue for microscopic examination and quantification of tissue proportions, vessel diameter and frequency (number/mm<sup>2</sup> area).

Fiber morphology was studied after macerating the fibers following the Franklins method – 1945 (Bhat and Bhat, 1984). Macerations were done using the small slivers, measuring up to a radial thickness of 2 to 3 mm, taken from two positions of radial wedges representing near the pith and near the bark. Fiber length with diameter, wall thickness and lumen width in midpoint between the two tips of 30 randomly selected fibers per segment were measured through projection microscope.

From the measured fiber properties, certain parameters were derived for comparison among the clone as shown below:

Fiber shape factor = d2-l2 / d2+l2

Where, d is fiber diameter, l is lumen diameter

Runkel ratio =  $2 \times \text{fw} / 1$ 

Where, fw is fiber wall thickness, I is lumen diameter

Vulnerability ratio = vd / vf

Where vd is mean vessel diameter, vf is number of vessels per mm<sup>2</sup>.

After preliminary assessment, selected potential clones were further subjected to T-tests in order to know effects of spacing, age, irrigation *vs*. rain fed plantations, cultural practices, coppicing, soil type (red *vs*. black).

# Statistical analysis

The general form of the model applied to test the significant influence of various factors on the dependent variable of the interest such as wood density, fiber length is

 $Y = \mu + \beta_1$  No. of trees per ha +  $\beta_2$  Age + Clone + Location + Error

Where Y is the dependent variable,  $\mu$  is the intercept parameter;  $\beta_1$  and  $\beta_2$  are the regressions coefficients associated with covariates.

The model fitting was implemented using ANOVA procedure in SPSS package. Using the above model, the mean values of dependent variables of interest for different clones were obtained after adjusting for the effect of the age of the trees, number of trees per ha and location. The significant difference between the mean values of the different clones was tested using Bonferroni test. The mean values of wood properties of selected and most potential clones were compared separately by T-tests to assess the specific effect of age, site conditions/soil type, irrigation, species, coppicing, etc.

#### **Results and Discussions**

The results obtained on 98 eucalyptus clones and 4 seedling control grown in varied site with differences in age, espacement and cultural practices and there influences on various wood properties are presented below:

#### A. Factors influencing wood properties

In order to identify the most suitable clone from among the widely represented four clones for further development of pulpwood plantations, adjusted mean values of various parameters which varied considerably fitting into the model are represented in Table1.

# Tree size/volume

The effect of the factors *viz.*, clone, location, age and spacing were examined. All the factors had significant effects on tree size. As number of trees increased per ha with closer spacing, tree size/volume decreased. As age increased tree volume increased. The adjusted mean value of clone 6 was significantly lower than clone 7, indicating that the clone 7 may yield the highest volume per tree with faster growth rate.

#### **Basic density**

The initial espacement alone did not explain significant variation in wood basic density. By and large wood density increased with age up to 9 years in all the clones studied. Clone 4 displayed densest wood (710 kg/m<sup>3</sup>) which was significantly different from clone 6 with lightest wood. As the basic density value higher than 600 kg/m<sup>3</sup> is not desired in eucalyptus. For better pulp yield trees, clones such as 3, 6 and 7 are better compared to clone 4. Among 98 clones studied, clones 10 (699 kg/m<sup>3</sup>) and 433 (676 kg/m<sup>3</sup>) also have high density. Bhat (1990) reported that aiming at faster growth do not necessarily affect the wood density.

Clone No.	Tree Vol.m³/tree	Basic Density kg/m³	Moisture content %	Heart wood %	Fiber length µm	Fiber dia. µm	Lumen width, µm	2 x fiber wall thickness μm	Vessels per mm <sup>2</sup>	Vessel dia. µm
3	0.238	575.8	31.8	20.3	0.88**	19**	8**	10**	2.2*	131
4	0.169	613.9**	18.8	34.8	0.81*	16*	7	9*	2.6	136
6	0.138*	573.1*	21.4	32.2	0.86	16*	7*	9	2.5	144
7	0.346**	580.7	30.4	17.8	0.84	18	8	10	2.7**	141

Table 1. Estimated adjusted means of clones with regard to selected wood propertie	Table 1	L.	Estimated	adjusted	means	of	clones	with	regard	to	selected	wood	propertie
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\* Significantly lower value, \*\* significantly higher value

# **Moisture Content**

Although the initial moisture content in wood increased with age neither the espacement nor the particular clone had any effect.

# Heartwood percentage

Though not related to espacement, heartwood percentage increased with age (For clone 3 - at 3 yrs 16%; 6 yrs 40% and 9 yrs 56%), after initiation at the age of 3 years in clone 3. No significant difference was noted among the clones.

# **Bark percentage**

Bark per cent varied from clone to clone and increased slightly with age (For clone 7 - at 3 yrs 12% and 9 yrs 15%), but the fitted model itself was not significant to explain the variation.

# **Fiber length**

All the factors had significant effects on the fiber length though the overall differences among the four clones were not statistically significant. It increases with the increase in number of trees per ha with closer spacing and age of the trees. The fiber length varied from 0.7 to 1 im and with age (for clone 3 - at 3 yrs 0.82 and 9 yrs 1im).

# Fiber diameter

The fiber diameter varied from 13 to 20 im. All the factors had significant effects on the fiber diameter and it decreased with the increase in number of trees per ha. With age increases fiber diameter also increases. The adjusted means of clones 3 *vs.* 4, 3 *vs.* 6 and 4 *vs.* 7 & differed significantly.

# Fiber lumen width

Lumen width varied from 6 to 9.74 im. Neither the number of trees per ha nor age had significant effects on fiber lumen diameter. With widest fiber lumen, clone

3 was significantly different from fiber lumen width of clone 6.

#### **Double fiber wall thickness**

The fiber double wall thickness varied from 6.66 to 10.98 im. All the factors had significant effects in fiber wall thickness as it decreased with the increase in number of trees per ha and increased with tree age. The adjusted mean of clone 3 was significantly different from clone 4.

#### Tissue percentage

Neither the clone, espacement nor the age influenced the percentages of vessels, fibers and parenchyma.

#### Number of vessels/mm<sup>2</sup>

The vessel frequency varied from 1.38 to 3.28 per mm<sup>2</sup>. Vessel frequency (number of vessels) per mm<sup>2</sup> area decreased with age although espacement had no effect. The adjusted mean of clone 3 was significantly different from clone 7.

#### **Vessel diameter**

The vessel diameter varied from 107 to 163 im. As number of trees per ha increased, the vessel diameter also increased although the adjusted means of clones were not significantly different.

#### **B. Between- Species differences**

Species to species comparison revealed that wood of *Eucalyptus camaldulensis* clone was significantly denser than *E. tereticornis* and eucalyptus hybrids. Wood was also denser with longer fibers in *E. tereticornis* than in eucalyptus hybrids wood was also denser with longer fibers in *E. torelliana* than in *E. urophylla* at the age of 5 years. The longest fibers were found in *E. torelliana* and *E. urophylla* while the densest wood was in *E. camaldulensis* which is not the most preferred species for pulping.

#### C. Clonal comparison

The general comparison of the wood properties and tree size among clones revealed that there could be significant differences in certain properties such as basic density, heartwood and bark contents, fiber dimensions (length, width, wall thickness) and vessel frequency (number per mm<sup>2</sup>). As a single factor, neither the

clone, nor the age and espacement (number of trees per ha) influenced the percentages (volume) of vessels, fibers and parenchyma. This implies that the clones sampled in the study are quite uniform in wood composition in terms of tissue proportions (Plate I & II).

However, if basic density is considered as selection criterion with an average value remaining within the threshold value of 600 kg/m<sup>3</sup>, two clones *viz.*, clone 3 and 7 are the most desirable ones for pulping in view of their relatively long fibers (with a range of 0.77 to 0.97 mm from 1 to 9 years) among the clones studied (Table 1). If the rotation age of 3 years is to be fixed, clone 3 appears to the most potential one as it merits most desired features such as growth rate (wood volume), basic density and fiber length. Clone 10 (699 kg/m<sup>3</sup>) and clone 433 (676 kg/m<sup>3</sup>) appear to be the densest woods among the clones sampled. The density and structure of hardwoods in relation to paper surface characteristics and other properties are similar to the studies conducted by Higgins, *et.al.*, (1973).

Comparison of wood properties of clones 3 and 7 in three age groups indicates that clone 3 is superior in tree size, basic density, fiber length at the age of 3 years although difference at the age of 4 and 5 years were not of practical value although overall growth was greater in clone 7.

# Fiber shape factor

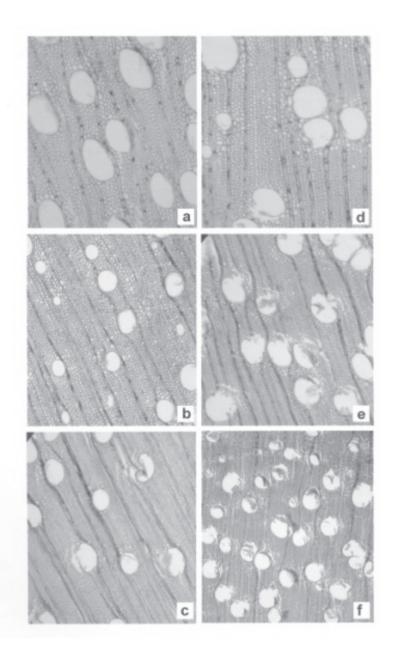
Cross sectional fiber shape  $(fd^2-ld^2/fd^2+ld^2)$  value was almost the same for all clones as clone 3 has 0.69 as against 0.67 of other three clones (4, 6 and 7). This implies that cross sectional shape is almost similar among the various clones (Plate II).

# **Runkel ratio**

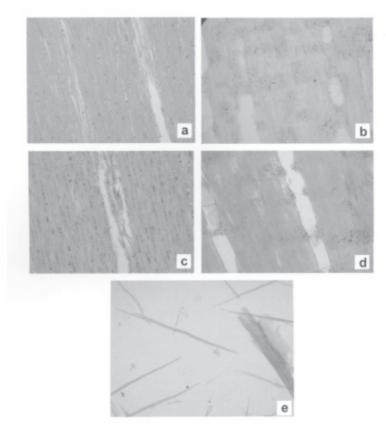
None of the clones had Runkel ratio value <1 as clone 3 and 7 displayed 1.25 as against 1.28 by clones 4 and 6. If this ratio is critical, clonal eucalyptus of young age may not meet the required fiber boding in pulping.

# D. Age effect

Among the various factors considered, age seems to be most crucial factor influencing the pulpwood quality of the clones up to 9 years. Basic density, heartwood percentage and fiber morphology (length, diameter and wall thickness) improved with age from 1 to 9 years in all clones while tissue proportions and vessel diameter did not vary significantly. Basic density and fiber length showed



**Plate I.** Transverse sections of clones showing anatomical changes with age. Clone 3: 1-year-old (a) x 80; 3-year-old (b) and 9-year-old (c) trees (x40) Clone 7: 1-year-old (d) x 80; 3-year-old (e) and 9-year-old (f) trees (x40)



#### Plate II.

Clone 3: Tangential longitudinal section (a), Radial longitudinal section (b) Clone 7: Tangential longitudinal section (c), Radial longitudinal section (d) and Macerated fibers (e)

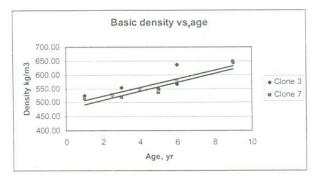
linear relationship with the age increasing consistently (Fig. 1 to 5). However, vessel frequency per mm<sup>2</sup> area decreased accompanying a small increase in vessel diameter with age during the initial years of growth.

Generally with age heart wood content increased and bark content decreased although variation was very high depending on the site conditions. Trees in Jangareddygudem sites showed a tendency of higher percentage of heartwood as seen in clone 3, 115 etc.

# Effect of rotation age

In order to ascertain the wood quality difference between 3 and 5 year rotations of most potential clones, wood properties were compared between clones 3 and 7.

Expecting a small difference in tree size, no significant difference was noticed in prime wood properties. This result implies that there is no economic advantage of extending the rotation age from 3 to 5 years in terms of wood quality improvement in these conditions.



**Figure 1.** The relationship between Basic density and age (r=0.82, 0.84 in clone 3 and 7).

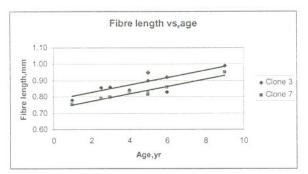


Figure 2. The relationship between fiber length and age (r=0.77, 0.96 in clone 3 and 7).

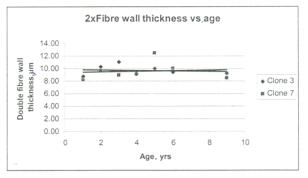


Figure 3. The relationship between fiber wall thickness and age (r =-0.15, 0.1 in clone 3 and 7).

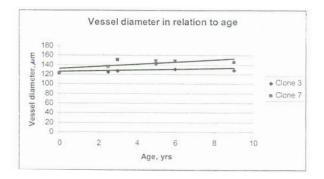


Figure 4. The relationship between vessel diameter and age.

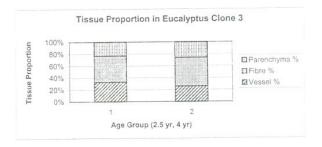


Figure 5. Tissue proportion in relation to age.

# E. Site effect

The comparison of clone 2011 and 2021 at the age of 4 years between fertile and poor sites revealed that wood property difference were not significant expecting lower moisture content and wider fiber lumen in wood from fertile site. (Moisture: Fertile = 24.4% and Poor = 20%; Lumen: Fertile = 8.87 and Poor = 6.96 im).

# Soil type and wood quality

When clones 3, 7, 115, and 413 were compared at the age of 4 to 6 years between the sites with black soil *vs.* red soil, the former promoted GBH with higher moisture and heartwood contents with wider fiber lumen and thinner fiber walls. This suggests that black soil condition is more favorable for better tree growth and thinner fibers although higher heartwood and moisture contents are not desired by the pulping industry. Similar earlier studies by Purkavastha, *et.al.*, (1979) and Singh, *et.al.*, (1986) on 5 different plantation sties of *E. tereticornis* in India indicated influence of locality factor in the variation in wood specific gravity and fiber/ vessel characteristics and their influence on surface properties of hard sheets.

Majority of clones have shown site specificity (areas with black soil and red soils) in respect of growth, adaptability and productivity. Hence, in the first phase, the site specific clones are shortlisted and then clones with best wood properties are promoted for large scale raising of plantations (Kulkarni, 2004).

# F. Irrigation/ fertilization effects

Clones 3 and 7 were sampled from 3 to 4 years old plantations to compare the wood properties from irrigated *vs.* rain fed conditions. As a response to irrigation, clones showed higher vessel percentage with larger vessels, for better conduction efficiency, and greater moisture content in the wood although the difference was significant only in clone 3 for the former and clone 7 for the latter due to small number of samples studied. This tendency of higher vessel percentage is in agreement with the index suggested by Carlquist and Hoekman (1985) with vulnerability ratio (vessel diameter/vessel frequency per mm<sup>2</sup>) value being lower toward the drought condition, as it decreased from 59 to 56 from the irrigated to rain fed plantations in clone 3. In 3 year old clone 3 from Jangareddygudem locality, the trend was clear for increased tree height, GBH, basic density, fiber lumen width, vessel percentage, vessel diameter and vessel frequency in irrigated condition. Bhandari, *et.al.*, (1988) in their studies on Kraft pulps of *E. tereticornis* bought out effect of locality on fiber/ vessel characteristics and strength/surface prosperities of paper.

# G. Coppice vs. main crop

Testing of 3 years old trees of clones 3 and 7 indicated that the differences where significant only for fiber with thinner walls with higher moisture content in coppice crops of clones 3 while those clones 7 had longer fibers and higher percentage of bark. The study needs to be continued with testing larger number of clonal samples to support the current indicative figures.

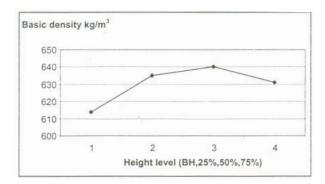
# H. Influence of tree height

Within the tree, tissue proportions did not show consistent variation from the base to the top. However, basic density, fiber length and heartwood percentage varied significantly. As shown in clone 3, basic density increased initially from the base upto 50% of the tree height before start decreasing to the top while fiber length increased only upto 25% of tree height level and then decreased towards the crown. Heartwood percentage decreased consistently from the base to top (Fig. 6 & 7).

# I. Superiority of clones over seedling control

The comparison of clone 3 with seedling crop at the age of 3 years indicates that clones have gained superiority in pulpwood quality over the seedlings in Sarapaka site. Basic density was higher by 13% and fiber length by 17% with increase in fiber diameter and wall thickness in clones 3 and 7 respectively while other parameters of fiber morphology were not significantly affected. At age of 6 years, the superiority was seen clearly in clone 3 with longer fibers and less dense wood having density value of 520 kg/m<sup>3</sup> than the seedling crop having density value of 648 kg/m<sup>3</sup>.

Based on the wood morphology, site/soil and silvicultural practices out of 98 clones studies, the below listed 31 potential clones (3, 6, 7, 10, 27, 99, 105, 122, 130, 222, 226, 265, 271, 272, 273, 274, 285, 286, 288, 316, 405, 411, 412, 413, 501, 2045, 2069, 2070, 2135, 2253 and 2254) of superior pulp and paper qualities are short listed for commercial cultivation in clonal plantation programme.



**Figure 6.** Basic density as a function of height level within the tree in 6-year-old clone 3.

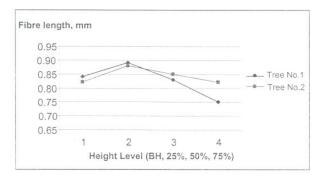


Figure 7. Fiber length Variation from base to top of two trees in 6- year-old clone 3.

#### Conclusion

Based on the results achieved the study draws the following conclusions.

1. Clones gained considerable superiority over seedling crop with optimal basic density and longer fibers. Wood quality is significantly influenced by various factors such as clone, age, site/soil type, spacing, irrigation etc. Age seems to be the most crucial factor that determines the pulp wood quality up to the age of 9 years.

2. Among the 98 clones tested clones 3, 4, 6, and 7 merit attentions in short listing the clones as most potential ones for commercial multiplication because of their relatively modest wood density, longest and widest fibers with wider lumen and thicker walls. However clone 4 has denser wood (>600kg/m<sup>3</sup>) and shorter fibers making it the least favored among the four potential clones identified.

3. Clones with denser wood showed the tendency of having shorter fibers.

4. Two clones *viz.*, clone 3 and 7 are the most desirable ones for pulping in view of their relatively long fibers and desired wood basic density around 576 to 581kg/m<sup>3</sup>.

5. Clone 7 yields more wood per tree (greater growth rate).

#### Acknowledgements

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### Preliminary first global NIRS models to predict chemical properties of *Eucalyptus* woods

Gilles Chaix

Global near infrared models to predict wood properties of Eucalyptus were developed using one hundred samples. Samples were provided from different country and location (Congo, Senegal, Brazil), including different species and hybrids (E. urophylla, E. grandis, E. camaldulensis, E. urophylla x E. grandis, E. urophylla x E. pellita) from different age (from 5 to 30 years old). The global models tested by cross-validation, based on our own reference data, shown encouraged fits for extractives (correlation coefficient R<sup>2</sup>cv = 0.98 and error of cross-validation SECV = 0.76 and ratio of performance deviation RPD = 7.2), lignin (R<sup>2</sup>cv = 0.91, SECV = 1.1, RPD = 3.3), cellulose (R<sup>2</sup>cv = 0.89, SECV = 1.2, RPD = 3.0), hemicelluloses (R<sup>2</sup>cv = 0.82, SECV = 1.6, RPD = 2.4). The high variability of chemical properties due to the sampling (for example extractive contents varied from 3 to 21%), associated to the good repeatability of reference measurements, provided high values of model parameters. These results suggest that global calibration could be useful in tree breeding processes and for different experiment trials from the fields, to rank genotypes for extractives, lignin, cellulose, and hemicelluloses. In order to get near infrared local models, we are improving our sampling in term of number and origin of wood.

ORAL SESSION 9. BIOTECHNOLOGY

### Genomic selection in eucalyptus breeding: assessing the gain per unit time in recurrent selection scheme for clone production

Marie Denis, Jean-Marc Bouvet

#### Keywords

Genomic selection, BLUP, eucalyptus breeding, dominance effect, additive effect, selection accuracy, genetic gain, simulation

#### **Background and aims**

In eucalyptus breeding, studies have shown the interest of early selection based on phenotypic traits and this approach has been applied in various commercial programs (Bouvet et al. 2004). However, juvenile selection is not very efficient under half rotation age and the experimentation costs remain high. Genomic selection (GS) has emerged as a new method for marker-assisted selection that can actually improve the performance of early selection in tree species (Grattapaglia et al. 2011). With the advent of high throughput molecular technology, numerous molecular markers distributed throughout the whole genome can be produced and used to characterize many genetic entries (Meuwissen et al. 2001).Genomic selection consists in following steps: (i) estimation of the effects of all markers in a 'training data set', where the individuals are phenotyped and genotyped; (ii) prediction of the genetic values of other 'evaluation' individuals by combining their marker genotypes with the estimates obtained in step (i).

In tree breeding the GS could significantly reduce the cost of genetic improvement schemes by limiting the size and number of field experiments; and facilitating the early selection at the nursery stage as showed the first studies (Bernardo et al. 2007). However the efficiency of this approach needs to be studied in different tree breeding contexts to fully understand the advantages of this new selection method.

The aim of this study is to investigate the performance of GS in the context of eucalyptus breeding when the selection is based on both breeding and total genotypic value. The proposed approach takes into account both additive and dominance effects. Six scenarios are simulated to assess the gain per unit time in the frame of recurrent selection scheme producing clones.

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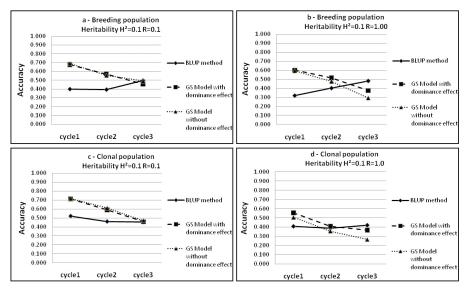
#### Methods

#### Simulation of Individual genome, genotype and phenotype

The genome of each individual was simulated using HaploSim package (Coster et al. 2009). The additive and dominance values at each QTL were simulated using a biallelic locus and the genetic quantitative model developed by Falconer and Mckay (1996). For the phenotypic value, we added to the genotypic value a random deviates from a normal distribution N (0,  $\phi_e^2$ ) where the variance  $\phi_e^2$  is defined using the broad-sense heritability H<sup>2</sup> definition. 44 QTL were simulated for each individual.

#### Simulation of breeding program

The eucalyptus breeding program was simulated using R software and HaploSim package. After 1000 generations of random crossings, a natural population with mutation-drift equilibrium and an effective size of 100 was simulated. Fifty parent trees were then pre-selected to start a recurrent selection scheme that was conducted during three cycles. At each cycle, a progeny test was implemented using a factorial mating design crossing16 females and 34 males to select the 25 best parents. They were crossed using a circular design to produce the following breeding population



**Figure 1.** Evolution of the accuracy over generations for the GS and BLUP selection methods: influence of the dominance to additive variance ratio R for breeding (a, b) and clonal population (c, d) with  $H^2=0.1$ .

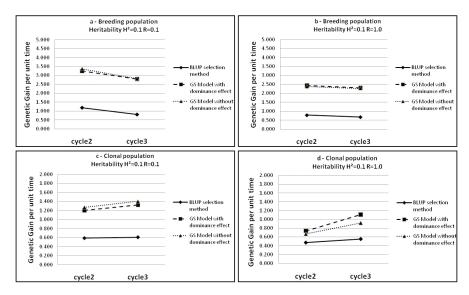
of 125 individuals and to start the second cycle. At each cycle, 10 % of the best trees of the progeny test were selected and were evaluated for clonal selection.

#### Training and candidate population

The 670 individuals of the progeny test and the 125 trees of circular design of cycle 1 were genotyped for 600 SNP markers equally-spaced across one chromosome of one Morgan and corresponding to an efficient marker density (Solberg et al. 2008). These both progeny tests constituted the training population. The candidate population of generation 2 and 3 were the progeny test for clonal selection and the circular design for parent selection.

#### Model for estimating marker effect and genetic value:

For genomic selection, the substitution effect and dominance effect at each marker was assessed using a mixed model (Toro et al. 2010). A Bayesian implementation of the Lasso method with the BLR package in R software (de Los Campos et al. 2009) was used to estimate the substitution and dominance effects for each of the 600 SNP. For phenotypic selection, breeding and total genetic values were estimated using BLUP theory and pedigree information using Asreml software (Butler et al. 2009).



**Figure 2.** Evolution of the genetic gain per unit time over generations with BLUP and GS methods : influence of the dominance to additive variance ratio R for the breeding population (a, b) and the clonal population (c, d) with  $H^2=0.1$ .

#### **Comparison of methods and scenarios**

Six scenarios resulting from the combination of three dominance to additive variance ratios (R=0.1, 0.5 and 1) and two heritabilities ( $H^2$ =0.1 and 0.6) were analysed.

Accuracy of selection methods were assessed by the correlation between GS or BLUP estimates and true breeding or total genotypic values. True genetic gains were calculated as the difference between clonal and breeding population estimated in cycle 2 or 3 and cycle 1. The gain per unit time was calculated supposing a cycle of (9, 15) years with BLUP for breeding and clonal population respectively and a cycle length of (5, 11) years with GS.

#### **Key Results**

GS accuracy increased with trait heritability and decreased with breeding cycles whereas BLUP method presented constant performances (Fig. 1 a,b,c,d). GS model including dominance effect performed better for the prediction of total genotypic value (selection of clones) and for the prediction of breeding values in breeding population when dominance effects are preponderant: dominance to additive variance ratio higher than R=0.5 (Fig. 1b, d). This trend was more pronounced with higher heritability (H<sup>2</sup>=0.6).

The genetic gains were much closed during the second and third cycles whatever the method, but the genetic gain per unit time was higher for GS for breeding and clonal population (Fig 2 a,b,c,d). The model with dominance effect led to a higher gain for clonal population (Fig. 2c, d).

#### Conclusions

Genomic selection appears very attractive in tree breeding for reducing cycle length and breeding cost. The GS model developed in the present study is particularly interesting for eucalyptus improvement dealing with hybrid populations where dominance effects are marked and clonal varieties are produced.

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# Towards elite *Eucalyptus dunnii* using conventional tree breeding applications and molecular technologies in South Africa

Andrea K Louw, Elize CL Ade and Arnulf Kanzler

#### Background

On Sappi landholdings in South Africa*Eucalyptus dunnii* is the most extensively planted temperate eucalypt species. Advantages of the species include fast growth on poor sites, good stem form, fast canopy closure and tolerance to termites. In addition, it has higher basic wood density and greater cold tolerance than *E. grandis*. This species has potential for rapid genetic gain due to early flowering and a good response to flower induction. The importance of *E. dunnii*has been elevatedin Sappi due to limitations in other commercial temperate *Eucalyptus* species.

#### Introduction

A second generation *E. dunnii* trial was established in 2004 at Piet Retief, in Mpumalanga, South Africa. The objectives of this study were to test the  $F_2$  open pollinated *E. dunnii* material in order to (1) forward select individuals in superior familiesas founders for the breeding population and for seed production purposes and (2) to identify high general combining ability individuals for seed orchard establishment.

#### Methods

Diameter-at-breast-height (dbh) was assessed at 54 months of age and wood properties (density and NIR predicted pulp yield) of designated families were completed at 69 months of age to quantify variation at the individual tree and family level. All statistical analysis was conducted using SAS® ver 9.1 (SAS Institute Inc. 2004). Genetic parameter estimates for the traits of economic importance were determined. Breeding values and corresponding gains for dbh were calculated for all families in the trial, excluding controls. Breeding values and gains for wood density and predicted pulp yield were calculated for 59 of the 121 families in the trial where wood samples were collected. Phenotypic selections were made in 89 families based on the traits of economic importance.

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A DNA fingerprinting parentage analysis study was implemented in order to enhance and complement the tree breeding activities. Xylem scrapings were collected from 40 open pollinated families (nine individuals per family) along with the 'related' maternal tree located in the  $F_1$  orchards and submitted to the Wood and Fibre Molecular Genetics Programme for analysis. The 40 families represented the top performers for the economically important traits.

#### **Results And Discussion**

The heritability for dbh at mid-rotation in the trial was 0.13. This estimate was lower than the heritabilities at mid-rotation (dbh) in two other  $F_2E$ . dunnii progeny trials planted on the same plantation, namely, 0.17 (66 months) and 0.24 (66 months); generally, growth traits range from 0.10 to 0.30 (Cotterill and Dean, 1990). The heritabilities for density and NIR predicted pulp yield were 0.16 and 0.23, respectively. Similarly, these estimates were lower than estimates in the two aforementioned *E. dunnii* progeny trials and to those reported in the literature (Raymond, 2002).

The results acquired from the DNA fingerprinting study authenticateidentity and genetic relationships of the individuals. Several misallocations and errors were quantified, and using the results further analysis was done with the corrected information.

#### Conclusion

Complementing traditionaltree breeding methodology with the application of molecular DNA technologies will benefit Sappi through increasing selection efficiency and improving genetic gains by increasing precision. Fingerprinting all genotypes in the F<sub>1</sub> orchards could be used to determine the level of out-crossing in the progeny trial, and estimate the level of external contamination.

#### Acknowledgements

This trial was designed and established by Wayne Jones and his team. Pulp yields were predicted from NIR data by Wesley Naidoo. WFMG analysed samples submitted for DNA fingerprinting. The authors also thank the Sappi staff involved in the measurement and maintenance of this trial.

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# Plant regeneration from somatic embryogenesis in *Eucalyptus* globulus: current status and future perspectives

Glória Pinto1\*, Clara Araujo2, Conceição Santos1, Lucinda Neves2

#### Background

Forest tree improvement programs will benefit from the emergence of new biotechnological strategies that join plant developmental biology, and discovery of genes associated with complex multigenic traits. Significant progress has been made during the past years in the area of plant regeneration via somatic embryogenesis (SE) for economically important tree species (e.g Populus, Pine, Eucalyptus). These advances have open up new scenarios for deployment of new high-performance clonally replicated planting stock to forest plantations and could also be a valuable tool for the development of efficient gene transfer techniques [1].

From 1999 under a protocol between University of Aveiro and Altri Florestal, a reproducible protocol for SE induction was developed in *E. globulus* from mature zygotic embryos (ZE) [2]. However, for the use of SE in *E. globulus* improvement programs, the frequency of SE initiation, maturation, germination and acclimatization needs to be improved and controlled. One of the greatest challenges today is the ability to extend this technology to elite germplasm, such that it becomes an economically feasible means for large-scale production and delivery of improved planting stock.

In this presentation we will address genetic control, as well as the influence of different environmental factors in the SE process (from media composition, antioxidants, light and PGR), from induction to plant acclimatization in both primary and secondary SE.

#### **Materials and Methods**

**Primary SE**: Half-sib and full-sibs seed of *E. globulus* produced in 2002 (and after) from Altri Florestal breeding program were used. Seeds were surface-sterilized and initiated in aseptic culture using standard protocols [2, 3]. Factors as explants age/ type and PGR effect on SE potential were tested [2] as also Medium and anti-

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browning agents [4]. Histocytological studies during induction were also implemented [5]. Embryogenic potential among families, variability in yearly production and the effect of genetic control during SE was examined using both open-pollinated and control-crossed families [3].

**Repetitive SE:** Secondary somatic embryos were obtained and ploidy studies implemented according to [6]. Factors as medium, PGRs and light were tested as [7]. Emblings were acclimatized according to [8].

#### Results

#### **Primary SE**

Somatic embryos were induced in the presence of NAA from callus derived from cotyledon explants and from mature ZE [3]. Later on, we investigated the importance of different culture media in the induction phase. MS and B5 were the best media for inducting SE and the addition of anti-browning compounds to control tissue oxidation had a negative effect on SE potential[4]. The genetic control of the SE induction process was also investigated, using 13 open pollinated families that were analyzed over 3 consecutive years in a diallele mating design with five parents. SE induction varied among families and over the years of seed production. Furthermore, it was shown that SE was under the control of additive genetic effects [3]. Finally in 2010 we emphasized the importance of reserve accumulation during SE induction with a comprehensive histocytological study [5].

#### Secondary SE

Repetitive SE in *E. globulus* was first reported in 2004 [6]. In order to evaluate the genetic stability and the true-to-type propagation via repetitive SE, eight month old somatic embryos were analyzed by flow cytometry and no major ploidy changes were detected [6].

Repetitive somatic embryogenesis was maintained in MS medium without PGRs. Reducing the levels of auxin (NAA) increased the proliferation of globular somatic embryos and allowed the maintenance of SE competence in the absence of PGRs. The addition of cytokinins (BAP and KIN) to the MS medium did not improve proliferation of globular secondary embryos, but was crucial during later stages of the SE process (germination and conversion). Depending on the SE stage, light also played an important role, influencing the quality of the process [7].

Successful acclimatization of Emblings was only reported for E. grandis, E.

*citriodora* and *E. tereticornis*. In *E. globulus*, the basic acclimatization procedure included a gradual reduction of the environmental relative humidity and the transfer to soil substrates. In 2011 the complete process from embling regeneration to acclimatization of *E. globulus* was described as well as histocytological changes that occur in leaves until successful acclimatization [8].

#### Conclusions

Somatic embryogenesis in *Eucalyptus globulus* was carried out from induction to plant acclimatization using OP and CP seeds from families with somatic embryogenesis potential. However, additional research is needed to overcome some of the current bottlenecks and devise a successful strategy to efficiently establish a SE system at the industrial level for plant propagation and as a tool for plant breeding. Testing new explants types and even adult material (floral buds related explants, shoot apices) can now take place, using trees from the identified families that have been shown to have embryogenic potential [9].

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# Plant growth regulator choice and properties dictate the rooting ability and root physiology of *Eucalyptus* spp. clones *in vitro*

M. Nakhooda1; M.P. Watt1; D. Mycock2

#### Background

Eucalyptus spp. forestry plantations are an important commercial industry in South Africa, which relies heavily on mass vegetative propagation techniques to ensure continuity, competitiveness, and maintenance of superior selected pure and hybrid lines (Watt et al 2003). These techniques include propagation through mini- and micro- cuttings, which offer a number of technical and economic advantages over propagation via stem cuttings (de Assis et al 2004). Microcuttings, for example, are known to root faster, display a better quality root system, and tend to be more uniform than stem- or mini- cuttings (Eldridge et al 1994; de Assis et al 2004), thereby reducing costs. The material for these microcuttings is sourced from micropropagated plants, which serve as mother plants in the nursery. While micropropagated plants are not directly deployed in plantations, the in vitro techniques have other specific potential applications, e.g. storage of valuable germplasm and propagation of new or transgenic eucalypt lines (Eldridge et al 1994). Further, in vitro vegetative propagation is a useful tool in directed fundamental research, by providing a highly controlled and easily manipulated growth environment. For example, an oft-encountered obstacle in the establishment of a large number of potentially economically-important eucalypt lines is the difficulty in rooting, and indeed, the ability to produce roots is paramount to the success of any vegetative propagation technique. In this regard, the in vitro environment offers an opportunity to understand the requirements of root induction and development, and subsequently translate this knowledge to nursery activities. Central to *in vitro* protocols is the empirical use of plant growth regulators to achieve the desired morphogenesis, i.e. shoot and root production. The present investigation was aimed at providing some insight into the plant growth regulator requirements of both easy- and difficult-to-root eucalypt clones, with the focus on root production and quality.

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#### Results

Based on their rooting ability as cuttings two clones were investigated, clone 1 being a good-rooter, and clone 2 a poor-rooter. Once transferred *in vitro*, the rooting response using a standard micropropagation protocol, which utilised IBA (indole-3-butyric acid) in the rooting stage, reflected this rooting potential, i.e. 87% and 45% rooting for clones 1 and 2 *in vitro*, respectively (Nakhooda *et al* 2011). This difference allowed for valuable insight, through prudent manipulation, into the phytohormone requirements of eucalypts with varying rooting abilities.

Shoot and root morphogenesis relies mainly on the regulation of the cytokinin and auxin groups of phytohormones, *in situ* and *in vitro*. A number of analogues of each of these phytohormones are currently utilised in eucalypt vegetative propagation programs. The present study showed that with the addition of the most commonly used auxin for rooting of eucalypts, IBA, a significant reduction in rooting ability of the good-rooting clone (1) followed *in vitro* (87%), compared with 100% without any IBA. This indicated that under *in vitro* conditions the auxins supplied during the pre-rooting culture stages were sufficient for root production in this clone. Reducing the auxins during multiplication and elongation for the good-rooter, by varying the combination of exogenous auxins, resulted in a corresponding decrease in the clone's rooting ability, as well as some loss in root gravity perception with decreasing IAA availability (Figure 1). These findings not only reinforced the role of auxins for root induction, but also highlighted its role in post-induction development (Nakhooda *et al* 2011).

While exogenous IBA reduced the rooting ability of clone 1, the tested poorrooting clone, on the contrary, could only root to 19% in the absence of exogenous IBA during the rooting stage. Realising the influence of the pre-rooting culture

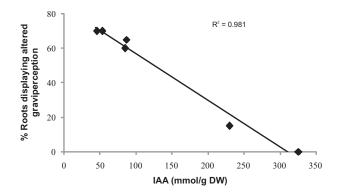


Figure 1. Relationship between shoot IAA and root graviperception.

stages on root induction, and considering the antagonistic relationship between auxins and cytokinins, an investigation into their influence on root induction in the poor-rooter was undertaken.

Since auxin is responsible for root induction, the poor-rooting clone was tested for auxin signal transduction and auxin conjugation, using ñchlorophenoxyisobutyric acid (PCIB) (Oono et al 2003) or dihydroxyacetophenone (DHAP). This revealed that clone 2 was not deficient in auxin signal perception, nor was auxin conjugation responsible for poor rooting. However, reducing the cytokinin content (kinetin) in the elongation stage in vitro, resulted in a significant increase in that clone's rootability, to over 80%. Using gas chromatography-mass spectrometry (GC-MS), a strong relationship between the shoot cytokinin:auxin and shoot rootability was realised ( $R^2 = 0.943$ ), suggesting that the cytokinins used for shoot production *in vitro* may persist and subsequently inhibit root production. Replacing kinetin with the less stable *trans*-zeatin, significantly improved clone 2 rootability, compared with kinetin treatment (from 19% to 46%). These findings were consistent when another established poor-rooter was tested, indicating that the pre-rooting treatment regimes could be the determining factor in a eucalypt clone's rootability.

#### Conclusion

The complex interplay between auxins and cytokinins, and the varied requirements for these key phytohormones between good- and poor- rooting eucalypts *in vitro*, may provide a suitable model for understanding mini- and microcutting requirements in the field. One such study in our laboratory has already shown that supplying IBA to the base of cuttings, as is the usual nursery practice, to a relatively good-rooting clone, retards root production compared with IBA-free cuttings, as shown *in vitro*. Current investigations are aimed at understanding the phytohormone requirements of poor-rooting *Eucalyptus* spp. mini-cuttings, using the results obtained *in vitro* as a model, with the aim of increasing the rooting potential of economically important, but difficult-to-root eucalypts.

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# Treeforjoules, a Plant KBBE project to improve eucalypt and poplar wood properties for bioenergy

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Recently, interest in sustainably produced bio-energy and bio-based products has skyrocketed due to efforts to reduce reliance on nonrenewable fossil fuels, decrease environmental degradation, mitigate climate change, and develop robust knowledge-based bio-economies. Concomittantly, there has been an increased interest in the utilization of lignocellulosic biomass from forest plantations for second-generation renewable bio-energy feedstocks as they are non-food crops and offer the potential for generating a lower carbon footprint than annually produced crops. Fast-growing tree species such as poplar and eucalypts grown as short-rotation coppice (SRC) represent one of the most appealing sources of renewable biomass feedstock for Northern/Western and Southern Europe as they are easy to establish, produce high yields of lignocellulosic biomass, and offer secondary benefits such low nutrient input. Since the chemical and structural composition of lignified secondary cell walls render woody feedstocks particularly recalcitrant to degradation, improved genetic material is needed to use these SRC as energy crops in an efficient manner. The first step to accomplishing this is to identify genes regulating relevant cell wall properties before moving on to identify the specific desirable allelic variants for breeding.

This is the overall goal of TREEFORJOULES an European Plant KBBE (Knowledge-Based Bio-Economy) project started in April 2011 and gathering 13 research groups from public and private organisations from France, Germany, Portugal, and Spain. The objective is to identify the major factors underpinning the physicochemical properties of cell walls, the recalcitrance of which remains a key scientific challenge

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for establishing highly efficient, sustainably produced, secondgeneration biofuels. This knowledge will be invaluable for breeding fast-growing elite trees such as poplar and eucalypts for improved down-stream processing and efficient degradation.

TreeforJoules specific aims are to:

• Identify and characterize the regulatory candidate genes (i.e. transcription factors and miRNAs) that control wood properties relevant to bioenergy through integration of existing and new transcriptomic resources, delineation of the transcriptional interactome, functional characterization of candidate genes (CGs) in transgenic wood sectors, assessment of environmental and seasonal impacts on CGs expression and correlation with biomass production of high-performing genotypes

• Develop high-throughput NIR spectroscopic methods for wood property measurements including all key cell wall constituants with impact on the saccharification potential of biomass polysaccharides for bio-ethanol production and bio-oil production from lignin.

• Delineate and characterise genomic regions in eucalypts and poplar that control wood properties valuable for efficient cellulosic bioenergy production through comparative analyses at both the structural (comparative genetic and physical mapping) and functional (comparative QTL mapping) levels.

### Cold-tolerance and initial growth of 38 Eucalyptus species in the Southeastern of the United States

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Eucalyptus species have very rapid growth in many parts of the world. Recent opportunities in using tree biomass as a replacement for fossil fuels have generated interest in growing Eucalyptus in the SE of United States. Previous attempts to introduce these species in the SEUS in the 1980s failed largely due to cold temperatures that occurred in the 1983 and 1985 winters. However, work has been done in the interim and there are extant Eucalyptus stands in the SEUS up to 10 years old. Also, additional varieties that may be more tolerant to cold are now available from sources around the world. This new genetic material as well as access to expertise in growing the species stimulated interest in this work, which has the following objectives: i. To identify cold-hardy Eucalyptus species that can survive and thrive in the SEUS; ii.To quantify the productivity of these species under different silvicultural treatments; and iii.To provide information for tree breeding strategies and hybridization. The project is being conducted by the Forest Productivity Cooperative (www.forestproductivitycoop.org).

Eucalyptus species from commercially available sources were planted in 2010 and 2011 in eleven sites across the southeastern US to screen for cold tolerance. Species were grouped as low, medium or high in likelihood for survival with 16 (4 x 4 grid), 32 (4 x 8 grid), and 64 (4 x 16 grid) trees planted per plot. Design replicates are the sites.Five sites were planted in 2010 and six in 2011, totalizing 38 species in 2010 (entries from five countries) and 63 species in 2011. The sites are located in Florida, Alabama, South Carolina, Georgia, Louisiana and North Carolina. The sites were planted between May and September of each year. Hourly temperature at each site was recorded from November 2010 to March 2011 with a HOBO thermometer.

The 2010 winter was colder than historical average and allowed an adequate screening of the 38 tested species planted in this year. Assessments of height and

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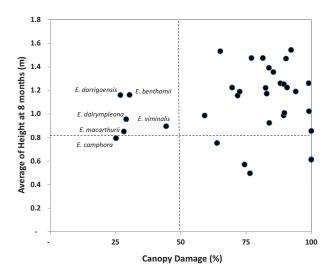


Figure 1. Canopy damage and height of 38 Eucalyptus species in April of 2011 in the SE US.

survival for the 2010 trials were completed in April of 2011 after the frost free date. Survival and growth were very good at all sites prior to the winter due to careful site preparation, fertilization and weed control. A Canopy Damage Assessment was developed for visual evaluation of the frost damage. All 2010 sites experienced subfreezing temperatures, and, as expected, the coldest site were in NC and the one with the mildest temperatures was in South of Florida.

Across sites the number of species that survived the winter decreased with the increasing events of subfreezing temperatures. So, sites in South Florida have a broad spectrum of Eucalyptus species to work with when compared with more temperate sites, like NC.

Overall, for all sites installed in 2010, five Eucalyptus species came up as potential genetic materials for cold-hardy tolerance and fast-grow (Fig. 6). *E. benthamii, E. dorrigoensis, E. viminalis, E. dalrympleana* and *E. macarthurii.* 

The development of these species during thesecond winter, in 2011, together with the new species/entries being tested, will allow a better evaluation and understanding regarding the potential of cold-hardy Eucalyptus species for the SEUS.

POSTER SESSION 1. FOREST ESTABLISHMENT AND REGENERATION

## Tree and stand growth for clonal *E. urophylla* x *grandis* over a 16 years following a range of fertilizer treatments in southern China

Chen, Shaoxiong<sup>1</sup>, Liu, Jiefeng<sup>2</sup>

A large spacing x fertilizer factorial field trial was established in 1993 with a hybrid clone of *Eucalyptus urophylla* x *grandis* in southern Guangxi province, China. This included six fertilizer treatments, involving varying rates of N, P and K ranging from  $N_{100}P_{50}K_{50}$  up to  $N_{300}P_{200}K_{200}$  (the subscript numerals denote the quantity of the element applied in kg ha<sup>-1</sup>) applied as pursuant fertilizer in a split dose at ages 2 and 38 months, and six spacing treatments ranging from 667 to 2222 trees ha<sup>-1</sup>. This report examines the results for the main effect of fertilizer treatments. Growth was assessed at various intervals up to age 192 months. Outer-wood wood properties were assessed at 192 months – pilodyn penetration measuring wood density and Fakopp measuring acoustic velocity as an indicator of modulus of elasticity. There were significant differences between fertilizer treatments for standing volume ha<sup>-1</sup> at all ages up to 144 months.

At each age of assessment, the highest volume yields were in the highest fertiliser treatment and the lowest fertiliser treatment had the lowest yields. The maximum differential between the highest and lowest fertiliser treatments was at age 99 months (8.3 yr) with a differential of 15% between the two (equating to 23.6 m<sup>3</sup> ha<sup>-1</sup> difference). Periodic annual increments peaked in all fertiliser treatments at age 42 months with a maximum of 43.6 m<sup>3</sup>ha<sup>-1</sup>yr<sup>-1</sup> for the highest fertiliser treatment. Mean annual increments peaked in all fertiliser treatments with a maximum of 31.1 m<sup>3</sup> ha<sup>-1</sup>yr<sup>-1</sup> for the highest fertiliser treatment.

It was not possible to partition the fertiliser effects between N, P and/or K – due to the treatment design the rates of each individual element were confounded with rates of the other 2 elements. Even so, partial regression coefficients obtained through multiple regression analyses showed that the level P had the strongest correlation with volume, indicating that P is the most important nutrient to be added in fertiliser.

Fertiliser level did not affect the outer-wood properties assessed at age 192 months using Pilodyn and Fakopp. Also, there were no significant interactions between fertiliser and spacing for any traits assessed at any age, indicating the effect of each of these two silvicultural variables is 'additive'.

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# Growth patterns in widely spaced clonal *Eucalyptus urophylla* × *grandis* plantations on different site classes up to age 16 years

Shaoxiong, Chen

The research focus on the growth of spacing 666 trees·ha<sup>-1</sup> on three site index (SI) of 22, 24 and 26, the results showed that: (1) The time to the target of middle & large diameter timber of SI 22, 24, 26 were 10,8 and 7 years respectively, and MAI were 21.6, 27.2 and  $31.1m^{3}\cdot ha^{-1}$  respectively at that time. (2) DBH growth and distribution: 1) the DBH growth decreased as time goes on, it was over 4 cm ·yr<sup>-1</sup>at first two years, 2 cm at 3<sup>rd</sup> year, and over 1 cm at 4-6<sup>th</sup> year; it were over 0.4cm till the final measurement at 16<sup>th</sup> year; 2) the DBH distribution only covered 2-3 diameter grades in the first two years; 7 diameter grades at 6th year; 10-11 diameter grades at 16th year; 3) Mean H growth decreased as time go on, it was over 3 m ·yr<sup>-1</sup> at the first three years, over 2 m ·yr<sup>-1</sup> at 4-5<sup>th</sup> year, over 1 m ·yr<sup>-1</sup> at 5-10<sup>th</sup> year, it were over 0.6m till the final measurement at 16th year; 4) CAI peak appeared at the 4th year, the values were 43.3, 52.23 and 54.69 m<sup>3</sup>·hm<sup>-2</sup> at 5<sup>th</sup> year and 7 m<sup>3</sup>·hm<sup>-2</sup> at 6<sup>th</sup> year; The cross points of CAI and MAI curves were appeared at the 7<sup>th</sup> year for all SI.

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### Growth and aboveground biomass of an *Eucalyptus regnans* stand, managed for high value timber production, located in the coast of Arauco, Biobío, Chile

Magdalena Lisboa<sup>1</sup>, Jorge Cancino<sup>1</sup>, Ruth Maturana, Fernando Muñoz<sup>1</sup>

#### Introduction

Global interest in genus Eucalyptus plantations for production of high value timber has been increasing due to the depletion of timber from natural forests, a growing interest of people in environmental issues that has led to the increased the protection of these forests and the possibility that wood from Eucalyptus plantations replace tropical hardwoods (Flynn 2003, Montagu et al. 2003; Smith et al. 2006). Moreover, it is expected that the future of the Eucalyptus wood is decorative applications such as furniture, flooring and paneling (Wardlaw et al. 2003). Several species of Eucalyptus have been established in various countries for this purpose.

Chilean lumber production is based almost exclusively on *Pinus radiata* D. Don, a smaller percentage in native species and a very small percentage in species of Eucalyptus genus, mainly *Eucalyptus globulus* Labill and *E. nitens* H. Deane et Maiden (INFOR 2011).

*E. regnans*, a species from the states of Victoria and Tasmania, southeastern Australia, showed potential to be cultivated for this purpose in Chile, It is commonly known as "Mountain Ash", and belongs to the group of "Ashes" with *E. delegatensis* and *E. fastigata*. Florence (2004) indicates that the species of the "Ash" group are one of the most productive forests of Eucalyptus in Australia.

Although the volume growth and accumulated biomass are the most important variables when deciding which species of trees to plant, as they are critical to the profitability of the forestry business and since they are excellent parameters to express the productivity of a forest ecosystem, growth and biomass studies on alternative species to currently used in commercial silviculture in Chile are limited.

*E. regnans* has enormous potential for timber production. This raises interest to study a stand of *E. regnans* located in the province of Arauco, Biobío Region, aiming to provide information on diameter, height and volume growth and aboveground biomass. The study also compares, broadly, the growth of *E. regnans* with the growth of *E. nitens* and *E. globulus*, the latter being the most widely used species of Eucalyptus in Chilean pulp industry.

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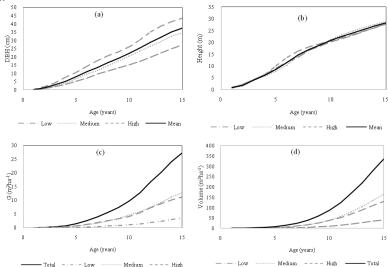
#### Methodology

A growth and aboveground biomass study was performed in a 15 years old *Eucalyptus regnans* stand, managed for producing high value timber, located on the coast of the province of Arauco with an approximate density of 300 trees per hectare. The stand was differentiated into three diameter classes: low medium and high. To determine growth a stem analysis was performed to 9 trees, three of each class, from which were obtained annual increments in diameter, height, basal area and volume. Also for each diameter class biomass values were obtained for each tree component (leaves, branches, twigs, bark and wood). Later biomass functions were fitted.

#### Results

At 15 years the trees had an average height near to 30 m and a mean diameter at breast height (DBH) exceeding 37 cm. The cumulative size in volume per hectare is 350 m<sup>3</sup> at 15 years (Figure 1), with an approximately mean annual increment (MAI) over 20 m<sup>3</sup>ha<sup>-1</sup>year<sup>-1</sup> and a current annual increment (CAI) of 55 m<sup>3</sup>ha<sup>-1</sup>year<sup>-1</sup>. The basal area increment culminates at 24 years exceeding 2.4 m<sup>2</sup>ha<sup>-1</sup>año<sup>-1</sup> and volume increment at 20 years with 28 m<sup>3</sup>ha<sup>-1</sup>year<sup>-1</sup>, showing an estimated maximum CAI over 60 m<sup>3</sup>ha<sup>-1</sup>year<sup>-1</sup> at age 14.

In the study area *E. regnans* growth is similar to *E. nitens* but higher than *E. globulus*, two species of great interest in the Chilean forestry due to its high growth rates.



**Figure 1.** Acumulated growth in (a) Diameter at breast hight (DBH); (b) Height; (c) Basal area (G); and (d) Volume.

Diameter Class											
Component	Low			Medium			High			Total per Hectare	
	Biomass (t/ha)	SD	(%1)	Biomass (t/ha)	ST	(%1)	Biomass (t/ha)	SD	(%1)	Biomass (t/ha)	(%2)
Stem Wood	15,528	2,87	88,7	81,486	4,74	88,0	81,034	7,29	89,5	178,048	88,7
Stem Bark	1,139	0,18	6,5	6,169	0,80	6,7	6,816	1,00	7,5	14,124	7,0
Stem Total	16,667	3,02	95,2	87,654	5,35	94,6	87,850	7,03	97,0	192,171	95,7
Branches	0,498	0,38	2,8	2,334	0,27	2,5	1,284	0,12	1,4	4,116	2,1
Twigs	0,134	0,07	0,8	1,219	0,28	1,3	0,741	0,14	0,8	2,094	1,0
Leaves	0,209	0,11	1,2	1,428	0,30	1,5	0,710	0,16	0,8	2,347	1,2
Crown Total	0,840	0,50	4,8	4,981	0,55	5,4	2,735	0,29	3,0	8,557	4,3
Tree Total*	17,508	-	100,0	92,636	-	100,0	90,585	-	100,0	200,728	

Table 1.	Biomass of ea	ch component pei	r hectare (t/ha) and	d percentage of each			
component by size class in relation to the total per hectare.							

SD: Standard Deviation.

%1: Percentage of each component per size class in relation to the total per hectare

%2: Percentage of each component per size class in relation to total per tree.

\* Total dry mass of the tree over the ground

Wood is the component that most contributes to total biomass (88.7 % of total), followed by the bark (7%) and then branches (2.1%) (Table 1). DBH was de variable that showed the greater correlation with most of the tree biomass components.

#### Conclusions

*E. regnans* has a greater growth in the studied site than in its origin country. In the study area, its growth is similar to *E. nitens* and greater than *E. globulus*.

Trees of the largest diameter class are those that provide more biomass being 1.7 and 2.6 times the contribution of trees belonging to the middle and lower diameter classes, respectively. The combination of DBH and the live crown length is the best predictor of crown biomass.

The high growth and high volume and aboveground biomass achieved in a short period of time demonstrate the enormous potential of this species in the study area.

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### Nutrient Export and Cycling at Harvest of Eucalyptus dunnii Maiden

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Recently, there has been an increase in the areas of Uruguay dedicated to forest plantations for pulp production, with Eucalyptus dunnii Maiden being one of the more common species used. It is known that Eucaliptus sp. require a high nutrient extraction. The actual amounts extracted depends on soil type, species and plantation age (Morais et al., 1990; Herbert, 1996; Goya et al., 1997; Santana et al., 2000; Laclau et al., 2000). The export and cycling of the nutrients also depend on the harvest and residue management method (Spangenberg et al., 1996; Gonçalves et al., 1997). In addition, decomposition rates are affected by climatic conditions and characteristics of the residues; in general, the woody materials decompose more slowly, (Rezende, 2001; Burgess et al., 2002). Since large scale forestry has recently been established in Uruguay, information about harvest residue decomposition for Uruguayan soils and climatic conditions is rather limited. The knowledge of the process will contribute to improve the residue management practices as well as plan the fertilizer applications for the next turn.

The objectives of this study were: a) to quantify nutrient export of Eucalyptus dunnii Maiden and b) to characterize decomposition of harvest residues and the potential recycling of nutrients to the soil. The study site was located in Algorta (Uruguay, longitude and latitude coordinates: 57°17′40″ W y 32°25′56″ S, respectively). The climate is classified as temperate with a mean annual temperature of 18°C, mean temperatures of 11.7°C in the coldest month (June) and 24.8°C in the warmest month (January), and mean annual rainfall of 1,218 mm. The soil was a fine, mixed, thermic Albic Argiudoll (Soil Survey Staff, 1999), with a 18 cm depth A horizon, and total exchangeable bases of 8.98 cmolc kg<sup>-1</sup>.

During harvest of a 9-year-old E. dunnii plantation (MAI 24.2 m<sup>3</sup> ha<sup>-1</sup> y<sup>-1</sup>), 24 representative trees were selected. The aerial biomass was calculated from the individual weight of logs, leaves, bark, and branches. From each fraction, samples were taken to analyze for N, P, K, Ca and Mg content.

For the decomposition study, samples of the harvest residues were utilized. One

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hundred grams of leaves, branches and bark were put into individual 30 cm x 30 cm mesh bags and placed over the soil.

This process, with one bag per sampling date, was replicated in three different areas of the harvested plot. Samples (one bag per area) were taken after 1, 2, 4, 6, 9, 12, 18 and 24 months. In the successive sampling dates, the remaining aerial biomass was estimated from the decomposition rate calculated during the period. The rate of decomposition was calculated using an exponential decay model:  $P/P_{inic}$  =  $e^{-kt}$ , in which  $P/P_{inic}$  is the proportion of residue material remaining at time t, measured in years, and k the decomposition constant. This model was also used to calculate the mean life (t1/2), in years, of the different components.

From the total aerial biomass (236 Mg ha<sup>-1</sup>, dry basis), 73 % corresponded to de-barked commercial logs. However, nutrient export, although variable, did not exceed 45% of the total biomass (range: 18-45%). When the logs were extracted with the bark, nutrient export increased to between 41and 81% of the total harvested nutrients. The bark contained the majority of Ca and Mg, while N and P were mainly in leaves. The values for K were similar in the different residues. The largest concentration variability corresponded to Ca, which varied from 1.7 g kg<sup>-1</sup> in logs to 27.3 g kg<sup>-1</sup> in bark. Because of the high nutrient content in bark, particularly for Ca and Mg, de-barking outside the harvest site increased Ca and Mg export from 18 and 31%, respectively, to 81 and 66% respectively.

After two years of decomposition in the field, the residue loss was, on average, only 38% of the original biomass, with a higher decomposition rate in the second year compared to the first. The leaves were easily decomposable (83% loss), due to their smaller size and higher soluble C content.

In contrast, the coarse residues (bark and branches) with high C/N ratios showed slower decomposition rates. The exponential decrease model adjusted for biomass decomposition for each component showed that the longest half life corresponded to bark (5.27 years), while leaves had the shortest half life (0.92 years).

Nutrient release from the harvest residues varied depending on decomposition patterns and the nutrient characteristics. After 2 years of decomposition, the amount of nutrients lost was 176, 18, 375, 460 and 92 kg ha-1 of N, P, K, Ca and Mg, respectively. This represented 42, 62, 83, 27 and 41% of the initial N, P, K, Ca and Mg, respectively.

From these results, it can be concluded that the potential use of the released nutrients for the subsequent plantation will depend on harvest method (with or without de-barking) as well as the decomposition rates. Differences in nutrient release represent different nutrient availabilities for the next turn. While K seems to be readily available, the other nutrients would be released at a slower rate. In this context, the residue management practices that accelerate the decomposition process, such as residue fragmentation and improvement of soil-residue contact will contribute to accelerated decomposition and nutrient release. It is important to remark that the released nutrients are not always available for the next turn. For example, N availability could be decreased by immobilization or by gaseous and leaching losses, while P availability depends on complex chemical and biological processes. In contrast, the cations (K, Ca and Mg) are more likely to be available when released from the residues. Slow decomposition is not necessarily negative from the standpoint of recycling because the nutrient release would progress with the tree growth and nutrient needs.

Keywords: Eucalyptus dunnii, Nutrient export, Nutrient cycling

### Analysis of the economical viability of *Eucalyptus* spp. coppice compared to replanting using genetically improved seedlings on different productivities sites in Minas Gerais State, Brazil

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Keywords: Coppice, Replanting, Genetic gain, *Eucalyptus* spp., Net Present Value

#### Background

A long standing forest conundrum is the doubt of when is it economically viable to replant a harvested stand using genetically superior material and when is it better to withhold the costs associated with planting and conduct a coppiced stand.

This work presents the results of a cash flow model used to compare coppice and genetically improved replanting options in different productivity sites for *Eucalyptus* spp. pulpwood and energy plantations in Minas Gerais State, Brazil.

#### Methods

The unimproved *Eucalyptus* spp. production values were obtained from Scolforo et al. (2008). This data represents mean volume growth of first rotation clonal *Eucalyptus* plantations located in the central region of Minas Gerais State. The data is divided into four productive classes represented by the mean dominant height at age 7 years: 19, 23, 27 and 31m.

The first step taken was to determine the rotation age in which the maximum aboveground wood volume would be attained for the different sites, thus determining the silvicultural rotation for the first cycle. A rotation age of 5 years was detected as possessing the maximum mean annual increment (MAI) in volume for the four site indexes used. The volume values obtained at age 5 years were: 119.7, 161.3, 203.1 and 229.2 m<sup>3</sup>/ha arranged from the lowest to the highest site indexes. These productivities values result in a MAI in volume ranging from 24.0 to 45.8 at age 5 years.

The costs assumed in the conduction of the plantation, replanting and coppice are from Rezende et al. (2005), reporting average 2005 values for the cerrado

region of Minas Gerais State. The costs in US dollars per hectare for plantation and replanting were: 600.00 for establishment, 151.65 in the first year, 58.46 in the second and 21.97 for the subsequent years. The coppice costs were: 260.08 in the first year, 63.24 in the second and 15.75 for the subsequent years.

Incremental net present value (NPV) was used to compare the two mutually exclusive options (Whittock et al. 2004), a plantation followed by replanting or coppice, assuming a planning horizon of 10 years (e.g. a cycle of two rotations). The incremental NPV is obtained subtracting the coppice 10 year NPV from the replanting NPV, where a positive incremental NPV indicates that the coppice option is more profitable whilst a negative incremental NPV indicates that the replanting option is more profitable. A genetic gain of 0 to 50% of the unimproved first rotation was used. The coppice volume production ranged from 70 to 130% of first rotation values.

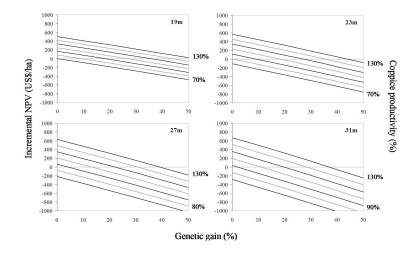
The following conditions were assumed in the simulation: discount rate of 10% p.a.; basic density of 446.8 t/m<sup>3</sup>; 5% reduction of the basic density of the coppice option in view of accelerated growth; 10% increase in the harvest costs of coppice do to the larger number of stems; the genetic improvement was divided as 90% in volume and 10% in higher basic density; a harvest cost of US\$ 3.00/m<sup>3</sup>; sale price of US\$ 50.36 per dry weight ton. No land or transportation costs were considered.

#### **Results and Discussion**

Figure 1 presents the results of the economical simulation to determine at what levels of coppice productivity and genetic gain is replanting of the stand the most viable option.

The results indicate that for more productive sites smaller genetic gains are needed to enable the replanting of the stand. Considering a coppice production value of 100% of the first rotation, the genetic gain needed to render the incremental NPV null (thus equaling the coppice and replanting options) are: 26.7, 18.2, 13.1 and 10.8% for site indexes 19 to 31m respectively.

These results indicate that for less productive sites the application of more intensive silviculture (e.g. replanting) is not justified. For example, at site 19m the replanting or coppice options at a coppice volume production of 70% of the first rotation yield the same economic benefits when no genetic gain is considered. For sites 23, 27 and 31m the values of coppice production that equal the economical benefits when no genetic gain is considered are 80, 85 and 88% respectively. Thus, when the coppice productivity levels are bellow the stated values the replanting of the stand should be conducted even if no genetically improved stock is available.



**Figure 1.** Incremental NPV considering different levels of genetic gain of the replanting material, coppice productivity and site indexes (expressed in meters), where a positive NPV indicates that the coppice option is more profitable and a negative NVP indicates the replanting of the stand.

The larger amounts of genetic gain needed to enable the replanting in less productive sites is consistent with the reality of Minas Gerais *Eucalyptus* plantations. Scolforo et al. (2008) presented the productivity of first rotation clonal and seed origin *Eucalyptus* stands in northern Minas Gerais for sites 19 and 23m. At age five years the clonal stands presented a volume production 38% higher than the seed origin stands for site 19m. For site 23m the difference was 11%, indicating that greater gains in poorer sites is achievable by planting genetically improved material.

#### Conclusions

For the costs and volume production data used, higher productivity sites are more sensitive to genetic gain values, where a smaller genetic gain is needed to make stand replanting viable when compared to less productive sites.

The application of more intensive silvicultural regimes (such as replanting) is more suitable in higher productivities sites, while in less productive sites the option of managing the coppiced stand is appropriate, except in cases of large genetic gains.

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# Effect of container type on root architecture of *Eucalyptus* globulus Cuttings

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The use of containers in plant production, has several advantages, both at the biological and economic level that range from root system protection, better nutrition control and easy handling both in the nursery and in the field. However, containers do restrict root growth which can have a negative impact on the growth of several species..Root deformation caused by the type of container used, can extend to the nursery growth phase and have a negative effect under field growth conditions, demonstrating the importance of developing appropriate growth conditions for forest plant production that avoid root deformation contributes to deficient water and nutrient absorption which can cause a disequilibrium between the root and aerial part, which become more important in marginal plantation areas, took Altri Florestal in collaboration with Serida, to develop the present project where different type of plastic containers were used for the production of *E. globulus* cuttings

The main goals of the present study was to evaluate the effect of different types of containers on the morphology and topology of the root system of Eucalyptus globulus cuttings, as well as the effect of air prunning in the growth and overall plant quality parameters. In this study, the development of the root system of two Eucalyptus globulus clones in five different plastic containers was followed during the nursery production phase. The substract mixture was composed of peat (25%) and vermiculite (75%). Rooted cuttings were evaluated at 12° days and the following parameters were recorded: root colar diameter, shoot heigh, root deformation level and plant biomass (root biomass, shoot biomass, leaf biomass and total biomass) and also the morphology and topology of the root system.

The different types of containers strongly influenced the morphology and topology of these two clones root system, showing that container design is na área that can provide significant improvements in plant production towards better

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quality plants. Air penetration that varies in all five containers analyzed, strongly influenced all parameters recorded.

The results reported here, on the use of plant containers having different air penetration areas for the production of *E. globulus* Labill. cuttings, show that the containers having larger areas of air penetration have a more uniform root system, less root deformation problems and better quality plants. Data on survival and growth of these plants in the field at five years will also be discussed.

# Genetic Diversity among different provenances of *Eucalyptus* spp with phenotypical markers base in greenhouses

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#### Introduction

Originally from Australia, genera *Eucalyptus*, is a forest representative of a country with high diversity on climate and soil. Thanks to an environmental variation and geographical isolation, a significant genetic variation took place, ending in a variety of species separation (Mayr, 1977). Nevertheless, it was necessary to develop adaptations strategies for different Australia's ecological niches, producing what it is known as subpopulations of the same species, also called provenances. That is the reason why within the same species of Eucalyptus, it is possible to find provenances with certain differences on diverse characteristics of economical importance as, increase of growth, and low weather and drought resistance (Eldridge et al., 1993).

Following Bueno (2001), the genetic diversity of species it is an important way to maintain the natural capacity to respond to climatic changes and any other types of biotic and no biotic stresses. Then, the introduction of Eucalyptus in a new region, the evaluation of species and provenances using morfological and phisiological markers is fundamental to initiate a genetic improvement program, allowing the establishing of strategies for similar program on this tree species looking for commercial plantations.

Actually, the commercial production of Eucalyptus' seedlings on most forestry enterprises, is made, almost totally, using vegetative propagation, or improved seeds allowing a better productivity and forestry quality, presenting simultaneously certain advantages on populations, better adaptation to local conditions ad increase of productivity.

Within the production system, the development and seedlings quality are influenced by different factors as, for example, genetic material, hydro and nutritional management, containers and different substrates.

The main objective of this experiment was to characterize three provenances of

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*Eucalyptus* spp using morphological and physiological markers and to evaluate the quality of the seedlings.

#### **Material and Methods**

The experiment was conducted in a tree nursery of the Federal University of Reconcavo da Bahia, at Cruz das Almas, State of Bahia, Brazil. It was conducted using the Totally Randomized Design and three repetitions.

Provenances' seeds was supplied by the Forestry Institute of Sao Paulo, coming originally from different regions of São Paulo and Maranhão States: *Eucalyptus pellita* (from Anhembi-SP); *Eucalyptus camaldulensis* (from Açailândia-MA), and *Eucalyptus citriodora* (from Restinga-SP).

Seedlings was produced in 55cc tubes and the substrate was the commercially fertilized known as Osmocote (15-9-12) using 1,5 kg m<sup>-3</sup> of substrate as dosage.

The planting process was manual and the thinning occurred after germination and the emersion of seedlings up to a pair of leaves, allowing the seedling to become vigorous.

Those seedlings were evaluated in relation with the collar diameter (D) and the aerial height (H) with 15, 30, 45, 75 and 90 days after the sowing. The dry mass of the aerial part (MSPA) was evaluated with 90 days, taking four seedlings for plot of each treatment and maintained in the stove at 70 °C for 48 hours for quantification.

The data was submitted to analysis of variance and the treatments' averages were compared by Tukey Tests at 5%.

#### Results

In relation with morphological and physiological parameters evaluation, some results are presented in Table 1 considering plants height, collar diameter and dried mass of the aerial part, from provenances of *E.camaldulensis, E. pellita* and *E. citriodora*, all of them with 75 days of age. In all treatments it was verified one adequated development in relation with height and collar diameter of the seedlings. In the case of *E. camaldulensis* (Açailândia-MA provenance) and *E. citriodora* (Restinga-SP provenance), they showed the greatest values in height with 75 days. All three species do not showed any differences in collar diameter and dry mass with the same age. It is possible to prove that the origin of this material come from improved seeds.

In the case of height, Gomes and Silva (2004), registered that depending of material origin, this parameter may show less results that expected because of the

Specie	Height (cm)	Diameter (cm)	MSPA (%)
E. camaldulensis	19,0 A	2,1 A	0,38 A
E. pellita	17,3 A	2,2 A	0,46 A
E. citriodora	13,2 B	2,0 A	0,42 A
CV (%)	9,22	8,87	19,38 A

Table 1. Height, collar diameter and dry mass characteristics with 75 days of age.

Averages followed by the same characters do not differ from Tukey Test at 5% probability.

natural difficulty of a non pure material to show the appropriated characteristics within an ideal period of development.

In relation with collar diameter, Sturion et al. (2000) and Gomes et al. (1996) set up values of 2 and 3.5 mm, for seedlings considered able for planting on the field.

# Conclusions

All three provenances used on this experiment showed appropriated development during the seedling production phase, giving the clue that those different provenances could be used in the Cruz das Almas region, to create forested areas of Eucalyptus.

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# Background on the growth and yield of *Eucalyptus globulus* coppice in chile, an opportunity for efficient forest management

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#### Summary

Traditionally in Chile *Eucalyptus globulus* plantations have been established from seedlings produced from improved seeds with elevated establishment costs within a rotation age from 12 to 15 years. On the other hand, small and mediumsize forest companies and forest landowners that have *Eucalyptus globulus* plantations require technological alternatives to increase profitability and to access to markets efficiently.

To reduce establishment costs and rotation age, *Eucalyptus globulus* growers can overcome this problem, by managing *E. globulus* coppice shoots. By using this strategy, some authors have reported shortening the rotation age from 8 to 12 years and at the same tine reducing the initial cost of establishment (selection and cut of coppices), increasing plantation profitability. Growing a plantation from shoots reports similar growth rates and yield to the original plantation for 3 to 4 rotations before replacing the original plant. This management strategy could be an attractive alternative to produce pulpwood in a short-rotation scheme, which is easy to trade and with clear market. This option is outlined with great interest, but needs to be validated for different site conditions in Chile.

Presents the main developments from a study of 8 years developed by the Forestry Institute of Chile, in relation to the growth of *E. globulus* coppice in different situations in Chile.

The study aims to generate models of forest management for economic use of Eucalyptus globulus coppice and make proposals for innovative forest management.

The general methodology used model analysis applicable management coppice stands of *Eucalyptus globules* associated with the measurement and maintenance trials in much of the country and the constant introduction of new tests. In addition, an analysis of information and development of models to estimate the growth and yield for Eucalyptus Coppice, and management

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proposals recommended.

The text stresses the importance of the cultivation of eucalyptus in the forest sector and that the current plantation area marks it as an established and growing industry.

The country can recognize two opposite situations. On the one hand, the advantages involved in the use of genetically improved and is a concept widely accepted by forest companies, which have shown a clear trend to replace the current use of seed by seed with some degree of improvement, according to the availability of this and the progress of their breeding programs at the other end, small landowners, which together manage important forest areas, usually unaware of the advantages of using seed improved, so tend to use only common seed.

The use of establishment and management schemes based on the bush, should relate to the progress of breeding programs and seed availability with degrees of improvement. Until there is better seed to be used on a farm given the option of coppice is reasonable to regenerate the plantation from the sprouts.

The economic costs associated with each form of regeneration, as well as the expected income of each, are the stuff that has not been sufficiently addressed and to which companies have expressed interest in that information is required. With this background it may be decided what kind of management and can be used to establish under what conditions it is convenient to the expense of replanting and what scenarios would be advisable to regenerate from sprouts.

There are degrees of uncertainty that can only be removed when it has the largest number of measurements in older ages, especially when information is available abundantly in the range of age from 8 to 12 years.

The results of this work allow history to have validated, which added to other developing countries, such as management models and estimation of growth and yield, are supportive of the efficient management of *Eucalyptus globulus* coppice, which even allow the estimation generation of biomass for wood energy, using short cutting cycles based on the rapid growth of coppice

One of the most important aspects of this research is the need of to maintain the measurement of experimental units to increase the database of work and analysis, covering also other geographical areas.

The conclusions of the paper states:

• It highlights the exciting growth in Chile in the *Eucalyptus globulus* coppice and the need to develop tools to support the efficient management of coppice with this species. • It is observed in stands of *Eucalyptus globulus* coppice growth in height is greater at first, but then is struck by the growth of the traditional plantation which has a greater height projection.

• Although the growth of the traditional plantation is greater from a certain stand age, the growth reaches the eucalyptus coppice for an option for pulp, generating an economic scenario of high interest to the owners

• Measurements must be maintained in order to establish whether the results obtained so far are maintained or change over time.

# Planting of cuttings eucalyptus with biodegradable containers

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# Background

The first use of tube revolutionized in the 80 nurseries, and nowadays even with disadvantages, is widespread throughout the forest sector. These polypropylene tubes, derived from petroleum, have two problems: use of non-renewable sources and waste disposal. An alternative is the substitution by biodegradable plastics, and the polyhydroxybutyrate (PHB) is one of them.

PHB, the leading member of the class of poly alcanoatos, has good biocompatibility, it is 100% biodegradable, is resistant to water, is a thermoplastic polymer, enabling the same applications of conventional polymers (LEMOGNIE, 1995 *apud* PACHEKOSKI, 2005), and is synthesized by microorganisms from renewable materials such as sugars or other carbon source.

The use of a tube or a similar packaging, and preferably biodegradable, viewed by Gomes and Pereira Couto (1985), is not yet reality. It is necessary to rethink the use of tube from non-renewable resources should remain in favor of maximizing profits at the expense of environmental impacts.

In this context, the objective is to evaluate the development of clonal eucalyptus planting with biodegradable containers.

# **Material and Methods**

Cuttings were planted in three tubes with a volume of 50 cm<sup>3</sup>, polypropylene, PHB composite with 20% (p p<sup>-1</sup>) of wood dust in normal color (brown) and PHB composite darkened in color, and minitubes with 33 cm<sup>3</sup>: polypropylene and composites of PHB in normal color. The tubes had six longitudinal grooves and the minitubes had four.

The study was realized in four commercial areas from forestry companies in the state of São Paulo, localized in Botucatu, São Miguel Arcanjo, Mogi Guaçu and Santa Branca. The spacing used was 3.0 m between rows and 2.0 m between plants, the design in three blocks with seven rows of thirteen plants. The establishment of

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forest plantations in the companies followed the business operating procedures, there is no differentiation in the activities.

According to the classification of EMBRAPA (1999), the soils were classified as Typical Dystrophic Red-Yellow (São Miguel Arcanjo, Mogi Guaçu and Santa Branca) and Psament (Botucatu).

In the field, height growth in ground level diameter (DAC) and biomass production, was measured at 15, 30, 60, 90, 120 and 150 days after planting. Measurements in these five plants from each plot were taken from the soil with hoes and aid of levers, taking care to remove most of the roots. These cuttings were measured, photographed and separated into leaf, stem and root. In the cuttings planted with the PHB tubes, these were removed in the root system and carefully collected. The drying of all plant material and the containers was accomplished using an oven temperature from 60 to 65°C until constant weight. Biomass to 150 days after planting was ground and sent for determination of macronutrients using the methodology described by Malavolta, Vitti, and Oliveira (1989).

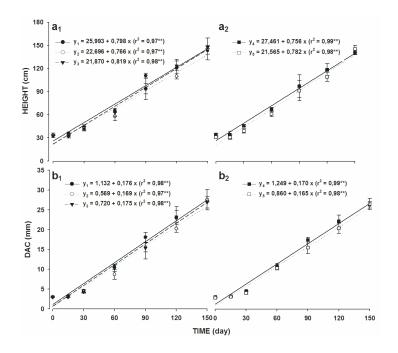
Temperature and precipitation were provided by weather stations located closest to the IAC sites. Data were tested for normality (Shapiro-Wilk test), homogeneity of variances (Box-Cox) analysis of variance (ANOVA) and means (Tukey). The analysis of the relationship between the dependent and independent variables was performed by analysis of correlation and regression.

#### **Results and Discussion**

At the site of Botucatu, the average increase in plant height was 128.8 cm in tubes and 134.3 cm in minitubes; of 145.5 and 144.0 cm, the site of Mogi Guaçu; of 144.0 and 146.5 cm, the site de São Miguel Arcanjo, and, of 97.4 and 96.8 cm at the site of Santa Branca. In the same sites, the average increase in DAC was 22.1 and 22.9 mm by 27.2 and 26.4 mm by 27.5 and 26.8 mm, and 15.9 and 16.2 mm, respectively.

At this age, the site of Botucatu, Mogi Guaçu and São Miguel Arcanjo, the growth in height and DAC did not differ among all treatments. At the site of Santa Branca cuttings grown in darkened color composite tube were smaller in DAC of the cuttings grown in tubes of polypropylene. At the site of Botucatu, the relationship between height growth over time would not allow setting, the relationship of DAC was sigmoid. These same relationships were linear in Mogi Guaçu site (Figure 1), and exponential at the site of São Miguel Arcanjo and Santa Branca.

At 150 days after planting, in the sites of Botucatu, Mogi Guaçu and São Miguel



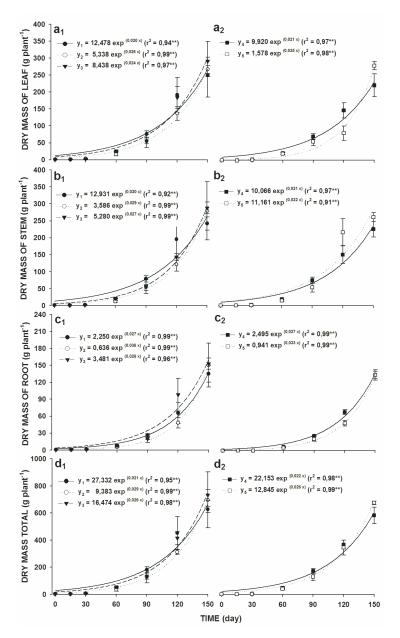
**Figure 1.** Growth in height (a) and ground level diameter (DAC) (b), 150 days after planting with cuttings grown in tubes (1) of polypropylene ( $y_1$ ), of normal color composite ( $y_2$ ) and darkened color composite ( $y_3$ ), and minitube (2) of polypropylene ( $y_4$ ) and minitube of composite ( $y_5$ ), in Mogi Guaçu site. The bar next to the mean is its standard error.

Arcanjo, the biomass production of all components did not differ. At the site of Santa Branca, the cuttings grown in darkened color composite tube, showed lower biomass of leaf, stem, root and total, than of cuttings grown in tubes of polypropylene, with no differences between the other comparisons. In the four sites, the relationship between biomass production of all components with time was exponential (Figure 2).

Overall, excluding the site of Botucatu, where rust occurred, there was no systematic positive or negative effect on the concentration of nutrients in different plant tissues, 150 days after planting in the different tubes and minitubes.

#### Conclusion

The tubes and minitubes of composite did not prevent the development of the root system. The roots grew through cracks and openings in the bottom hole of the



**Figura 2.** Dry mass of leaf (a), stem (b), root (c) and total (d), the planting to 150 days, of the cuttings grown in tubes (1) of polypropylene  $(y_1)$ , of normal color composite  $(y_2)$  and darkened color composite  $(y_3)$ , and minitube (2) of polypropylene  $(y_4)$  and minitube of composite  $(y_5)$ , in Mogi Guaçu site. The bar next to the mean is its standard error.

tube, with no differences in growth and biomass production observed between the different containers.

The development of cuttings in minitubes was satisfactory, when comparable to the tubes, especially after planting.

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# Production of cuttings in biodegradable containers

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#### Background

The Brazilian forestry sector has a growing importance for the country and nowadays it has more than 4.0 million hectares of *Eucalyptus* plantations forests (ASSOCIAÇÃO BRASILEIRA DE PRODUTORES DE FLORESTAS PLANTADAS – ABRAF, 2009)

The success formation of high yielding forests largely depends on the standard quality of the cuttings. To achieve the necessary quality, the production of cuttings in containers is the most used system, mainly to allow a better control of nutrition and protection of the roots, as well as providing the most appropriated management at the nursery, in transportation, in distribution and in the field plantation. (GOMES et al., 2003).

Since the 80's, the most used recipients were the polypropylene container, made by one of the several synthetic plastics that derivates from petroleum. The use of synthetic plastics has been causing problems because it is a product of a nonrenewable source and it generates large amounts of waste for disposal. The low degradability in the environment, allied to other environmental problems such as intoxication, accumulation of toxic compounds and the death for plastic granules of marine animals has made the scientific community think about alternatives. One of this alternatives is the replacement of the conventional polymers by a similar biodegradable (PICCOLI, 2000), that completely deteriorate by microbial attack in a short time, under appropriated conditions of environment.

The objective was to evaluate the use of biodegradable containers of composite of polyhydroxybutyrate plus sawdust in eucalyptus cuttings.

#### **Material and Methods**

Three tubes with 50 cm<sup>3</sup> of capacity were used: polypropylene, composite of PHB with 20% (p p<sup>-1</sup>) of sawdust in normal coloration (brown) and the darkened PHB composite; and, two minitubes with 33 cm<sup>3</sup>: polypropylene e PHB composite in

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normal coloration. The tubes had six longitudinal grooves and the minitubes had four.

The study was realized in four nurseries from forestry companies in the state of São Paulo, localized in Bofete, Ibaté, Mogi Guaçu and Jacareí. The experiment was arranged in four randomized block design with one hundred containers in each plot.

The growth in height and ground level diameter were measured in 50 cuttings in each plot, 90 days after cutting, with the assistance of one graduated ruler and a digital caliper. The biomass was measured randomly in ten cuttings in every plot. The cuttings were separated in leaf, stem and roots, and they were dried in a dry kiln in 60 to 65°C until constant weight.

The information went thought normality tests (Shapiro-Wilk), variances homogeneity (Box-Cox), variances analysis (ANOVA) and means (Tukey). The analysis of relation between the dependent and independent variables was performed by analysis of correlation and regression.

# **Results and Discussion**

In the four nurseries, with 90 days after cutting, the average growth in height from the cuttings was 27.8 cm in the tubes and 26.2 cm in the minitubes; and, in ground level diameter, of 2.76 and 2.61 mm, respectively. The largest growth was obtained in tubes and minitubes of polypropylene, compared to the composite (Table 1).

Visually the cuttings showed no symptoms of nutritional deficiency (Figure 1). The exposure of the substrate resulted in drying and inhibition of root growth. As a consequence there was a decrease in growth and biomass production of the cuttings produced in containers of composite compared to the polypropylene.

The average rate of decomposition of the composite at 90 days after cutting, in four nurseries was 8.0; 14.0; and 11.0%, respectively for the tubes in normal coloration, darkened coloration and for the minitube.

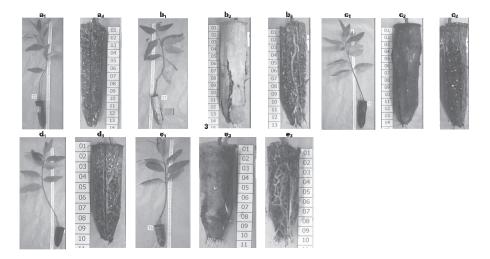
In nurseries from Bofete, Mogi Guaçu, Ibaté and Jacareí, the average rate of decomposition from composite was 2.9; 13.5; 12.0 and 15.6% respectively. The larger rate of containers decomposition occurred on the highest average temperature nurseries.

The equations that relate the decrease in mass with the environmental conditions of the nurseries presented high coefficient determination (Table 2). The minimum and maximum temperature were the environmental factors that most

**Table 1.** Average growth in height<sup>1</sup> in ground level diameter<sup>1</sup>, and biomass production<sup>1</sup> of cuttings in different components at 90 days after cutting, in four nurseries. The standard error of mean is shown in brackets.

Container and composite	Bofete	Mogi Guaçu	Ibaté	Jacareí	Mean	Bofete	Mogi Guaçu	Ibaté	Jacareí	Mean
container and composite			height				grou	nd level diameter		
			cm					mm		
Polypropylene	24,5 (0,88) a	33,0 (3,07) a	33,8 (0,52) a	27,9 (1,26) a	29,8	2,29 (0,04) a	3,06 (0,16) a	3,34 (0,04) a	2,57 (0,02) a	2,82
PHB normal	23,6 (1,03) a	33,3 (0,96) a	24,9 (1,26) bc	28,3 (2,50) a	27,6	2,33 (0,06) a	2,99 (0,09) a	3,18 (0,04) abc	2,54 (0,11) ab	2,76
PHB darkened	21,4 (0,52) ab	32,7 (0,43) a	23,6 (1,12) c	26,3 (2,11) a	26,0	2,35 (0,07) a	2,93 (0,06) a	3,03 (0,04) c	2,49 (0,11) abc	2,70
Mini polypropylene	22,9 (1,17) a	35,5 (0,85) a	28,1 (0,95) b	24,5 (1,07) a	27,7	2,25 (0,12) ab	3,02 (0,05) a	3,20 (0,04) ab	2,25 (0,06) bc	2,68
Mini PHB	19,2 (0,47) b	31,9 (1,26) a	25,1 (0,89) bc	22,2 (1,59) a	24,6	2,01 (0,03) b	2,87 (0,12) a	3,07 (0,04) bc	2,23 (0,06) c	2,55
Mean	22,3	33,3	27,1	25,8		2,25	2,98	3,17	2,42	
C.V. (%) 2	7,1	7,9	6,5	10,7		5,6	5,8	2,3	5,5	
			leaf					stem		
					g cutt	ting				
Polypropylene	0,50 (0,01) ab	1,15 (0,06) a	1,25 (0,08) a	0,64 (0,03) a	0,88	0,27 (0,01) ab	0,64 (0,02) b	0,65 (0,04) a	0,79 (0,06) a	0,59
PHB normal	0,61 (0,05) a	1,05 (0,02) a	1,04 (0,09) ab	0,50 (0,10) a	0,80	0,31 (0,03) a	0,79 (0,04) ab	0,44 (0,04) b	0,64 (0,07) ab	0,55
PHB darkened	0,48 (0,01) ab	1,01 (0,08) a	1,04 (0,11) ab	0,43 (0,10) a	0,74	0,24 (0,01) ab	0,72 (0,06) b	0,48 (0,05) b	0,61 (0,04) ab	0,51
Mini polypropylene	0,56 (0,02) ab	1,17 (0,09) a	0,89 (0,03) b	0,68 (0,13) a	0,83	0,29 (0,01) a	0,68 (0,08) b	0,41(0,01) b	0,56 (0,07) ab	0,48
Mini PHB	0,47 (0,03) b	0,98 (0,06) a	1,04 (0,11) ab	0,56 (0,10) a	0,76	0,21 (0,01) b	0,91 (0,02) a	0,42 (0,04) b	0,52 (0,05) b	0,51
Mean	0,53	1,07	1,05	0,56		0,26	0,75	0,48	0,62	
C.V. (%)	2,5	11,8	14,2	30,1		10,3	21,6	14,0	17,9	
			root					total		
					g cutt	ting				
Polypropylene	0,36 (0,01) ab	0,50 (0,004) b	0,46 (0,03) a	0,48 (0,05) a	0,45	1,13 (0,02) abc	2,29 (0,08) a	2,36 (0,15) a	1,90 (0,12) a	1,92
PHB normal	0,38 (0,02) a	0,62 (0,02) a	0,39 (0,03) ab	0,35 (0,05) a	0,44	1,29 (0,09) a	2,47 (0,07) a	1,88 (0,16) ab	1,49 (0,21) a	1,78
PHB darkened	0,29 (0,01) cd	0,50 (0,03) b	0,44 (0,05) ab	0,38 (0,03) a	0,40	1,02 (0,02) bc	2,23 (0,15) a	1,96 (0,21) ab	1,42 (0,11) a	1,66
Mini polypropylene	0,32 (0,01) bc	0,56 (0,05) ab	0,32 (0,01) b	0,37 (0,08) a	0,39	1,17 (0,03) ab	2,41 (0,22) a	1,62 (0,05) b	1,61 (0,29) a	1,70
Mini PHB	0,25 (0,01) d	0,59 (0,02) ab	0,38 (0,04) ab	0,32 (0,06) a	0,38	0,93 (0,06) c	2,48 (0,07) a	1,84 (0,19) ab	1,40 (0,19) a	1,66
Mean	0,32	0,55	0,40	0,38		1,11	2,38	1,93	1,56	
C.V. (%)	6,8	8,7	14.8	26.0		8.0	10,2	14.3	21,1	

<sup>1</sup> Means followed with the same letter do not differ significantly by Tukey test (p=0,05); <sup>2</sup> C.V. – Coefficient of variation



**Figure 1.** Appearance of the cuttings (1), composite tubes and composite minitube (2), root systems (3), 90 days after striking, grown in tube of polypropylene (a), normal composite tube (b), darkened composite tube (c), polypropylene minitube (d) and composite minitube (e), in the nursery of Mogi Guaçu.

contributed to explain the variances of the values of mass reduction of the composite.

The darkened composite absorbed more sunlight and suffered the effect of

Composite	Equation	R <sup>2</sup>	S <sub>yx</sub>	Ν
Normal	y = 1,128 . Tmax + 1,584 . Tmin - 2,552 . Tmed	0,99*	0,037	4
Darkened	y = - 0,841 . Tmax + 1,130 . Tmed	0,98*	0,353	4
Minitube	y = 0,118 . Tmin - 0,003 . P	0,97*	0,230	4
Normal, darkened e minitube	y = - 0,533 . Tmax + 0,720 . Tmed	0,87**	0,575	12

**Table 2.** Equations that estimate the decrease in mass (g tube<sup>-1</sup>) in the different tubes and minitubete of composite, according to various independent variables.

<sup>1</sup>Tmax, Tmin, Tmed = Maximum temperature, minimum temperature and average temperature of environment, P = precipitation; \* Significant at 5% of probability, \*\* Significant at 1% of probability

thermal expansion and contraction, causing more quickly photodegradation and fragmentation than in other containers. This resulted in bigger rate of decomposition, lower growth in height and ground level diameter and lower biomass production.

There was a management trouble in the composite containers caused by cracks.

# Conclusion

The composite containers reduced the growth rate in height and in ground level diameter, and the aerial part and root biomass in some nurseries.

The darkened composite tube does not favor the cutting development.

The cuttings produced in tubes and minitubes had a similar development.

Adjustment in the mixture of the composite is required to prevent a fast degradation and management damages.

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# Impact of mechanized logging on physical properties of forest soils

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#### Background

Mechanization allowed productivity and quality increase of the cultivation of forests of *Eucalyptus sp.* and others forestry species. However, it may be responsible of environmental impacts, such as soil compaction, which is the results of traffic, mostly during logging operations.

The compaction consists of pressures applied on the soil, reducing the volume occupied by the pores, increasing the bulk density and soil strength (Reichert et al., 2010). Consequently, the smaller pore sizes reduce hydraulic conductivity, leading to a slower water infiltration and movement (Dickerson, 1976), increased runoff and reducing the aeration, resulting in impediments to growth and activity of roots (Greacen and Sands, 1980).

With the possibility of production losses, forestry companies have expressed interest in quantifying and minimizing this problem. Therefore, understanding the traffic effects on the soil provides support to the operations planning, conciliating sustainability, improvement in production and reduction of costs.

The objective of this study was to evaluate the impact and the extent to which physical properties of two forest soils are affected by traffic of logging machines.

### **Materials and Methods**

The research was conducted in areas with stands of *Pinus taeda L*. of a forestry company located in northern Santa Catarina State, Brazil. The evaluated soils were a Humic Cambisol (HC) with sandy clay loam texture and organic soil carbon content (C-org) of 19.86 g dm<sup>-3</sup>, and a Litholic Neosol (NL) with sandy clay texture and C-org of 21.01 g dm<sup>-3</sup>

The logging system evaluated was Full-Tree, composed of Feller-buncher (FB) CAT 522 with crawler tracks, and Skidder (SD) CAT 545 with tire tracks.

In experimental areas the wood was harvested without machine traffic, and then were established two blocks with plots of 5 x 40 m. In blocks, each plot represented a treatment, and these were made according to the simulation of different

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traffic intensity. The treatments were: without traffic (WT), one pass of FB (FB), one of FB more one pass (FB + 1SD), three passes (FB + 3SD), five passes (FB + 5SD), ten passes (FB + 10SD) and fifteen passes (FB + 15SD) of SD. In HC, the SD drive with an extraction load of 3.55 tons and the average volumetric water content (è) of soil was 0.464 m<sup>3</sup> m<sup>-3</sup>, and in NL the extraction load was 3.2 tons and the è was 0.342 m<sup>3</sup> m<sup>-3</sup>.

In the plots, sampling of soil was done by with Koepeck soil cores (100 cm<sup>3</sup>) at five points in the depths of 0 to 15, 15 to 30 and 30 to 50 cm for dry bulk density (BD), total porosity ( $\dot{a}_{,i}$ ) and air-filled porosity ( $\dot{a}_{,ir}$ ) of soil.

Penetration resistance (PR) was measure using a penetrologger until the depth of 80 and 50 cm to HC and NL, respectively. The measures were taken in skid trails and in horizontal positions at intervals of 25 cm in five points for each wheel track per treatment per block, and then the following positions were evaluated: within the wheel tracks (WH), between the tracks (BT), outside of wheel tracks at 25 cm (OUT25), 50 cm (OUT50), 75 cm (OUT75) and 100 cm (OUT100) away.

The experimental design was randomized blocks. The variables values, except PR, were submitted to one-way ANOVA and means comparisons were using Tukey's test with  $\dot{a} = 0.05$ . We further applied GLM to related PR to treatments, positions and depths. WT-values were not incorporated in this analysis, in order to obtain correct interpretation of interaction between factors. And finally, the means comparisons were using Tukey's test with  $\dot{a} = 0.05$ . Although, the results of PR were processed graphically using GS+ 9.0.

# **Results and Discussion**

The traffic caused changes in physical properties in all treatments (Table 1 and Fig. 1). In HC the FB + 15SD was the cause of greatest impact, but significant changes were observed in the FB + 5SD. For the NL the levels of traffic did not cause sharp differences in terms of changes in the soil, showing that the lower levels of traffic already resulted in changes.

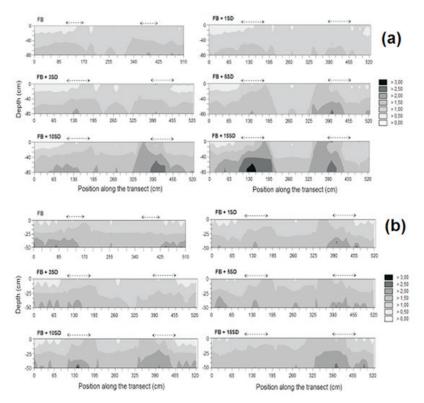
The behavior of soils was affected by texture, C-org, extraction load at the moment of the traffic and, specially, water content (è) which affected the load support capacity ( $\dot{o}_p$ ). In HC, the è was close to the field capacity ( $\dot{e}_{cc} = 0.487 \text{ m}^3 \text{ m}^3$ ), and the  $\dot{o}_p$  was 43.12 kPa, estimated according to Dias Júnior et al. (2004); while in NL the è was equivalent to 63% of  $\dot{e}_{cc}$  (0.540 m<sup>3</sup> m<sup>-3</sup>) and the  $\dot{o}_p$  of 150.21 kPa, thus justifying the difference between of soils when subject to traffic, once that forestry tractors apply pressures between 400 to 600 kPa (Horn et al., 2004).

**Table 1.** Mean bulk density, total and air-filled porosity values (±standard deviation) by treatment and depth.

, Depth (cm)	F-ratio				Treatment			
		WT	FB	FB + 1SD	FB + 3SD	FB + 5SD	FB + 10SD	FB + 15SD
			Bulk c	lensity (Mg r	n⁻³)			
HC					,			
0 - 15	5,19**	0.90 b	0.96 ab	0.95 ab	0,98 ab	1,00 a	1,03 a	1,04 a
		(±0.04)	(±0.03)	(±0.04)	(±0,10)	(±0,08)	(±0,08)	(±0,02)
15 – 30	6,56**	1.01 c	1.02 bc	1.08 abc	1,09 abc	1,10 ab	1,11 ab	1,16 a
		(±0.07)	(±0.04)	(±0.06)	(±0,10)	(±0,05)	(±0,06)	(±0,04)
30 – 50	4,96**	1.05 b	1.12 ab	1.13 ab	1,16 a	1,13 ab	1,17 a	1,18 a
		(±0.07)	(±0.07)	(±0.02)	(±0,09)	(±0,05)	(±0,05)	(±0,05)
LN								
0 - 15	13,28**	1.25 c	1.33 b	1.38 ab	1,42 a	1,38 ab	1,41 a	1,41 a
		(±0.04)	(±0.04)	(±0.03)	(±0,02)	(±0,11)	(±0,03)	(±0,02)
15 – 30	2,75*	1.36 b	1.39 ab	1.42 ab	1,40 ab	1,44 a	1,41 ab	1,43 ab
20 50		(±0.05)	(±0.02)	(±0.03)	(±0,03)	(±0,09)	(±0,06)	(±0,03)
30 - 50	5,50**	1.39 c	1.42 bc	1.44 abc	1,47 ab	1,45 ab	1,47 a	1,47 ab
		(±0.04)	(±0.03)	(±0.05)	(±0,04)	(±0,04)	(±0,06)	(±0,04)
			Total	porosity (m <sup>3</sup>	'm⁻³)			
НС					,			
0 - 15	2,46*	0.625 a	0.555 ab	0.582 ab	0,558 ab	0,570 ab	0,551 ab	0,537 b
		(±0.020)	(±0.042)	(±0.039)	(±0,068)	(±0,065)	(±0,087)	(±0,043)
15 - 30	2,83*	0.584 a	0.543 ab	0.505 ab	0,531 ab	0,507 ab	0,496 ab	0,482 b
		(±0.051)	(±0.048)	(±0.065)	(±0,069)	(±0,076)	(±0,073)	(±0,051)
30 - 50	3,11*	0.561 a	0.524 ab	0.542 ab	0,501 ab	0,523 ab	0,497 ab	0,485 b
		(±0.039)	(±0.052)	(±0.048)	(±0,048)	(±0,045)	(±0,040)	(±0,060)
LN								
0 - 15	12,38**	0.517 a	0.482 ab	0.446 bc	0,432 c	0,447 bc	0,424 c	0,434 c
		(±0.028)	(±0.016)	(±0.027)	(±0,027)	(±0,042)	(±0,026)	(±0,022)
15 – 30	7,69**	0.479 a	0.440 ab	0.410 b	0,436 b	0,403 b	0,437 b	0,408 b
		(±0.016)	(±0.020)	(±0.042)	(±0,021)	(±0,045)	(±0,030)	(±0,015)
30 – 50	5,68**	0.454 a	0.436 ab	0.440 ab	0,423 abc	0,412 bc	0,420 abc	0,382 c
		(±0.029)	(±0.024)	(±0.029)	(±0,021)	(±0,039)	(±0,036)	(±0,033)
			Air-fille	ed porosity (I	m <sup>3</sup> m <sup>-3</sup> )			
HC					•			
0 - 15	6,88**	0.151 a	0.021 b	0.030 b	0,047 b	0,039 b	0,058 b	0,027 b
		(±0.061)	(±0.015)	(±0.016)	(±0,006)	(±0,026)	(±0,025)	(±0,015)
15 - 30	2,72*	0.131 a	0.076 b	0.077 ab	0,115 ab	0,084 ab	0,089 ab	0,100 ab
		(±0.051)	(±0.048)	(±0.065)	(±0,069)	(±0,076)	(±0,073)	(±0,051)
30 - 50	5,76**	0.122 a	0.031 b	0.031 b	0,083 ab	0,089 a	0,076 ab	0,032 b
		(±0.055)	(±0.012)	(±0.018)	(±0,042)	(±0,047)	(±0,058)	(±0,010)
LN								
0 - 15	7,08**	0.152 a	0.143 a	0.105 ab	0,051 b	0,097 ab	0,077 b	0,104 ab
		(±0.030)	(±0.033)	(±0.038)	(±0,027)	(±0,058)	(±0,052)	(±0,035)
15 – 30	3,86*	0.163 a	0.080 b	0.072 b	0,117 ab	0,058 b	0,087 ab	0,083 b
		(±0.068)	(±0.045)	(±0.047)	(±0,022)	(±0,044)	(±0,081)	(±0,028)
30 – 50	3,60**	0.136 a	0.119 ab	0.110 ab	0,039 b	0,082 ab	0,072 ab	0,035 b
		(±0.097)	(±0.046)	(±0.038)	(±0,022)	(±0,058)	(±0,057)	(±0,026)

Means marked with same letter in the row are not statistically different each other by Tukey's test ( $\dot{a} = 0.05$ ); \* $\dot{a} = 0.05$ ; \*\*  $\dot{a} = 0.01$ ; ns not significant.

Analyzing the critical BD based on the least limiting water range (BDc LLWR = 1.65 and 1.49 Mg m<sup>-3</sup> to HC and NL, respectively), estimated according to Reichert et al. (2009), it is verified that in the HC the BD has not been exceeded, but in the NL this occurred, which could hamper the development of plants.



**Figure 1.** Effect of treatments along of the profile of Humic Cambisol (a) and Litholic Neosol (b) in isolines of penetration resistance. The legend indicates values of penetration resistance. The arrows indicate the position of the wheel tracks.

Both  $\dot{a}_{air}$  was more sensitive, and were detected reductions below 0.10 m<sup>3</sup> m<sup>-3</sup>, that can cause aeration problems.

The GLM analysis for PR in the HC has showed that there was interaction between treatments and positions (F-ratio of 6.33, p-Value <0.001), suggesting that the increased traffic increased the PR distinctly in all positions. For the NL the interaction between treatments and positions was not significant (F-ratio of 0.97, p-Value >0.05), because the increased of traffic not increased the PR distinctly.

The isoline graphs of PR (Fig. 1) show that in HC there were changes in soil, vertical and horizontally in relation to the skid trail, also showing increased of effect with increased of traffic, which can be seen for the NL in lower magnitude.

#### Conclusions

The increased of traffic caused changes in soils physical properties, and the

most of changes occurred after the first passes of machines, affecting vertical and horizontally the soils.

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# Initial growth of Eucalyptus saligna smith in function of tillage and soil characteristics

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# Background

With the expansion of the forest in Rio Grande do Sul there was an increase in the area covered of forest plantations, occupying areas previously predominated by livestock and the crops. These regions need studies and research to evaluate the soil conditions that contributes to the development of technologies for soil management, which aim to ensure the full establishment of *Eucalyptus* sp. and land use in a sustainable way.

Under conditions of high natural bulk density or by artificial compaction, there is increased soil strength, decreased development of the root system and reduction in the rate of absorption of water, oxygen and nutrients which limits the growth and development of plants. However, soil tillage is intended to promote the establishment and early growth, improve soil physical conditions and increase forest productivity (Fonseca, 1978).

This study aimed to identify compacted areas, quantify the effect of compaction on physical properties and evaluate the methods of tillage in the early development of *Eucalyptus saligna* Smith.

# Materials and Methods

This study was conducted in an area dedicated to growing *Eucalyptus saligna* Smith, owned by Celulose Riograndense Brazil (CMPC), located in the municipality of São Gabriel, Rio Grande do Sul, Brazil, inserted in the physiographic region of the Central Depression.

The climate, according to the classification of Köppen, is Cfa, subtropical humid. The annual average temperature and precipitation are respectively 18° C and 1355 mm, (Moreno, 1961). The soil is classified as a sandy loam Paleudalf.

The sieve analysis indicated average values of 182.18 g kg<sup>-1</sup> clay, 420.98 g kg<sup>-1</sup> of silt and 396.85 g kg<sup>-1</sup> sand (sandy loam) to a depth of 0.6 m. The experimental area was set aside for more than one year, with coverage of *Baccharis* sp. (broom), *Eragrostis plana* (grass) and *Bachelor trimera* (broom).

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In the area sought to detect different states of compaction (EC). Therefore was marked a regular grid of 140 x 100 m (14,000 m<sup>2</sup>) and determined penetration resistance (PR) at points apart from each other every 5 m, totaling 609 points. The PR was measured using a digital electronic penetrometer (Model CP 20 Ultrasonic Remik Cone Penetrometer), having conical tip with 30° angle of penetration. Readings were taken every 0.015 m up to 0.60 m of depth.

The PR data were separated between the depth intervals of 0–0.10, 0.10–0.20, 0.20–0.40 and 0.40–0.60 m. Each depth data was adjusted by semivariograms with the Software GS + 9.0. The model was chouse by the higher coefficient of determination ( $R^2$ ) and spatial dependence (SD) using cross-validation technique, continuing with kriging of data and preparation of maps.

At the same time disturbed samples were collected to determine average soil gravimetric moisture (U) in the same depths, which was 0.19 kg <sup>1</sup>kg<sup>-1</sup>.

For the isoline maps of PR were characterized three states compaction, and then were distributed three sampling plots of 30 x 30 m for each EC. In each plot, soil samples were collected with preserved structure in metallic cylinders of diameter 0.06 m and 0.04 m in height in four layers (0–0.10, 0.10–0.20, 0.20–0.40, 0.40–0.60 m). Where determined: dry bulk density (Ds), microporosity (Mic), macroporosity (Mac) and total soil porosity (Pt), according to EMBRAPA (1997).

In each EC were installed the following treatments of tillage: subsoiling up to 0.50 m of depth and rotary tiller (S50), subsoiling up to 0.50 m of depth and ridge (S50R) and subsoiling up to 0, 70 m of depth and rotary tiller (S70). After that, there was the planted of seedlings of *Eucalyptus saligna* Smith spacing of 3.5 x 2.6 m, totaling 96 plants in each plot, divided into 8 rows with 12 plants in each row. The survival of seedlings evaluated 30 days after planting.

The experimental design was randomized blocks. The values of BD, Mic, Mac and Pt were subjected to one-way ANOVA and means compared by Tukey's test with a = 0.05.

## **Results and Discussion**

Analyzing the isolines maps of PR can be noted great variation of this property, characterized thus different states of compaction, as represented by the isoline map of the RP from 0.40 to 0.60 m depth (Fig. 1). The adjusted semivariogram was the exponential with R<sup>2</sup> of 0.975 and SD of 0.791.

The most of the area showed PR values above 2 MPa, which is the value were initiate the restrictions to root growth of various cultures (Silva 2003), but values

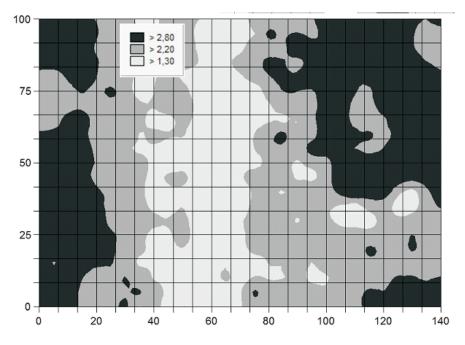


Figure 1. Soil penetration resistance (MPa) isoline map in the depth of 0.40 to 0.60 m, identifying the compaction states.

close to 3 MPa are also present in the area, which is the critical value for the development for the root system of forestry species (Zou et al., 2000).

In the layers with higher Ds (Table 1), Pt was lower, as this is dependent on the Ds and particles density. The Pt ranged from 0.33 to 0.49 cm<sup>3</sup> cm<sup>-3</sup>, which according to Suzuki et al. (2007) is within the proper range of porosity. Although there is a wide variation in relation to Pt, some values commonly found in sandy soils range from 0.33 to 0.40 cm<sup>3</sup> cm<sup>-3</sup>.

The volume of Mac, for the three states of compaction can be considered low in relation to the amount of Mic, an ideal soil should be 1/3 of Mac and 2/3 Mic, moreover, and volume of pores with higher than 0.10 cm <sup>3</sup>cm<sup>-3</sup>(Taylor & Ashcroft, 1972; Hillel, 1998).

The higher survival of seedlings occurred on treatment S50R. Probably, this increased survival of seedlings is due to greater soil tillage, due mainly to a better use of environmental conditions on early development. Fonseca (1978), analyzing the results of research on soil tillage with *Eucalyptus grandis*, found increases in survival and development of plants according to the method of soil inversion used.

EC	BD Mg m <sup>3</sup>	Mic	Macro cm cm <sup>3</sup>	Тр
		0.0 -10		
EC1	1.49 a	0.35 a	0.06 a	0.41 a
EC2	1.53 a	0.28 ab	0.06 a	0.34 ab
EC3	1.53 a	0.24 b	0.08 a	0.33 b
		0.10-0.20		
EC1	1.46 a	0.32 a	0.08 a	0.40 a
EC2	1.47 a	0.39 a	0.10 a	0.49 a
EC3	1.47 a	0.36 a	0.08 a	0.44 a
		0.20-0.40		
EC1	1.42 a	0.34 a	0.11 a	0.45 a
EC2	1.47 a	0.28 a	0.11 a	0.40 a
EC3	1.51 a	0.38 a	0.06 b	0.44 a
		0.40-0.60		
EC1	1.49 a	0.32 a	0.09 a	0.41 a
EC2	1.47 a	0.36 a	0.10 a	0.46 a
EC3	1.54 a	0.35 a	0.05 b	0.40 a

**Table 1.** Average values of bulk density, microporosity, macroporosity and total porosity by state of compaction and depth.

**Cenclusions** ith same letter in with depth interval are not statistically different each other by Tukey's test (á = 0.05).

The RP is important to identify compacted areas. Physical impairments and soil compaction are situations that affect the soil properties. The layers with the highest bulk density had the lowest porosity.

The treatment of subsoiling up to 0.50 m depth and ridge (S50R) was the treatment that had the highest survival seedlings of *Eucalyptus saligna* Smith.

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# *Eucalyptus* seedlings *urophylla* in different concentrations of hydrogel and irrigation time

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## Introduction

Obtaining seedlings of high quality planting before the final condition is important when the goal is the formation of productive forests. (Novaes, 2002). Considering the high consumption of water in the production of eucalyptus trees and the need for rationalization. Thalheimer et al. (2010) report that the alternative to increasing the availability of water, reduce losses by percolation and leaching is the use of hydrogels. To IPEF (2004), this product is a polyacrylate-acrylamide neutralized with potassium hydroxide and ammonia, insoluble in water but with high capacity to absorb it. It is free of waste, and once in contact with water, expands rapidly forming a gel with the capacity to store water and soluble nutrients to the environment. Volkmar and Chang (1995) report that most research on the topic in question point to the use of polymers, with the major advantage, the best use of water. Given the above, the present study was to evaluate the effects of different concentrations of hydrogel and times of irrigation on the quality of seedlings of Eucalyptus urophylla.

# **Materials and Methods**

The experiment was conducted at the State University of Southwest-UESB Vitória da Conquista, Bahia, in the coordinates 14  $^{\circ}$  51'S and 40  $^{\circ}$  50'W with an altitude of 930 meters. For the production seedlings was used to model conical vial with 50 cm<sup>3</sup>, containing the specific substrate for these containers and fertilized with 3.0 g of Osmocote fertilizer. We adopted 15 treatments arranged in 3 x 5 factorial, with three times of irrigation (10, 15 and 20 minutes) and four doses of hydrogel incorporated into the substrate (1, 2, 3 and 4 g / liter of substrate), including the control (no hydrogel), or T1-10 minutes of irrigation + 0 g of hydrogel / liter of substrate, T2-10 minutes of irrigation hydrogel + 1 g / liter of substrate, T3-10 minutes of irrigation + 2 hydrogel g / liter of substrate, T4-10 minutes of irrigation hydrogel + 4 g / liter of

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substrate, T6-15 minutes of irrigation + 0 g hydrogel / liter of substrate, T7-15 minutes of irrigation + 1 g of hydrogel / liter of substrate, T8-15 minutes of irrigation hydrogel + 2 g / liter of substrate, T9-15 minutes of irrigation + 3 g of hydrogel / liters of substrate; T10-15 minutes of irrigation hydrogel + 4g / l of substrate, T11-20 minutes of irrigation + 0 g of hydrogel / liter of substrate, T12-20 minutes of irrigation + 1 g of hydrogel / liter of substrate ; T13-20 minutes of irrigation + 2 g of hydrogel / liter of substrate, T13-20 minutes of irrigation + 2 g of hydrogel / liter of substrate, T14-20 minutes of irrigation + 3 g of hydrogel / liter of substrate, and T15-20 minutes of irrigation + 4 g of hydrogel / liter of substrate. The experimental design was completely randomized design with four replications. Four irrigations were performed daily. We evaluated the height of the air (H), stem diameter (D) and total weights of fresh and dry. The Means were compared by Tukey test at 95% probability.

### **Results and Discussion**

According to Table 01, it was observed that the highest average height of shoots were obtained from treatments that contained 4.0 grams of hydrogel/liter of substrate and time of 15 minutes, although there was no statistical difference when compared with the treatment of hydrogel containing 3.0 g/liter of substrate in both irrigation, which also showed no statistical difference when compared to the time of 20 minutes. As the variable stem diameter, was found the same results. According to Vieira and Pauleta (2009), added to the polymer substrate provided reserve water retention which in practice can help reduce water consumption in the nursery. Azevedo (2000), studying coffee seedlings (*Coffea arabica* L) found that the substrate, the effect of the polymer allows to extend the intervals between irrigations without compromising plant growth by water deficit.

#### Conclusion

Seedlings grown in substrate containing hydrogel 4 g / liter had the highest average for the studied parameters, allowing the reduction of irrigation time.

**Table 1.** Mean valuesof shoot height and root collor diameter of seedlings ofEucalyptus. urophylla, 95 days after sowing.

Tempo (min)				h	ydrogel (g/l	iter)				
		sho	ot height (	:m)			root colle	or diamet	er (mm)	
	0	1	2	3	4	0	1	2	3	4
10	19,67 bC	17,27 bD	20,64 bBC	22,04 bB	23,75 bA	2,05bC	2,11 aBC	2,36aAB	2,51aA	2,54 aA
15	22,27 aB	18,05 bC	22,23 aB	26,27 aA	26,93 aA	2,32aBC	2,10aC	2,17aBC	2,41aAB	2,64 aA
20	18,04 cD	19,91 aC	22,02 aB	25,37 aA	26,04 aA	2,10bC	2,19 aBC	2,29aBC	2,39aB	2,69 aA

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POSTER SESSION 2. ENVIRONMENTAL STRESS

# Carbon isotope discrimination and differential drought tolerance in eucalypt clones

Flancer Novais Nunes<sup>1</sup>, Ivo Ribeiro da Silva<sup>2</sup>, Nairam Félix de Barros<sup>2</sup> Roberto Ferreira de Novais<sup>2</sup>

# Abstract

In Brazil, eucalypt plantations have expanded to the Cerrado (savannah type of vegetation) region, where the soil is poor in nutrients and the dry season lasts from 4 to 6 months or more, imposing severe nutrient and water restriction for a long period of time during the year to the trees. Differential drought tolerance has been observed among eucalypt clones, resulting in reduced survival and growth rate. Thus, the objective of this study was to evaluate the  $\delta^{13}$ C of sensitive or tolerant eucalypt clones to water deficit. Four year old eucalypt plantations, located in two sites (Brasilia de Minas – site 1, Bocaiuva – site 2) in North of Minas Gerais State, Brazil, were assessed at the end of the 8th-month dry season of 16 2007. Plots of a sensitive (SS) and a tolerant (TL) clone to water deficit, were selected. Trees were cut down and the third or fourth leaf of apical branches was collected from four branches of the middle third of the canopy for water potential determination. Stemwood and leaf (third or fourth fully expanded leaf) samples were collected from each tree for  $\delta^{13}$ C determination. The wood productivity of living and dead trees (total productivity), productivity of living trees and survival rate in plots where trees were planted in different spacing were determined in March 2009, i.e., 6.4 years after planting. The more tolerant clone to water deficit showed higher ä13C values, i.e. less discrimination of <sup>13</sup>C in samples of leaf and wood, indicating a better stomata control, maintaining higher photosynthetic rate, survival rate and wood production than the sensitive clone. It is suggested that  $\delta^{13}$ C can be used as an indicator of eucalypt clones tolerance to water deficit.

#### Introduction

In Brazil, eucalypt plantations have expanded to the Cerrado (savannah type of vegetation) region, where the soil is poor in nutrients and the dry season lasts from 4 to 6 months or more, imposing severe nutrient and water restriction for a long period of time during the year to the trees. Differential drought tolerance has been

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observed among eucalypt clones, resulting in reduced survival and growth rate. Different physiological processes may be involved in plant drought tolerance, such as osmotic adjustment, greater root/shoot ratio, and lower stomata conductance, among others. Stomata closure can maintain tissue hydration but can reduce productivity because prevents  $CO_2$  absorption. However, there are genotypes that allow partial stomata opening, reducing water loss and maintaining the internal  $CO_2$  concentration, resulting in a photosynthetic rate which conduces to a greater efficiency of water use (WUE) (Condon et al., 2004). Thus, eucalypt clones with higher WUE have a lower water requirement per unit of dry matter produced.

The isotope discrimination of <sup>13</sup>C ( $\delta^{13}$ C) has been used as a criterion for the selection of genotypes tolerant to water deficit and correlates with WUE by plants (Impa et al., 2005; Monneveux et al., 2007; Duan et al., 2009). The establishment of relations between photosynthetic rate and stomata conductance with  $\delta^{13}$ C and the WUE (Farquhar et al., 1989) allowed the use of  $\delta^{13}$ C as a tool for selecting genotypes with increased tolerance to water deficit (Read et al. 1991; Sheshshayee et al., 2003, Condon et al. 2004; Ducrey et al., 2008). Thus, the objective of this study was to evaluate the  $\delta^{13}$ C of sensitive or tolerant eucalypt clones to water deficit.

#### **Material and Methods**

Four year old eucalypt plantations, located in two sites (Brasilia de Minas – site 1, Bocaiuva – site 2) in North of Minas Gerais State, Brazil, were assessed at the end of the 8th- month dry season of 2007 (Figure 1). Plots of a sensitive (SS) and a tolerant (TL) clone to water deficit, were selected based on visual symptoms

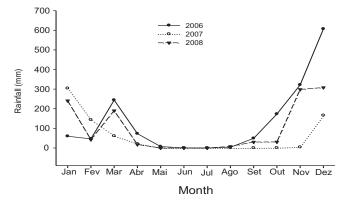


Figure 1. Average rainfall for sites 1 and 2 during 2006, 2007 and 2008.

Clone	Ψ	$\delta^{_{13}}$ C	(‰)	
	MPa	Leaf	Wood	
Site 1				
Tolerant	-2,00Aa	-26,16Aa	-26,27Aa	
Sensitive	-1,40Ba	-27,27Ba	-27,36Ba	
Site 2				
Tolerant	-1,40Ab	-26,28Aa	-26,86Aa	
Sensitive	-1,20Aa	-27,53Ba	-27,33Aa	

Table 1. Isotopic discrimination of $^{\rm 13}\text{C}$ ( $\delta^{\rm 13}\text{C}$ ) in leaves and wood samples of eucalypt
clones 14 sensitive and tolerant to water deficit collected from sites 1 and 2 15.

Capital letters compares (F test at 5%) clone effect in each site. Lower case compares site effect for each clone.

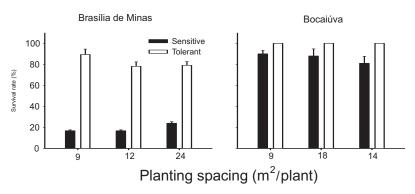
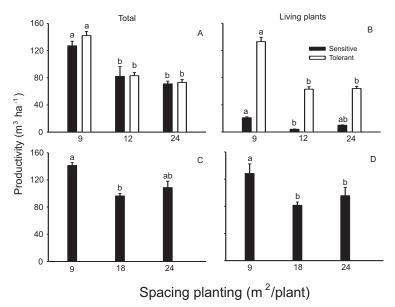


Figure 2. Survival rate of eucalypt clones (6.4 year old) as affected by spacing, in sites 1 and 2.

(dried branches and leaves from the base to the top) and survival. In each plot, were selected four trees (spaced of 3 x 3 m) without visual symptoms of water deficit. Trees were cut down and the third or fourth leaf of apical branches was collected from four branches of the middle third of the canopy for water potential determination. Wood and leaf (third or fourth fully expanded leaf) samples were collected from each tree for  $\delta^{13}$ C determination. The wood productivity of living and dead trees (total productivity), productivity of living trees and survival rate in plots where trees were planted in different spacing were determined in March 2009, i.e., 6.4 years after planting.

## **Results and Discussion**

Leaf water potential ( $\Psi_{wf}$ ) of the tolerant clone was lower in site 1 than in site 2 (Table 1), suggesting lower water deficit in the later site. Soil water potential (0 to 1.6 m depth) was similar between the two sites (-1 68 and -1.66 MPa, respectively).



**Figure 3.** Total (live + dead trees, A and C) and living trees (B and D) wood productivity of eucalypt clones, 6.4 year old, as affected (Tukey test at 5%) by planting spacing in sites 1 (A and B) and 2 (C and D).

Therefore, the lower  $\Psi_{wf}$  in the former site may be attributed to a higher vapor pressure deficit. The tolerant clone showed smaller values of  $\Psi_{wf}$  (more negative) with the largest differences between clones observed in site 1 (Table 1). This result, smaller  $\Psi_{wf}$  for tolerant clone, was not expected, since the mechanisms that promote greater tolerance of plants to water deficit, as lower stomata conductance, are related to the maintenance of higher  $\Psi_{wf}$  values (DaMatta et al., 2003).

The lowest  $\Psi_{wf}$  of the clone tolerant to drought, in site, was associated with higher 38  $\delta^{13}$ C values (Table 1). Low  $\Psi_{wf}$  leads to lower photosynthetic rates and consequently higher al., 2008).

The more tolerant clone to water deficit showed higher  $\delta^{13}$ C values, i.e. less discrimination of  $^{13}$ C in samples of leaf and wood (Table 1). The tolerant clone signals for a better stomata control, maintaining higher photosynthetic rate, and presents a strategy to use water more conservatively (Li, 2000).

The tolerant clone showed a higher survival rate (Figure 2) associated with lower 7 value of ä13C (Table 1). In site 1, the tolerant clone, in the 3 x 3 m spacing, presented  $\delta^{13}$ C of -26.28 ‰ and survival rate of 82.3%, whereas the sensitive clone showed -27.34 ‰ and 16, 8%, respectively. These results support the hypothesis that tolerant clones have lower stomata conductance associated with maintenance

of photosynthetic rate, contributing to lower isotope discrimination. We observed a higher wood productivity and lower  $\delta^{13}$ C in both sites (Figure 3, Table 1). In site 2, for example, the productivity of the tolerant clone was 191 m<sup>2</sup> ha<sup>-1</sup> and  $\delta^{13}$ C in the leaf of -26.28 ‰ while the sensitive was 141 m<sup>2</sup> ha<sup>-1</sup> and  $\delta^{13}$ C of -27.53 ‰. The positive correlation between  $\delta^{13}$ C and productivity and may be due to lower stomata conductance associated with maintenance of photosynthetic rate by the tolerant clone (Ducrey et al., 2008).

Thus, based on  $\delta^{13}$ C and survival rates, in both sites, and in the literature, it is suggested that  $\delta^{13}$ C can be used as an indicator of eucalypt clones tolerance to water deficit.

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# Cold acclimation for plant cryopreservation: some physiological and biochemical responses of *Eucalyptus grandis* to different nonfreezing low temperatures and exposure periods

C.H. Ting, K. Padayachee, D.J. Mycock

#### Background

*Eucalyptus grandis* is an important asset in the forestry industry for its superior wood and growth properties, consequently breeding and improvement are essential components of forestry programmes. Integral to this is the requirement for the maintenance of a broad genetic base. Plant cryopreservation is an economical option to maintain such a genetic base, as it allows storage of vegetative materials at sub-zero temperatures, while maintaining its juvenility (Padyachee *et al.*, 2009). However, cryopreservation of this sub-tropical species has been limited by its sensitivity to freezing, dehydration and desiccation. This has restricted its success in cryopreservation which exposes selected storage materials to all of these stresses.

Cold acclimation investigations, however, has demonstrated that *Eucalyptus* and many other plant species have increased freezing tolerance in response to exposure to low but nonfreezing (chilling) temperatures prior to freezing (Khayal *et al.*, 2006). This adaptive mechanism which can occur over a period of days at a specified chilling temperature is a result of molecular, metabolic, biochemical, and physiological responses that contribute to the cold tolerance (Khayal *et al.*, 2006). In addition, transcriptional and genomic evidence have suggested that similar biochemical processes could function in both dehydration- and cold-stress responses (Shinozaki *et al.*, 2003).

Thus, the aim of this study was to understand some of the physiological and biochemical responses of *E. grandis* to different chilling temperatures and exposure periods, and to determine the appropriate cold acclimation regime for the cryopreservation process.

#### Methods

*E. grandis* shoot clusters (4-8 leaves and 2-5 axillary buds) that were pre-cultured for 3 days at 25°C on *in vitro* tissue culture medium devoid of plant growth regulators,

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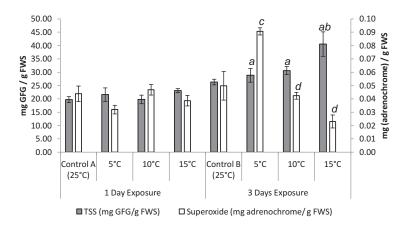
were exposed to the chilling temperatures of 5°C, 10°C and 15°C for 1 and 3 days (chilling treatments). After the chilling treatments, physiological and biochemical responses were evaluated and determined. The physiological responses evaluated were actual water content (AWC), viability (percentage of surviving explants), and vigour (i.e. the number of visible axillary buds and shoots produced over 2 weeks). The biochemical responses that were measured were the concentrations of: 1) total soluble sugars (as a glucose, fructose and galactose equivalent, GFG) (Chow *et al.*, 2004), 2) starch (Megazyme Total Starch Assay Kit), 3) phenolic acid (as a gallic acid equivalent, GAE) (Tabart *et al.*, 2007), and 4) superoxide, .O2 - (as an oxidation of (-)-epinephrine into adrenochrome equivalent) (Misra *et al.*, 1972).

#### **Results and Discussion**

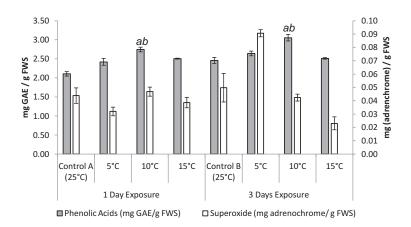
At the physiological level, AWC was not significantly different (P>0.05), and viability was 100% for all chilling treatments. However, while not significantly different (P>0.05), vigour rates at 10°C were the highest. Assessment of the proliferation of visible axillary buds showed 11.70±1.20 visible axillary buds/ week after 1 day cold exposure and 11.90±0.60 visible axillary buds/week after 3 days, while assessment of shoot growth rate was 2.60±0.100 visible shoots/week after 1 day cold exposure and 3.10±0.200 visible shoots/week after 3 days exposure to cold. This showed that after exposure to 10°C, growth was promoted while at 5°C and 15°C vigour rates remained similar to that of the controls.

From a biochemical perspective, total soluble sugar (TSS) concentrations after 3 days exposure to all three low temperatures were higher than after 1 day exposure (Fig 1). The highest TSS level was seen after 3 days at 15°C (40.55±4.59mg GFG/g fresh weight of shoots, FWS) (Fig 1). Starch levels, however, were not significantly different for all treatments (*P*>0.05). Phenolic acid accumulation was the highest at 10°C (after 1 day 2.74±0.06mg GAE/g FWS; after 3 days 3.05±0.09mg GAE/g FWS) where 3 day exposure was significantly greater than 1 day exposure (*P*<0.05) (Fig 2). With regards to superoxide ( $\cdot O_2^{-1}$ ), after 3 days exposure at 10°C (after 1 day 0.047±0.003mg  $\cdot O_2^{-1}$ /g FWS; after 3 days 0.042±0.003mg  $\cdot O_2^{-1}$ /g FWS) and 15°C (after 1 day 0.039±0.004mg  $\cdot O_2^{-1}$ /g FWS; after 3 days 0.023±0.005mg  $\cdot O_2^{-1}$ /g FWS) levels had decreased but for 3 days at 5°C,  $\cdot O_2^{-1}$  level was the highest level assayed (0.091±0.003mg  $\cdot O_2^{-1}$ /g FWS) (Fig 1 and 2).

These results suggested that TSS was not derived from starch but rather from an alternative carbohydrate metabolic pathway responsive to prolonged (3 days) exposure to non-freezing low temperatures. In addition, at 10°C, phenolic acids



**Figure 1.** Biochemical response of total soluble sugars (TSS) and superoxide from *E. grandis* exposed to 5°C, 10°C and 15°C for 1 and 3 days, with standard errors. *a*: All three low temperatures showed increased TSS levels after 3 days exposure. *b*: TSS was the highest after 3 days exposure at 15°C. *c*: Superoxide was the highest after 3 days exposure at 10°C and 15°C superoxide levels decreased.



**Figure 2.** Biochemical responses of phenolic acids and superoxide from *E. grandis* exposed to 5°C, 10°C and 15°C for 1 and 3 days, with standard errors. *a*: Phenolic cids were the highest at 10°C. *b*: Phenolic acid accumulation after 3 day exposure was significantly greater than after 1 day exposure (p<0.05).

and TSS may have a combined role in decreasing  $.O_2^{-}$  after 3 days exposure while the decreased  $.O_2^{-}$  level for 3 days exposure at 15°C may have involved TSS. Furthermore, the stress of 3 days at 5°C showed the highest  $.O_2^{-}$  level which could not be alleviated by increased TSS and phenolic acid levels.

#### Conclusions

The biochemical response of *E. grandis* was varied at the different low temperatures and exposure periods: TSS accumulated after 3 days exposure at 15°C, while phenolic acid accumulation was noted after 10°C. The increased levels of TSS and phenolic acids may have contributed to the decrease of  $O_2^-$  at 10°C while at 15°C mainly TSS, acting as low temperature stress responsive biomolecules. However, in the 5°C 3 day treatment, the high levels of  $O_2^-$  could not be alleviated by the accumulation of TSS and phenolic acids. In addition, *E. grandis* responses showed that after 10°C exposure, growth was improved as indicated by vigour rates. With consideration of both physiological and biochemical responses of *E. grandis*, it was inferred that a 3 day exposure to 10°C might be an appropriate cold acclimation condition for the preparation of the material for the cryopreservation process.

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### Tools for early selection of drought tolerant eucalyptus

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In recent years, as an output of the breeding programs new *E. globulus* plantations have used clones selected for growth and higher pulp yield. However in regions of Mediterranean climate, like Portugal, it is desirable that selected clones are well adapted to the summer drought, as this is the main environmental constraint to productivity.

In previous studies we observed that clones least susceptible to drought showed a greater allocation of biomass to roots and higher hydraulic conductance, which allowed to prolong the water-stress-free period for active carbon assimilation. These developmental changes, which maintained the balance between transpiration and absorption areas when soil water availability declined, seemed to be the key determinant of performance under drought condition. As early selection of individuals with a desirable phenotype allows increasing genetic gain per time unit, and also that breeding programs require detailed physiological information of the stress-response of the clones selected. The objective of this study was to identify traits and adapt methodologies to assess - quickly, inexpensively, and preferably in a not destructive system - the clones efficiency in capture and use of water.

In order to achieve this goal we compared 8 clones previously classified in relation to their drought sensitivity (I: more tolerant to VIII: more sensitive) according their performance (survival and growth) in field trials through physiological (transpiration, stomatal conductance; predawn and midday leaf water potentials, plant temperature) and morphological (height, leaf area ratio, specific leaf area and leaf-to-root area ratio, biomass) characteristics of 8 months container-grown plants; Six cuttings per clone were assigned to either a well-watered regime (WW; water supplied to equal transpiration losses) or a water-stress regime (WS; water supplied equal to 40% of transpiration losses) for 83 days.

The low water availability affected all traits evaluated and significantly reduced the growth of all clones. Thermo-imaging technique does not require the destruction of the plant material, it is easily used in the field or in studies under controlled conditions; it showed significant differences between clones.

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# Evaluation of climate effects on *Eucalyptus grandis* Hill Ex Maiden provenances tests

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#### Abstract

The paper analyzes climate variables influence on *Eucalyptus grandis* growth. Provenances tests were planted in seven locations and average growth were analyzed with multivariate statistic. Summer and winter rainfall were the most important factor to explains growth traits among locals.

#### Introduction

It is well known and recognized that local conditions affect growth and survival of eucalypts planted outside their natural distribution, because populations of forest trees are genetically adapted to their natural distribution environments. Most of plantations established nowadays are resulted of species and provenances selection established on field trials and selections aimed wood production to supply forest industries; therefore, they were based on productivity per planted area.

A considerable number of eucalypts species and provenance seed lots were introduced in Brazil last century and *Eucalyptus grandis* pointed out as one of the most important species. During the period of 1986-1990, a network of field trials with 10 provenances was established in EMBRAPA in collaboration with private companies, universities and forest research institutions (HIGA; RESENDE; SOUZA, 1991; HIGA et al., 1997).

As pointed out by Matyas (1994), comparative studies of the growth and productivity of populations in different environments provide a valuable tool for examining the nature of changes in biomass production and stability of forest ecosystems as a result of an altered climatic environment. Although these field experiments were not set to analyze climate, they can provide useful information on that, mainly considering that climate change is one of the biggest threats faced by forestry and adaptation plays an important part. Many questions have been made regarding climate change and forests, such as, what environmental variables

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determine growth rates? How existing material will react to climate changes? How are they vulnerable to climate changes?

Basic ecophysiological studies have been going on during the last decade aiming to identify *Eucalyptus* growth limitations. Although many progresses have been achieved, still many remains unknown (LINDER, 2008). Number of variables related manly to soil and climate, genetic variability, management practices and their interaction imposes a great level of difficulty in understanding growth completely. Nevertheless, main factors can be detected and used for modeling and to predict climate change impact for some wood species.

This paper aimed to discuss some of the climate effects on *Eucalyptus grandis* Hill Ex Maiden growth at distinct climate area.

#### Methods

Seeds from 10 provenances within *E. grandis* natural distribution were planted in seven localities Brazil (Table 1). The experimental design used was randomized blocks, with six plants per linear plot, and variable replication number. Provenances growth performances were measured at 5 years of based on total height (H) and diameter at breast height (DBH).

Multivariate regression analyzes were performed to evaluate the relationship between growth (provenances average) and climate data of planted area, using R<sup>®</sup> software. A factorial analysis was performed to obtain meaningful factors from climate variables; afterwards it was fitted to a multiple multivariate to explain the relationship between growth variables and factors.

#### **Results and Discussion**

Summer rainfall showed to be the environment factors most related to growth

	Local	LAT.(S)	LONG.(E)	ALT.(m)
PROVENANCE	Baldy State Forest 194-Atherton-QLD	17°18'	145°25'	1000-1200
	12 km S. of Ravenshoe-Mt Pandanus-QLD	17°42'	145°28'	860-940
	Bellthorpe St. forest-QLD	26°52'	152°42'	500
TRIALS	Telêmaco Borba (PR)	24 °32'	50 °62'	736
	Barra Ribeiro (RS)	30 °29'	51 °30'	10
	Aracruz (ES)	19 °82'	40 °27'	56
	Guanhães (MG)	18 °78'	42 °93'	811
	Ipatinga (MG)	19 °47'	42 °54'	304
	Caçapava (SP)	23 °10'	45 °71'	569
	Boa Esperança Sul (SP)	21 °99'	48 °39'	571

 Table 1. Eucalyptus grandis seed origin (Australia).

QLD - QUEENSLAND; NSW - NEW SOUTH WALES.

in all localities tested in Brazil. Summer rainfall and winter rainfall can be responsible for growth differences observed among the locals (Table 2). These results confirm that related by Ryan et al. (2008), who found that increased resource availability, mostly water, increased canopy photosynthesis and changed carbon allocation. Water availability combined with soil physical properties and temperature seems to be the most important factor for all tropical trees productivity. Increases in the frequency and duration of drought could also change growth patterns and increase physiological stress and interactions with other disturbances such as pest outbreaks and fire (ALLEN et al., 2010).

As pointed out by Anderson and Chmura (2009), plant populations may adjust to climate change in three ways: 1) by expressing an inherent capacity for environmental tolerance by alterations in physiology and development; 2) by migrating to new habitats; or 3) by adaptation in place through natural selection on genetic traits important to survival in the dynamic climate. The effects of natural environmental characteristics on provenances growth, as observed in this study, exemplify the adaptation mentioned as the third way. However, the relative roles of tolerance or adaptive processes will depend on the biological characteristics of the individual species and the time frame considered.

The results of this study may also suggest a reflection related to selection and breeding, especially concerned to plantation using clones of selected genotypes. Usually, high productive clonal forestry presents reduced genetic variation, which increase risks to overcome an unusual climatic event. Most of clonal selection is done based on growth and wood characteristics, not considering the climate effects during that growth period. Trees average approximately 68% more polymorphic loci and 45% higher genetic diversity (He) within populations than herbaceous species (HAMRICK, 2004) The author also pointed out that available data indicate that trees also maintain high genetic diversity within their populations for quantitative traits, many of which may be adaptive. Generally narrow-sense

	Factor 2	Factor 3	DBH	Н
Factor 1	-0.24	-0.16	-0.23	-0.27
Factor 2		0.17	0.26	0.23
Factor 3			0.74	0.71
DBHP				0.97

Factor 1 – Maximum and minimum temperature

Factor 2 – Winter rainfall

Factor 3 – Summer rainfall

heritability values are at least moderate for trees indicating that sufficient genetic variation may be available for populations to respond to environmentally imposed selection pressures (HAMRICK, 2004).

The increasing concern with climate change should make trials set, especially genetic material testing, more aware of climate information, with local detailed data. Not only climate data, but also soil variables which interactions are complex, and knowledge on this issue is essential to clarify growth trends.

Seed sources have distinct adaptive traits and may show different reactions in response to climate changes. Next step will be to use more accurate analyzes based provenance performance and climate from the origin.

#### Conclusions

The effect of climate changes on tree growth and adaptation is a challenging issue, but an important and necessary task to support long-term planning of a species and provenance selection. This issue requires better understanding of the role of climate in forest ecosystem processes. It is important to known the key process underlying tree species growth and relate then to the expected climate. Tree breeding programs should consider current and future climate trends and focus more heavily on other traits such as drought hardiness.

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## Effects of saline stress over mineral nutrition on *Eucalyptus* seedlings

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#### Introduction

In Brazil, where different species of *Eucalyptus* are planted extensively for different uses, there are few records about its behavior on areas affected by salinity.

Salinity may result in water deficiency (osmotic stress), toxicity (mainly by Na and Cl) and nutritional unbalanced, difficulting the evaluation of everyone of those reduction effects on plant growing process, mostly because of the great number of factors involved, as it is the case of ion concentrations on substrate, time of exposure, plant specie, variety, state of plant development and environmental conditions, among others (MARSCHNER, 1995).

In order to inhibit the planting process on area with high salinity, it is necessary to know the grade of tolerance of those species, as well as the way they grow and use nutrient during their development. This knowledge helps to define the management practices for saline environment. Though, the objective of this study was to evaluate the effect of increasing NaCl concentration over the rest of nutrients and to measure the Na concentration on aerial section of the *Eucalyptus tereticornis* seedlings.

#### **Materials and Methods**

The experiment was conducted in a greenhouse at UENF – Universidade Estadual do Norte Fluminense, in Goytacazes, Rio de Janeiro, Brazil. Pots of 11,5L capacity were used, filled with river sand, washed with conventional treated water (100L) and 10L of deionized water. The substrate was dried first within the greenhouse. Finally, seedlings were transplanted into the pots. Seedlings were produced in plastic tubes of 50cm<sup>3</sup>.

The experiment was statistically randomized: one specie (*E. tereticornis*) and 5 levels of electrical conductivity ((1,41; 2,50; 4,50; 6,45 e 8,33 dS m<sup>-1</sup>) with 5

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repetitions, one seedling by each repetition.

At the moment of transplanting, every pot received 1.8L of nutritive solution, according to the treatment, quantity enough to accomplish, approximately, 60% of the water retention capacity.

The nutritive solution received more NaCl (2M) in 5 different levels of salinity: for 1,41dS m<sup>-1</sup> of salinity, no NaCl solution was added; for 2,50 dS m<sup>-1</sup>, 5ml was added; for 4,50 dS m<sup>-1</sup>, 15ml; for 6,45 dS m<sup>-1</sup>, 25ml and, for 8,33 dS m<sup>-1</sup> the addition was 35ml.

Definition of those levels considered the base of value of 4 dS m<sup>-1</sup> of electrical conductivity, because, following SHANNON et al. (1994), concentrations over 2600 mg.L<sup>-1</sup> can diminish productivity on most of the plant species.

During the conduction of this experiment, a daily irrigation was done at 8:00, 12:00 and 17:00 hours, with non mineral water, keeping, approximately, 60% of the water retention capacity.

At the 75<sup>th</sup> day after transplantation on sandy soil, plants were collected and selected by leaves, stems and roots, washed and submitted to dry between 65 and 70 °C , for 48 hours. Leaves and stems were mashed and chemically analyzed to determine levels of nutrients such as N, P, K, Ca, Mg, S, Fe, Cu, Mn, B and Na.

To calculate the index of use efficiency (IUE) of macronutrients on aerial plant elements, it was used the following expression: IUE = (aerial plant dry matter)<sup>2</sup>/ accumulated aerial plant nutrient in  $g^2g^{-1}$  (SILVA et al., 2000).

Data was submitted to sequential regression.

#### Results

Levels of Na, N, K and Cu on leaves, and P,K,Ca, B, Cu and Mn on stems, responded to increasing on NaCl concentration.

On leaves, level of Na increased with the increase in salinity, following an exponential relation, while the level of N followed a linear relation. Levels of K and Cu (Cu =  $0,5418Ce^3 - 9,3578Ce^2 + 45,65Ce - 40,932$ , R<sup>2</sup> = 64,1) showed a cubic polynomial regression, in relation with the electrical conductivity.

On stems, levels of B (B = 8,9539-0,1508Ce,  $r^2$  = 87,95) showed lesser values with the increased of salinity, while level of K presented a quadratic polynomial regression, with its minimal around 5,5 dS m<sup>-1</sup>. Levels of Cu and Mn (Mn = -0,0025Ce<sup>3</sup> + 0,0404Ce<sup>2</sup>-0,2155Ce + 0,8724 R<sup>2</sup> = 100) adjusted on cubic polynomial regression, while Ca, showed regression of the fourth grade, in relation with the electrical conductivity.

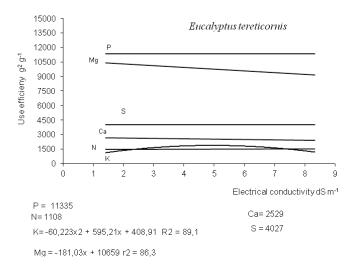


Figure 1. Use efficiency of macronutrients on seedlings' leaves of *Eucalyptus* tereticornis.

The efficiency of using N, P, Ca and S by *E. tereticornis* was not influenced by salinity. Nevertheless, for K had increasing of values of use efficiency up to 4,9 dS m<sup>-1</sup> of electrical conductivity, reducing itself from this mark. The use efficiency of Mg was reduced with the increasing of electrical conductivity of the substrate. The index of use efficiency is a good indicator of the grade of tolerance to salinity. For *E. tereticornis* only the use efficiency of K and Mg were affected. (Figure 1).

#### Conclusions

Seedlings of *E. tereticornis* maintain the use efficiency for nutrients as N, P, Ca and S, through tested levels of salinity between 1,41 a 8,33 dS m<sup>-1</sup>, showing certain tolerance to high salinity conditions.

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# Low temperatures risk analysis for eucalyptus introduction in south east us

Omar Carrero, Lee Allen, José Stape, José Alvarez

#### Background

Biomass production for energy generation has become an important theme. In the SE US, Eucalyptus forests can be a good alternative to help meet this demand in a fast-growing short rotation management. Previous attempts to introduce Eucalyptus in US did not succeed, and low temperatures were identified as the main cause. However, in the last 30 years continued Eucalyptus selection has improved cold tolerance and silviculture practices have improved also. Site selection could be as important as the species selection, in consequence the average of minimum temperature in the coldest month could be used as an indicator for site selection. However, trying to model the extreme temperatures could be even more useful, since the average could hide the extreme values. Two different places could have the same average of the minimum temperature, however in one of them the probability of getting extreme events could be higher. Going farther, the occurrence of extreme events when occurs drastically, could be more critical than just the occurrence of a extreme events whether the plants have had a acclimation period, which make the reaction of plants to frost temperature, site dependent (Hallam, 1989). Cold acclimation could drop the temperature that causes damage, and then it permits the plant to be more resistant, increase the growth length period, and in consequence to have higher survival rates. Almeida et al (1994), related frost resistance with cold hardening period, using different Eucalyptus species and hybrids, founding that hardened plants were more resistant than unhardened plants. Hardened plants had a higher osmotic pressure as a consequence of having a higher concentration of soluble sugars, which drops the freezing point making the plants more tolerant to low temperatures. Levitt (1980) and Steponkus (1990) arrived to the same results. Pitz (2008), studied the relation between hardening period and frost resistance, founding that hardened plants are more resistant to frost, and those plants had a higher concentration of soluble sugars.

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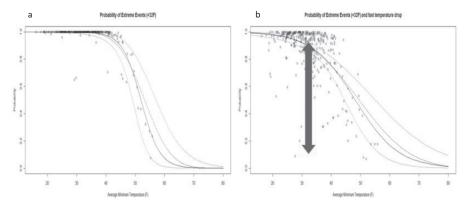
Our objectives were: 1) to assess the usefulness of using average minimum temperatures to indicate frost risk, 2)to determine the main factors influencing the risk of extreme temperature fluctuation events in Southeast US, 3)To identify sites with the lowest risk of extreme temperature events in South East US.

#### Methods

385 weather stations distributed in southern states were selected based on data availability for daily records for total monthly precipitation, minimum and maximum temperature during the last forty years. For each one and for every month the minimum temperature average and the probability of occurrence of 1 or more extreme events were calculated using the Poisson distribution. The probability of occurrence of 1 or more events was calculated as **1-P(0,ë)**.

Two different kind of extreme events are considered in this study. The first type occurs when the minimum temperature drops below a defined value 32 F. The second type of event occurs when, beside the previous condition, the temperature drops form one day to the next in more than 30 F.

Logit and probit functions were used, to understand the relationship between the probability of extreme events and average of minimum temperature, as suggested by Faraway (2006) and Schabenberger and Pierce (2002). Other variables such as: latitude, longitude, elevation, minimum distance to the coast, were also included in the analysis as covariables to assess their influence on risk of extreme events. Akaike Information Criteria (AIC) was used as a measure of goodness of fit.



**Figure 1.** a) Relationship between events under 32 F and b) events under 32 F and a fast drop in temperature with average of type minimum temperature.

An interpolation was performed using ARCMAP v9.3.1, using the average and the probabilities of extreme events to recognize spatial patterns and to identify areas with lower risk for Eucalyptus plantations establishment. Ordinary kriging was performed to interpolate probability of extreme events in the south east US. There were generated different surfaces for the coldest month:

- Average of minimum temperature.
- Probabilities of extreme events (<32 F)
- Probabilities of extreme events (<32 F) and fast drop in temperature

#### **Results and Discussion**

Average minimum temperatures are a good indicator of extreme events risk when events under 32 F are considered ( $R^2=0.78$ ). However, when considering a fast drop in temperature, average minimum temperature alone is inadequate to explain the risk of extreme events ( $R^2=0.53$ ) (Include Figure 1a and 1b). For the same average minimum temperature there is a large variability in the risk of a rapid drop in temperature. Consequently, site selection should include examination of average minimum and risk of rapid drops in temperature for the best site selection.

Precipitation and distance to the coast were statistically significant (p < 0.0001) I. The spatial distribution of frost risk for the two kind of events are quite different (Include Figure 2a and 2b). More southerly sites do not necessarily have lower risk when considering rapid temperature drops. Sites in FL, South Louisiana, GA, and SC coast are the most attractive sites to establish Eucalyptus in SE US.

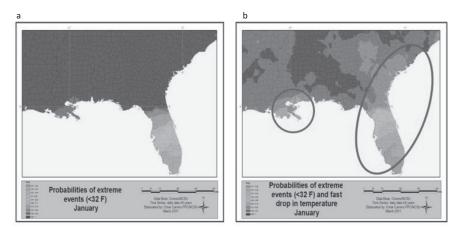


Figure 2. a) Exteme events probabilities under 32 f; b) Extreme events probabilities when fast drop in temperature is considered.

#### Conclusions

• Two different sites could have the same average minimum temperature but different risk.

• Average minimum temperature is not a good indicator for extreme events risk.

• Other variables like Average Precipitation and Distance to the Coast have an influence on risk.

• Areas with lower risk are located in FL, LA and coastal areas in GA and SC.

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## Standardization and optimization of core sampling procedure for carbon isotope analysis in eucalyptus and variation in carbon isotope ratios across species and growth conditions

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#### Background

Plants discriminate against the use of heavy isotope of carbon during photosynthesis (O'Leary, 1981). Theory linking carbon isotope discrimination ( $\Delta^{13}$ C) with plant water use efficiency has been well established (Farquhar et al., 1989) and is extensively being used as a time averaged surrogate to screen for water use efficiency in plants. However, accuracy of the results depends to a large extent on the procedure adopted in sampling. In trees,  $\Delta^{13}$ C has been shown to vary within the canopy, length of the branch, segment of the core collected from the trunk (Waring & Silvester, 1994). Therefore, as an initial step towards assessing clonal variations in water use efficiency among eucalyptus clones/germplasm lines, it is essential to quantify the variability amongst genotypes and standardize the sampling procedure appropriate for the determination of the carbon isotopic ratios of the species.

#### Objectives

The main objective was to standardize and optimize the sampling procedure for carbon isotope analysis in eucalyptus. The other objective was to determine the variability for carbon isotope fractionation across species and growth conditions.

#### Methodology

Two experiments were conducted to address the above objectives. In the first experiment, two species namely, *Eucalyptus grandis* and *E. urophylla* with five half sib families were selected to represent each species. Each family was replicated by taking three trees that were five years of age. Two core samples were collected at breast height from each of the trees spanning periphery to periphery (the stem diameter). Core samples were made in North-South and East-West directions and divided into half to represent North, South, East and West aspects. A sub- sample

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from each tree was further divided into 5 segments of equal length to approximately represent each growing year.

In the second experiment, 3 clones of *E. camaldulensis* and one clone of "*E. urograndis*" represented by 5 trees each were selected from separate plots grown under well irrigated and rainfed conditions. The samples were drawn as mentioned above and were oven dried, homogenized and used for the determination of carbon isotopic ratio using a continuous flow Isotope Ratio Mass Spectrometry (IRMS). Data was analyzed using SAAS/STAT software, version 9.1 of the SAS system for windows

#### **Results and Discussion**

The  $\Delta^{13}$ C values differed significantly among the families of *Eucalyptus grandis* and *E. urophylla*. Similarly, the fragments of the core samples also showed considerable variations in each family of both the species for  $\Delta^{13}$ C (Table 1). These results indicate genetic variation in  $\Delta^{13}$ C. Since the genotypes within each half sib family were derived from seeds, diversity amongst the trees is expected. Such family and genotypic differences for  $\Delta^{13}$ C was also observed by Osorio and Pereira (1994) and Li (1999 and 2000) in *Eucalyptus microtheca* and *E. globulus* respectively. The results also revealed that, genetic composition as well as the growing conditions significantly influenced the carbon isotopic signatures. However, the  $\Delta^{13}$ C values of the core samples taken from each aspect did not differ significantly indicating that the direction of the core had no influence on the  $\Delta^{13}$ C values (Table 1 & Fig 1).

This finding was further confirmed by sampling clonally propagated trees of *E.* camaldulensis and "*E.* urograndis" grown under well watered and rainfed conditions. The  $\Delta^{13}$ C values displayed significant clonal effects and the rainfed trees showed

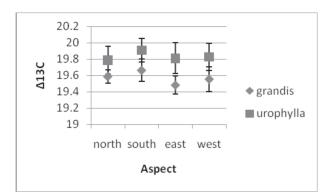


Figure 1. Carbon isotopic ratios at different aspects for E. grandis and E. urophylla.

generally lower  $\Delta^{13}$ C values than the irrigated trees (Fig 2). Interestingly, one of the clones (clone 6) displayed significantly lowered  $\Delta^{13}$ C values under rainfed conditions, perhaps an indication of its higher water use efficiency. In this experiment, there were also no significant differences observed between the directions of the core samples (Fig 3). The results therefore clearly indicated that the core samples can be taken from any direction at breast height for sampling eucalyptus trees for carbon isotope measurements.

Variable	N	Level of significance detected
Species	2	P<0.05
Family within Species	5	p<0.0001
Genotype within Family	3	p<0.0001
Aspect	4	NS
Fragment within Tree	5	p<0.01

**Table 1.** Summary of effects of variables on  $\Delta^{13}$ C values of core samples.

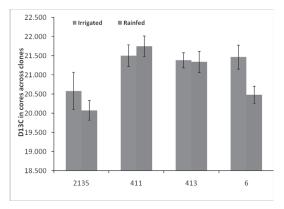


Figure 2.  $\Delta^{\rm 13} \rm C$  values across different clones both under irrigated and rainfed conditions.

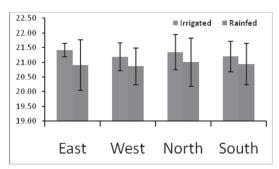


Figure 3. Core sampling aspect and mean  $\Delta^{13}$ C signatures across clones of Eucalyptus grown under irrigated and rainfed conditions.

#### Conclusion

Although there are species, family, growth conditions and genotypic differences in  $\Delta^{13}$ C values, the isotopic signatures are not influenced by the aspect of the sample. Hence, it can be inferred that the core sampling in Eucalyptus can be made from any direction at breast height.

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## Inter-clonal variability in fine root distribution in very deep soil layers: a poorly explored criterion for tree breeding in regions prone to water stress

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#### Summary

Eucalypt seedlings planted on highly weathered soils without impediment to root growth are likely to exhibit a very fast displacement of the root front. A chronosequence study showed that the deepest roots were at a depth corresponding to about 75% of the mean stand height in seedling plantations established in southern Brazil (Christina et al., 2011). Very deep roots can have an important role to supply water during dry periods (Christina et al., 2011; Mendham et al., 2011), and tracer applications showed that they can have a higher capacity than superficial roots to take up analogues of potassium and calcium (da Silva et al., 2011). Moreover, exceptional droughts can lead to the mortality of large plantation areas (several hundred hectares) for clones with growth strategies unable to make sparing use of water resources. Much attention has been paid on leaf characteristics (in particular water use efficiency at the leaf scale) to select clones for dry regions. However, inter-clonal variability in root development making it possible to take up large amounts of water stored in deep soil layers after clearcutting is poorly known. The objective of our study was to assess the inter-clonal variability in root density down to the root front in 2-year-old clonal tests set up in the EUCFLUX project (http://www.ipef.br/eucflux/). Three positions at different distances from trees were sampled down to a depth of 13.5 m for 3 clones (hybrids of *E. grandis*) selected by 3 companies for contrasted climates in Brazil, and 1 improved *E. grandis* seedling. Gravimetric soil water contents were determined every 0.5 m at each position to estimate the capacity of the different clones to withdraw water from deep soil layers.

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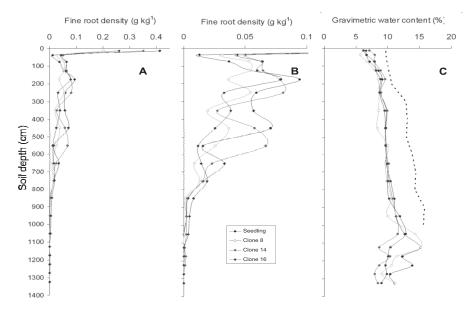
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**Figure 1.** Fine root distributions (A) down to the root front for 3 clones and 1 seedling at age 2 years in southern Brazil (Eucflux projet). Mean values for 3 independent positions (3 distances to different trees) are indicated. The scale in figure B has been changed to improve the visibility in very deep soil layers. Gravimetric water contents are indicated down to a depth of 13.5 m and a dotted line shows the values on 14/02/2010, during the first rainy season after clearcutting (C).

Fine root densities dropped from 0.2-0.4 g kg<sup>-1</sup> in the upper soil layer to values < 0.1 g kg<sup>-1</sup> below a depth of 25 cm, irrespective of the genetical material (Figure 1). Large inter-position variability led to non-significant differences between genetic materials, whatever the soil layer. Seedlings and clones exhibited a similar pattern of fine root development in deep soil layers. However, a trend of higher mean fine root density for the seedlings and clone 14 than for clone 8 was observed. Soil water content profiles showed that all the genetical material had the capacity to withdraw water down to depth > 9 m the 2 first years after planting. Clones 16 and 14 seemed to take up water down to 11.5 m whereas the maximum depth seemed to be 10 m for the seedlings and clone 8. This pattern was consistent with the maximum depth where roots were found for each genetic material. However, the variability in soil water content below a depth of 10 m also might reflect differences in soil texture among the sampled plots. The capacity for certain clones to withdraw water in deeper soil layers than other clones might be useful to take into account in the breeding programs. Such a strategy might contribute to explain differences in survival among clones during exceptional droughts.

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POSTER SESSION 3. GENETICS

## The influence of tree improvement and cultural practices on dry timber production of *Eucalyptus* pulpwood plantations over a rotation in temperate South Africa

Jacob Crous<sup>1</sup>, Louisa Burger<sup>2</sup>, Giovanni Sale<sup>3</sup> and Wesley Naidoo<sup>4</sup>

#### Background

In order to remain competitive on a global scale it is necessary for forestry companies to increase the yield per hectare and work towards reducing the unit cost of timber (Pallet and Sale, 2004). Operational gain is a concept that incorporates components of the deployment process (Pallet and Sale, 2002) that can be used to minimize environmental stress and maximize resource utilization to increase the final stand yield. These include efficiencies in tree breeding, propagation, site-species-genotype matching, plant use efficiency, stand density, nutrition and vegetation control. The aim of this study was to investigate the effects of and interactions between silvicultural practices and the deployment of improved genotypes on dry timber production over a rotation across a range of sites (Boreham and Pallett, 2009).

#### Methods

A trial series with a 2<sup>4</sup> factorial design with two replications at each site was established across five sites within the temperate areas of KwaZulu-Natal in South Africa. These sites were selected to cover various productivity classes. Two of the trials were destroyed before they reached clearfelling age. Of the three trials that reached clearfelling age, Hodgsons and Tweedie were located on SQII sites (above average) and Highflats on a SQIV site (below average). The four main treatments, each tested at a superior and inferior level, were species selection (a first choice species, based on operational site-species matching criteria relative to an alternative species), genetic improvement (improved genetic material relative to unimproved material), silvicultural intensity (intensive treatment receiving 2 litres water, starter fertilizer and two additional weeding operations during the first 48 months relative to the less intensive treatment) and level of site capture (1667

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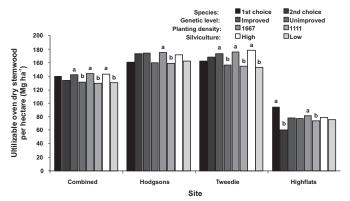
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stems ha<sup>-1</sup>relative to 1111 stems ha<sup>-1</sup>). Tree height and diameter at breast height was regularly assessed. At rotation age wood core samples were collected from selected trees. The basic wood density from these cores was used to calculate utilizable dry timber production at rotation age. ANOVA was used to investigate main treatment effects and first-order interactions.

#### **Results and Discussion**

The combined results of the three trials indicated that basic wood density was significantly (p=0.013) greater at 0.498 g cm<sup>-3</sup> for the first choice species compared to 0.486 g cm<sup>-3</sup> for the second choice species. None of the other main effects had a significant effect on basic wood density.

The utilizable oven-dry stemwood production data from the combined dataset indicated that except for the species choice the superior level of all three other main treatment effects significantly (p<0.05) outperformed the inferior level (Figure 1). Analysis of individual trial datasets indicated that at Hodgsons the planting density had a significant effect (p<0.05) on stemwood production. The effect of species selection and genetic improvement was only significant at p<0.10. Both *E. dunnii* (first choice) and *E. grandis* (second choice) are suited to the growing conditions at Hodgsons and market preference was the main driver for the species selection. At Tweedie all the main effects except species selection significantly affected stemwood production (Figure 1). The two species planted on this site, *E. smithii* (first choice) and *E. dunnii* (second choice) are also equally suited to the site



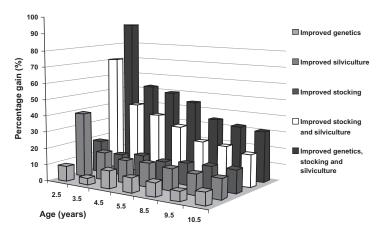
**Figure 1.** Mean oven-dry utilizable timber production at rotation age (10.5 years) across three sites as affected by site-species matching, tree improvement, planting density and silvicultural intensity. Different letters indicate significant differences between treatments within each site at the 5% level.

and can explain the lack of species response at this site. At Highflats, the low site quality site, species choice and planting density had a significant effect (p<0.05) on dry timber production at the end of the rotation (Figure 1). A significant (p=0.04) species x silviculture interaction was observed at this site as *E. dunnii* produced 10% more dry timber on the intensive silviculture plots compared to the less intensive treatment, while the *E. macarthurii* dry timber production was not affected by the silvicultural treatment.

The mean gains associated with genetic improvement, silvicultural intensity and level of site capture were investigated over time in the three trials that reached rotation age. The combined gain associated with the superior level of these three factors decreased from 93% at the age of 2.5 years to 31% at the age of 10.5 years (Figure 2). At rotation age improved genetics resulted in an 8% gain, improved silviculture in a 12% gain and improved stocking in a 14% gain in dry-timber production. Over time the gains associated with silviculture decreased, while that from genetic improvement and stocking remained relatively constant. Thus, expressed as a relative proportion of gain the relative contribution of genetic improvement and stocking increased over time.

#### Conclusions

The positive growth responses associated with genetic improvement, intensive



**Figure 2.** The mean gains in oven-dry utilizable volume per hectare over a rotation across three sites associated with improvements in genetics, silviculture, stocking plus silviculture and genetics plus stocking plus silviculture. The base (check) is unimproved genetic material established at 1111 stems ha<sup>-1</sup> that received a low level of silviculture (no fertilizer at planting and less frequent weed control).

silviculture and optimum planting density was maintained to rotation-age although the relative gain from the various treatments decreased over time. The trial series clearly demonstrated the additive response to treatments that minimize stress and maximize resource utilization. There was in general an absence of significant interactions between the treatments.

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# Performance of association genetics and genomic selection of wood physical traits in large populations of Canadian mature spruces

Jean Beaulieu\*,\*, Trevor Doerksen\*,\*, John MacKay\*, and Jean Bousquet\*

Spruces extend over most of Canada's boreal biome and are the most important forest resource (53% of wood volume used) for the Canadian forest industry. About 200 M spruce seedlings are planted yearly, which represents close to 60% of the total reforestation effort. There are active spruce breeding programs in many Canadian provinces with over 20 breeding populations assembled. In the past, emphasis has been put on improvement of growth and adaptive traits and the inclusion of wood quality traits in selection criteria is just beginning. Given the time delays and high costs for evaluating these traits, genomics-assisted selection could contribute positively in increasing gains per unit of time. In order to develop molecular breeding tools for wood quality in white spruce, one of the main reforested species in Canada, a pilot study has recently been completed to test for associations between single nucleotide polymorphisms (SNP) of candidate genes and wood traits. A discovery population of 1700 white spruces has been assembled from a 30-yearold provenance-progeny trial comprising 215 open-pollinated families. Increment cores were collected to assess wood physical traits using SilviScan technology. A subset of these trees were assayed for SNPs discovered in 550 candidate genes. A small proportion of the SNPs were found to be significantly associated with wood traits and the percentage of variance explained reached up to 11% with approaches combining several SNPs. Transcript accumulation levels were also determined for genes containing SNPs significantly associated with wood traits. In some cases, significantly different transcript levels were found among genotypic classes. Xylempreferential RNA accumulation was shown for the majority of these genes, which indicates that expression data were relevant for selecting candidate genes.

A larger genotyping assay including SNPs belonging to about 2500 candidate genes was developed to genotype the entire population. A total of about 7000 SNPs were retained for analysis as they met quality and minor allele frequency criteria. Association studies were carried out using a mixed linear model approach on

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single and multiple SNPs. Results of SNP-by-SNP analyses showed that between 100 and 200 SNPs were significantly associated (P d'' 0.01) with single traits related to earlywood, latewood or total wood. The variation explained by most significant SNPs for each trait varied between 1.1 and 3.3%. However, the total number of significant SNPs after correction for multiple testing for all the traits was reduced to 66 with a false-discovery-rate of 0.10. Multilocus Bayesian mixed models analyses were also tested and results indicated that about 20 to 25% of the phenotypic variation could be explained using 40 to 60 SNPs.

Prediction of genetic effects was also tested with models that include 1) polygenic information (pedigree information) only, 2) genomic information only, and 3) both polygenic and genomic information, using a Bayesian framework from posterior distributions with Gibbs sampling. Predictive value of the three models was estimated by cross-validation with within-family samples. Two parameters were estimated: predictive ability (correlation between estimated and observed phenotypes) and accuracy (correlation between individual genetic values and their estimates). Results will be presented and discussed in the context of the current breeding programs.

# Genetic variation for growth in a *Eucalyptus pellita* breeding population in Colombia

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#### Objectives

*Eucalyptus pellita* is one of the most important species in the tropical savanna regions of the world, especially due to its fast growth and sanity. In Colombia it has been studied more systematically since the 90s especially by the company Refocosta in collaboration with CONIF (Nieto and Gasca, 2010). The present study reports a genetic analysis of growth based on a two year old progeny trial which includes open pollinated families of PNG origin, and bulked seedlots from Australia. The study is complemented by an adjacent clonal trial using cloned plus trees previously selected from material from the same PNG sources. These trials are currently the main source of genetic material for further genetic improvement in Colombia.

#### Metodology

The progeny trial includes 13 open pollinated families of Papua New Guinea origin, collected from an older arboretum established in Colombia and four bulk seedlots from Australian provenances (Table 1).

The clonal trial comprises clones of 32 plus trees, which have been previously phenotypically selected from commercial plantations. These plantations were derived from seed from the AUS+PNG arboretum as the families in the progeny trial.

Progeny and clonal trials are located close to the city of Villanueva (Orinoquia region, Colombia). The progeny trial was established as randomized complete

			1 1.	0 7
Code	Locality	Lat	Long	Alt
18750	Wonga (Daintree, AUS)	16 16 S	145 2 2 E	15
14916	Nth Kuranda (AUS)	16 49 S	145 38 0 E	400
18313	Starcke Stn (AUS)	15 05 S	145 12 0 E	30
13826	Bloomfiend (Daintree, AUS)	10 04 S	145 19 E	200
All others families	Local arboretum originally of mainly PNG origin	Unknown	Unknown	Unknown

Table 1. Details of the provenances and families tested in E. pellita progeny trial.

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replicates, with 18 replicates. Each family was represented in each replicate by a 6-tree row plot, with a 3 x 3m spacing. The clonal trial had no formal experimental design and clones were established as unreplicated plots of 8 to 164 trees, each one of at least 4 rows wide. Only the measurements made on the plot's inner trees were included in the analysis to avoid effect of competition. A commercial seedlot was included.

Trees were measured for diameter at breast height at age 24 months in the progeny trial, and at age 19 months in the clonal trial. The individual tree data from the progeny trial were analyzed using a mixed linear model with replicates considered fixed and families and plots considered random. Family variance was only fitted to the 13 open pollinated families. Given the regular grid structure of the trial, the residuals were decomposed in a spatially dependent term and a spatial independent residual. A covariance structure assumed independent first-order autoregressive processes for rows and columns. The unreplicated clone trial was analyzed using a simpler linear model, where only clones were fitted, as random effects. The program AsRemI was used to obtain the variance components and solutions for the two models. Narrow sense heritability was calculated assuming an intra-class correlation between open pollinated family members of r=1/3.

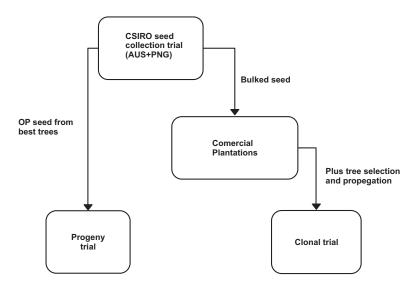


Figure 1. Origin and relationship between the progeny and clonal material tested in the current study.

#### **Results and Conclusions**

Survival was 81% at 2 years of age and average tree size was 8,5cm diameter and 8,7m of height at age two. In the progeny trial, the four seedlots of Australian origin were inferior to the average of the PNG families (Table 2). This provenance difference is consistent with other studies (e.g. Harwood, 1998). However, it may be enlarged here since families have been previously selected from the arboretum.

In the clonal trial, growth at 19 months was 12cm in diameter, with an overall survival of 95%. The comparison between the control seedlot and clonal average provides an empirical measure of the realized gains from clonal mass selection. The results indicate clones were 8% better in diameter growth which corresponds to approximately a 20% gains in volume. The analysis of variance indicated low additive genetic variance in this population, with h2 =0,04. Clonal heritability (amongst clones) was higher, with H2=0,21, suggesting there is a significant amount of non-additive genetic effects in this species (Table 3).

Spatial autocorrelation and replicate effects were near zero in the progeny trial suggesting site conditions were very homogeneous. The slight negative AR indicates that inter-tree competition may have already started. The homogeneous site

Source	Locality	Dbh
18750	Wonga / Daintree	7,16
14916	N. Kuranda	8,28
18313	Starcke STN	6,23
13826	Bloomfiend / Daintree	8,31
	Families (PNG origin)	8,83
	SEdiff	0,38

**Table 2.** Mean provenance effects and corresponding standard error of differences, for height and diameter measured at age 24 months.

 Table 3. Variance components, heritability and spatial autocorrelation parameters

 for each variable (height and diameter) measured at age 24 months.

Term	Dbh
Progeny trial	
Fam	0,105
Plot	0,350
Residual	7,671
AR(col)	-0,03
AR(row)	-0,12
h²	0,04(0,03)
Clonal trial	
Clone	0.664
Residual	2.489
H <sup>2</sup>	0.21 (0.06)

conditions indicate the clonal trial, which was adjacent to the progeny trial, should provide reasonable estimates of clone effects and variances, despite not being replicated.

The results confirm a significant realized gain has been obtained from the previous plus tree selection and propagation program carried out by Refocosta. However, the existing genetic resources, such as the progeny trial analyzed, seem to have low exploitable genetic variation and an infusion with unrelated material seem desirable.

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## Genetic diversity within and between subpopulations of Handroanthus heptaphyllus (vell.) mattos by microsatellite markers

Neide Tomita Mori<sup>2</sup>, Mario Luiz Teixeira de Moraes<sup>3</sup>, Caroline Midori Morita<sup>4</sup>, Edson Seizo Mori<sup>5</sup>

### Introdução

Handroanthus heptaphyllus (Vell.) Mattos is known by ipê-roxo, belonging to the Family Bignoniaceae. It is an important Brazilian forest tree species, because of their excellent wood quality, and medicinal products. It has been used in reforestation, landscaping, restoration, and reforestation in riparian forests, because the species develops well on humid soils. (MOREIRA & SOUZA, 1987; ETTORI, 1996).

The objective of this study was to investigate the genetic diversity within and between subpopulations of *Handroanthus heptaphyllus* by microsatellite molecular markers allowing the use of strategies in breeding program and germplasm conservation (BROWN, 1978).

### **Material and Methods**

 $\hat{A}_e = \frac{1}{1 - \hat{H}}$ 

We used DNA of 192 seedlings by seeds from 30 trees of *Handroanthus heptaphyllus*, through Botucatu region, São Paulo State, Brazil. In the total were analyzed eight polymorphic microsatellite loci using primers transferred from *Handroanthus aureus* developed by Braga et al. (2007).

Through the analysis of microsatellites loci were estimated allele frequencies ( $\hat{p}_i$ ), the average number of alleles per locus ( $\hat{A}$ ), obtained by arithmetic average of studied loci; effective number of alleles per locus (; Nei, 1987); observed heterozigosity ( $\hat{H}_o = 1 - \sum P_{ii}$ ); expected heterozigosity ( $\hat{H}_e = 1 - \sum p_i^2$ ), and fixation index ( $f = 1 - \frac{\hat{H}_o}{\hat{H}_e}$ ), based on Weir (1996). The genetic structure between populations was estimated based on statistics of Nei (1978), where:  $F_{IT}$  is the total genetic diversity;  $F_{ST}$  is the genetic differentiation among subpopulations, and  $F_{IS}$  is the genetic diversity within subpopulations, estimated by TFPGA software (Miller, 1997).

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### **Results and Discussion**

The numbers of alleles per loci ranged from six (TAU22 locus) to 14 (TAU12, TAU30, and TAU31 loci). The average of expected heterozygosity ( $H_e$ ) for the six subpopulations was 0.7318, the observed heterozygosity ( $H_o$ ) was 0.6183, and the average of fixation index (f) ranged between subpopulation from -0.082 (subpopulation 4) to 0.255 (subpopulation 3), with average of 0.152. The observed heterozygosity ( $H_o$ ) was less than expected heterozygosity ( $H_e$ ) for practically all subpopulations (Table 1). The exception occurred for subpopulation 4, that has shown a higher amount of heterozygosities, indicating inbreeding.

The average of fixation index (*f*) ranged between subpopulation from -0.082 (subpopulation 4) to 0.255 (subpopulation 3), with average of 0.152. The genetic differentiation among subpopulations ( $F_{st}$ ) was low (0.0538), indicating that the most of genetic diversity is within subpopulations (94.62%). Then, to collect germplasm it would be interesting a bigger sample into the subpopulations, and smaller samples of subpopulations to well sampled the species. The subpopulations have shown there is enough genetic diversity to support programs of genetic breeding and germplasm preservation.

When we observe the fixation index ( $\hat{f}$ ), almost all subpopulations have presented inbreeding, because they showed positive values with average of 0.1520; the excep was the subpopulation 4 that presented slight negative value (-0.0819), meaning to exist excess of heterozygous into the population. In relation to all populations, the inbreeding average was 0.2051.

The total diversity ( $F_{_{1T}}$ ) was 0.2229 (± 0.0195), the genetic differentiation among subpopulations ( $F_{_{ST}}$ ) was 0.0538 (± 0.0081), and the diversity within subpopulations ( $F_{_{sT}}$ ) was 0.1787 (± 0.0183).

Subpopulation	$\hat{H}_{e}$	$\hat{H_o}$	$\hat{f}$
1	0,7726	0,6249	0,1911
2	0,7371	0,6119	0,1698
3	0,7071	0,5271	0,2546
4	0,6748	0,7301	-0,0819
5	0,7613	0,6108	0,1977
6	0,7380	0,6048	0,1805
Average	0,7318	0,6183	0,1520
All Subpopulations	0,7858	0,6246	0,2051

**Table 1.** Value of expected heterozygosity  $(\hat{H}_e)$ , observed heterozygosity  $(\hat{H}_o)$ , and fixation index  $(\hat{f})$  of six *Handroanthus heptaphyllus* subpopulations.

### Conclusões

The most genetic diversity is within subpopulations (94.62%) and to collect *H. heptaphyllus* germplasm it will be interesting to do a bigger sample into the subpopulations, and smaller samples between subpopulations, to sample it well.

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# Estimates of genetic parameters for *Eucalyptus globulus* in Colombia

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### Background

Eucalyptus globulus finds excellent growing conditions in some parts of the Colombia, especially around the so-called Altiplano Cundiboyacense, in the Andean regions NE of Bogotá, at altitudes ranging between 2000 and 2800m asl. In 2008, a series of Eucalyptus globulus progeny trials were established at three locations in the region of Boyacá (Andean plateau, NE of Bogotá, Colombia). The open pollinated families were collected from a seedling seed orchard, also located in Colombia (La Florida, in Cundinamarca region). This orchard was formerly a progeny trial which included 106 families from 15 native provenances from Tasmania and Bass Strait Islands, and 52 families from three Colombian locations and considered here as a local land races. The three progeny trials studied here include 129 families from the La Florida Seed Orchard, 80 from mothers of Australian origin and 49 from mothers of Colombian land race origin. Four control seedlots were also included (one from CMPC, Chile, one from Forestal Probosque, Chile and two bulked seedlots from the La Florida orchard, Colombia).

### Objectives

To compare the merit of different seed sources relative to the local land race and estimate genetic parameters (heritability and correlations between traits and sites) for growth at three locations, from ages ranging between 9 and 36 months). The results will support the breeding effort for the species in Colombia.

#### Metodology

Open pollinated families were collected at La Florida orchard and were established at three locations (Santa Rosa, Firatoba and Tuta; Table 1). In each

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trial, families were established as randomized complete replicates with 15 replicates and single tree plots per replicate. Plants were established at a spacing of 3 x 3m and fertilized at planting with 250g of a local organic fertilizer and 100g of Superfosfate. Maintenance included control of weeds using manual and chemical control (Roundup) every six months.

Variables measured included height at 9, 12, 24, 31 and 36 months DBH and volume at age 36 months. Volume at age 36 months was estimated using the equation  $V = 0.0003D^{1.82810} \times H^{1.17322}$  (Gilabert, 2010). Families were grouped in subraces according to the classification by Dutkowski and Potts (1999). Details of family backgrounds are given in Table 2.

Sitio	Santa Rosa	Firavitoba	Tuta
Departmento	Boyacá	Boyacá	Boyacá
Latitud	5º 51′N	5º 43′ N	5º39′N
Longitud	72º59' W	73º 00 W	73º09' W
Elevation (m)	2724	2914	2800
Rainfall (mm)	972.9	663	827.1
Area (ha)	2.5	2.5	2.5
Establishment date	May 2008	May 2008	May 2008
No. Of Families	129	127	128
Control seedlots	4	4	4

Table 1. Details of the progeny trials.

 Table 2. Genetic grouping of families in the trials, according to race, sub race (viz Dutkowski and Potts, 1999), and locality.

Raza	Subraza	Origen	No. de Familias
Furneaux	Flinders Island	South Flinders Island	3
King_ls	King Island	King Island	5
NE Tasmania	Inland NE Tasmania	Jericho	2
		Pepper Hill	3
	NE Tasmania	Denison	2
		Seymour	6
	St. Helens	St. Helens	6
SE Tasmania	SE Tasmania	Chanel	10
		Rheban	9
		Uxbridge	3
Southern Tasmania	Southern Tasmania	Bruny Island	8
		Geeveston	7
		Leprena	7
Western Tasmania	Western Tasmania	Henty River	5
		Macquary Harbour	4
Colombia	Boyaca	Boyaca	11
	Cundinamarca	Cundinamarca	33
	Nariño	Nariño	5
Chile 1	Chile 1	CMPC	1
Chile 2	Chile 2	Probosques	1
Florida	Florida	Mezcla Huerto Fams 1, 130, 83, 7	76 1
Florida	Florida	Mezcla Huerto	1

Variances and corresponding heritabilities were estimated separately for each site and age, based on a mixed linear model considering subrace and replicates as fixed and families as random effects. A second multisite analysis was performed for height, DBH and volume at age 36 months, having previously normalized data to a constant phenotypic standard deviation. Site, replicate within site and subrace were fitted as fixed, and family and family x site interaction were fitted as random effects. In both analyses, the control seedlots were initially excluded. A subsequent BLUP analysis was conducted where the control seedlots were included, to test for significance of effects. Analyses were carried out based on REML, using SAS software.

#### **Results and Conclusions**

Growth rate differs significantly amongst the three sites, with Santa Rosa > Firavitoba > Tuta (means heights at 3 years of age was 11, 7 and 5m respectively). However, the study found no significant native or local race effects at any age and site. Therefore, current breeding population can be considered a homogeneous group for growth. The fact that the native open pollinated families have been pollinated by a mixed source of pollens may have contributed for the small race effects detected.

Heritability estimates for height at each site, between 9 and 36 months, had a tendency to improve with age, from 0,08-0,20 at 9 months to 0,15-0,33 at age 36 months. At 36 months, heritability for volume was 0,25 in Tuta, 0,20 in Firavitoba and 0,17 in Santa Rosa (with typical standard errors of 0,05). Genetic (type-B) correlation for volume amongst sites was 0,73 suggesting a stable genetic expression of growth across the three sites.

This is the first thorough genetic analysis carried out for this species in Colombia. The trials and the results obtained so far are part of an ongoing collaborative breeding effort between Smurfit Kappa Cartón de Colombia S. A., Reforestadora El Guásimo, Reforestadora del Caribe S. A. S, Corporación Nacional de Investigación y Fomento Forestal – CONIF, Universidad Distrital - Francisco José de Caldas, and the Ministerio de Agricultura y Desarrollo Rural.

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### Did you ask for something small? The microRNAs power in a Eucalyptus tension world! (the microEGo project)

Paiva, Jorge A. P.

Wood is a complex and highly variable tissue, which anatomical, chemical, physical and technological features are ontogenic and environmentally controlled. In response to a gravitropic stimulus, Angiosperms differentiate a tension wood. This type of wood shows specific characteristics such as the presence of a gelatinous layer (layer-G), with high cellulose content (~95%) presenting high crystallinity, and their microfibrils are oriented nearly parallel to axis of the fibers. Tension wood is considered an excellent model to study the regulation of the formation of xylem cell walls (eg biosynthesis and deposition of cellulose, lignin and other biopolymers) and how this regulation is reflected in the final properties of wood. The ability to drive wood quality is fundamental for Portuguese economy. Portugal is one of the largest producers of pulp derived from eucalyptus (E. globulus), with exports accounting for about 5% of GDP. It is also relevant that *E. globulus* represents about 1/3 of the Portuguese forest and that the use of adequate genotypes will optimize the exploitation of available areas. The understanding of the molecular mechanisms involved in the biosynthesis of the cell wall is of great importance not only for future production of pulp and paper, but also for the production of biofuels and bio-materials. Many of the genes involved in wood formation have been catalogued, but the mechanisms to regulate this process of development are still far from being elucidated. The discovery of microRNAs (miRNAs) and their functions opened new frontiers for a better knowledge on the mechanisms of regulation of many developmental processes, including cambium differentiation. However, of the approximately 8,600 miRNA available in the database miRbase (http:// microrna.sanger.ac.uk/sequences/), none of the entries relate to the most economically relevant Portuguese forest species, such as *Pinus pinaster*, *Quercus* suber and Eucalyptus globulus.MICROEGO project aims identifing and characterize E. globulus miRNAs involved in the regulation mechanisms of wood formation and their target genes, using as a model the tension wood forming tissues. The long

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term aim is to use this information to devise new ways to control the quality of wood produced by *E. globulus* and to provide the breeding programs with tools to direct their work to the selection/production of genotypes with desired wood qualities. This 3 years project combines the phenotypic characterization of the developing xylem with the latest genomic strategies/tools available, such as next generation sequencing technologies, gene expression analysis, bioinformatics, physical mapping, and functional analysis. The specific objectives of this project include:1) Development and application of new non-destructive technologies and methodology to chemical characterize wood forming tissues;2) anatomical characterization of wood forming tissues subject to a gravitropic stimulus;3) Production of expressed sequences catalogues, in particular miRNAs catalogues, and to characterize de genomic sequences of these transcripts;4) validation of miRNA candidates and their target;5) To provide spatial expression information for selected miRNA and targets; 6) to map target genes and miRNA onto physical maps;7) to provide new insights trough functional analysis in transient and stable transgenic system;8) To boost the competitiveness of national institutions in national and international scientific and technical panorama, by reinforcing and establishing new national and international collaborations with industry and academia. The project will benefit from the collaboration of an important Portuguese industrial partner (ALTRI FLORESTAL SA, Portugal) and internationally renowned laboratories(UMR5546, France). Their inclusion in this project will not have additional costs, and will be very important for their technical and scientific expertise, the availability of certified clones and installation of the experimental device. This project will also greatly benefit from technical and scientific expertise and synergies resulting from the collaboration between the different national and international partners, IBET, IICT, INESC-ID, UMR5546 and ALTRI FLORESTAL SA in the areas of phenotype characterization, functional genomics, bioinformatics, molecular biology and forest genetics and . Advanced training are being promoted in form of PhD and Master thesis. The project has as international consultants Dr. Francis Martin, and Dr. Tamas Dalmay with renowned expertise on the field of Eucalyptus genomics and miRNA regulation.

POSTER SESSION 4. ECOPHYSIOLOGY AND HYDROLOGY

## The effect of propagation methods on some growth and physiological characteristics of seed- and vegetatively propagated Eucalyptus varieties

Oscar Mokotedi<sup>1,2</sup>, Paula Watt<sup>2</sup> and Norman Pammenter<sup>2</sup>

The purpose of this study was to deepen our understanding of how propagation methods (seed- vs. micro- vs. macro-propagation) influence root characteristics (hydraulic conductance and anchorage) and above-ground performance (survival, growth and gas exchange). Seed-propagated Eucalyptus grandis and E. nitens, as well as micro-propagated and macro-propagated *E. grandis* xnitens, were grown in the field and maintained according to standard silvicultural practices. During the field trial, there was prolonged drought and occasional frost, and under these conditions micro-propagated plants showed the lowest survival rate. Responses in leaf gas exchange (instantaneous, light- and CO, –response curves) were similar between micro-propagated and macro-propagated plants, but differences were recorded between seed-propagated *E. grandis* and *E. nitens*. When normalised by leaf area, specific root hydraulic conductance was similar across all plant types, although roots of macropropagated plants were 32% more efficient in conducting water to the leaves than those of the micropropagated ones. At 16 months, vertical uprooting resistance was significantly lower in micro-propagated than in macropropagated and seed-propagated trees. Half of the uprooted macro-propagated trees produced an equivalent of the tap-root (i.e. 'tap-sinker') which increased uprooting resistance significantly, none of which was found in micropropagated plants. All vegetatively propagated trees without tap-sinkers showed little resistance during vertical extraction, and their roots were generally asymmetrical and shallow in the soil. Vegetatively propagated trees produced few and thick I-beam shaped, longer lateral roots, compared with the thin and numerous T-beam shaped roots of seed-propagated trees. The number of roots as well as root cross sectional area at the root-shoot junction had a significant effect on the maximum force required to vertically extract roots. These findings may have implications in terms of plantation practices, for example planting vegetatively propagated clonal eucalypts at higher density than seedlings may improve anchorage by facilitating

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rapid root ground cover. Furthermore, the current *in vitro* methods used for producing micro-propagated plants may need to be reconsidered for improved *ex vitro* root characteristics; at present micropropagated plants may be unsuitable for planting across sites with strong winds.

# Interaction between rainfall and potassium availabilities on the cycles of C, H<sub>2</sub>O and nutrients in *Eucalyptus grandis* plantations. Preliminary results on tree growth.

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### Summary

Many Eucalyptus plantations in tropical countries are located on potassiumdeficient soils and in regions subject to water deficit. The interactions between water and potassium (K) deficiencies on the growth and ecophysiology of *Eucalyptus* grandis stands are studied in an experiment manipulating water and K at the scale of the ecosystem in southern Brazil (Itatinga, São Paulo state). A split-plot experiment, with 3 complete blocks and plots of 10 x 12 trees, was set up in 2010 with 4 treatments: two water supply regimes (100% of rainfall and 66% of rainfall, with artificial exclusion from 1700 m<sup>2</sup> of transparent plastic sheets) and two types of fertilizations (0 and 4,5 kmol ha<sup>-1</sup> of K). Ecophysiological and biogeochemical studies are carried out over the 3 first years after planting, including: 1) annual biomass and nutrient accumulation in above- and below-ground tree components, 2) soil CO<sub>2</sub> effluxes measured fortnightly, 3) soil water content monitoring down to the water table (depth 17 m), 4) dynamics of leaf potential, photosynthesis and respiration, 5) litterfall and forest floor accumulation. Moreover, a <sup>13</sup>C-CO<sub>2</sub> pulse labelling in situ will be made in 2012 to assess the influence of the environment (water and K availabilities) on the dynamics of C allocation in *E. grandis* ecosystems. The fluxes of <sup>13</sup>C in the organs of the labelled trees, the microbial biomass and the fluxes of respiration will be quantified inside and at the surface of the soil after in situ labelling. The experiment was set up in June 2010 and biomass accumulation in above- and below-ground tree components one year after planting are presented here.

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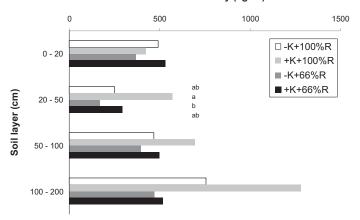
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Whilst trees strongly responded to potassium application, the exclusion of one third of rainfall over the first year of growth did not influence total stand biomass at age 1 year (Table 1). By contrast, dry matter allocation patterns were modified by the rainfall exclusion, with lower fine root biomass in the plots where one third of rainfall was excluded. Soil exploration by fine roots was influenced by the treatments, with lower fine root densities in the rainfall-excluded plots than in normally rainfed plots, whatever the K input treatment (Figure 1). The highest fine root density in the 1-2 m soil layer was found in the plots with K application and normally rainfed. Reversed plastic sheets installed in 3 plots without rainfall

**Table 1.** Dry matter accumulation in above- and below-ground tree components at age 1 year. Means and standard deviations are indicated (kg ha<sup>-1</sup>).

	100%	Rainfall	66% R	ainfall
	+176 kg ha <sup>-1</sup> K	+0 K	+176 kg ha <sup>-1</sup> K	+0 K
Leaves	3833 ±311 a	2300 ± 710 b	3243 ±47 a	1868 ± 302 b
Branches	2323 ±215 a	1718 ±497 b	2380 ±121 a	1688 ± 117 b
Stem wood	2011 ± 182 a	970 ± 470 b	2357 ±87 a	1172 ± 249 b
Stem bark	520 ±41 a	238 ±108 b	579 ±19 a	279 ±58 b
Coarse roots	1385 ± 62 b	997 ± 103 c	1571±36 a	1075 ±82 c
Medium size roots	708 ± 481 a	568 ± 285 a	1125 ± 495 a	1199 ± 752 a
Fine roots	2971 ± 709 a	1966 ± 134 ab	1846 ± 91 ab	1408 ± 230 b
Total	13751 ± 1137 a	8756±1859 b	13102 ± 673 a	8689 ± 1747 b

Different letters in the same row indicate significant differences (P < 0.05) between treatments.



#### Fine roots density (kg/ha)

**Figure 1.** Fine root densities (kg ha<sup>-1</sup>) down to a depth of 2 m at age one year in 4 treatments: without K application and rainfall exclusion (-K+100%R), with 4.5 kmol ha<sup>-1</sup> K applied and without rainfall exclusion (+K+100%R), without K application and exclusion of 1/3 of the rainfall (-K+66%R), with 4.5 kmol ha<sup>-1</sup> K applied and exclusion of 1/3 of the rainfall (+K+100%R). Different letters indicate significant differences (P < 0.05) between treatments.

exclusion showed a similar effect on tree height growth as rainfall exclusion and suggested that the different allocation patterns observed were mainly a result of modifications of the microclimate below the plastic sheets (rise in temperature). Soil water content monitoring suggested that the lack of impact of rainfall exclusion on total biomass accumulation up to age 1 year might be explained by the storage of water in the soil down to the water table after clearcutting, providing a large amount of water available to support the early tree growth (date not shown). Intensive monitoring in progress in this experiment should contribute to gain insight into the interactions between nutrition and water supply on eucalypt ecophysiology and, therefore, to understand better the consequences of climates changes on eucalypt ecosystem functioning.

# Improving the soil water content in the 3-PG model to estimate growth in Eucalyptus plantation

Cristiane Lemos<sup>1</sup>, Jose Luiz Stape <sup>2</sup>, Omar Carrero<sup>3</sup> Rodrigo Hakamada<sup>1</sup>

#### Background

The 3-PG model (Physiological Principle Predicting Growth, Landsberg; Waring, 1997) is a process-based model that has become more widespread for predicting forest growth. It is driven by intercepted radiation with the maximum canopy quantum efficiency, which is limited by the temperature, vapour pressure deficit, available soil water, stand age and site fertility, assimilating the carbon in the forest; supported by physical and ecophysiological processes, as soil water balance.

The soil water balance at the root zone in 3-PG model is represented by a single layer, monthly time step, limited by the maximum plant available water specified by the modeler, along with two empirical parameters that describe the relationship between the relative transpiration rate and the volumetric water content for different soil texture classes (Feikema et al, 2010); the water is supplied by precipitation and irrigation and the output is done through evapotranspiration and runoff.

Some modelers have associated the 3-PG model with other hydrological models (Almeida et. al, 2007; Feikema et. al, 2010) to obtain more detailed multilayered information and calculate the water balance on a daily time-step.

However, 3-PG was idealized to be a practical tool, accessible to forest managers, as well as to scientists, and because of this reason it was described to use simplified biophysical processes, combined with empirical relationship (Landsberg; Waring, 1997).

Based on this approach and, we decide to improve the soil water content in 3-PG model taking in count the root zone distribution in the soil profile and monthly time step.

### **Material and Methods**

Our study was developed in one site of BEPP (Brazil *Eucalyptus* Potential Productivity) network localized in Mogi-Guaçu, São Paulo State. It was conducted over 2001 to 2008, with two treatments: irrigated and rainfed, replicated in four

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blocks. The weather is Cwa Köeppen with a mean annual temperature range from 20°C to 22°C and an average yearly rainfall between 1,200 and 1,300 mm. The soil is red clayey Oxisol (40% clay).

Tree inventory of height and diameter per plot was done every three months, and the bole biomass was estimated based on allometric equations. Leaf area index was estimated every month for ages between 12 to 77 months, using Ceptometer AccuPaR. Destructive samples were done three times during the forest rotation, which permitted us to determine the biomass for each plant compartment and fit allometric equations to parameterize the model. Other parameters were taken from literature.

First of all, we calibrate the 3-PG model for the irrigation treatment, using LAI, bole and root biomass as output; and after that we apply the same parameters to evaluate the *Eucalyptus* growth for rainfed treatment. Only the measured parameters were changed. Regional weather data was using as input.

Statistical analyses were applied to evaluate the model performance – model efficiency (EF) and root mean square error (RMSE).

To infer about root zone distribution in the soil profile, we use Christina et al. (2011) root growth equation and root distribution evaluation for *Eucalyptus grandis* plantation in São Paulo State.

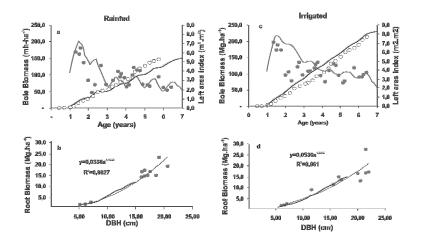
### **Results and Discussion**

During the calibration of 3-PG model, the bole biomass prediction in the rainfed treatment was underestimated (Figure 1). We suspected that available soil water to the plant was limiting the *Eucalyptus* growth. The same observation was done by Campion et al. (2005); evaluating a *Eucalyptus grandis* experiment in South Africa where they manipulated the water and nutrition regime.

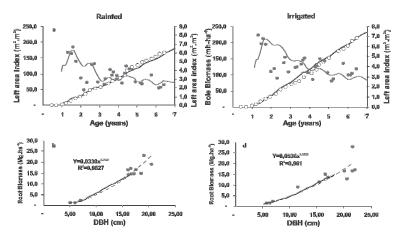
We investigated why the 3-PG model was underestimating the bole biomass, and we suspected the soil water content was limiting too much the canopy quantum efficiency and consequently it decreases the gross primary production and the bole biomass.

To solve this issue, we modified the maximum soil water content assumed as a constant value (maximum water available to the plant) by a value which is a variable value based on root growth and its distribution on the soil profile as the stand ages, as proposed by Christina et al. (2011)

Those authors determined the root dynamics from the development of fastgrowing tropical trees of Eucalyptus plantation. They found that root front was



**Figure 1.** Comparison between the measure data (represented by point) of leaf area index (m<sup>2</sup>.m<sup>-2</sup>), bole and root biomass (Mg.ha<sup>-1</sup>) and 3-PG estimation of those (represented by line) for Rainfed (a,b) and Irrigated (c,d) treatment before the soil water content was modified.



**Figure 2.** Comparison between the measure data (represented by point) of leaf area index (m<sup>2</sup>.m<sup>-2</sup>), bole and root biomass (Mg.ha<sup>-1</sup>) and 3-PG estimation of those (represented by line) for Rainfed (a,b) and Irrigated (c,d) treatment after the soil water content was modified.

85% of the stand height in his chronsequence, up to a mean tree height of 20m. And the depth corresponding to 95% of the fine root mass remained at depths < 5m throughout stand growth.

Using those data, we programmed the 3-PG model to variety the maximum soil water content along the root depth growth: 85% of *Eucalyptus* growth in height was the root growth, until it reaches a soil depth of 5 meters or the maximum soil depth, after that point the maximum water available became a constant value for all stand cycle. The initial soil water content was calculated as mm of water per cm of soil.

This modification improve our bole biomass estimation (Figure 2), the model EF of rainfed irrigation increase from 0,74 to 0,95 and the RMSE from 41,43 to 18,34; for irrigated treatment those statistical parameters didn't change.

### Conclusion

The soil water content modification improves the 3-PG model estimation for fast-growing tropical trees of Eucalyptus plantation for Brazil soils conditions, using monthly time step.

Further work should be directed to validate the 3-model soil water content adaptation for other Eucalyptus plantation.

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420 - Poster Session

### Project Nenus: a 3PG version that run in diary bases

Silva, Mariano<sup>1</sup>, Ribeiro, Aristides<sup>2</sup>

Based on 3PG (Physiological Principles Predicting Growth by Landsberg and Waring) is Nenus a version that allows different resolutions temporal that the original version, use more detailed weather information, use terrain information and a more detailed water balance. Some of these features were taken from the 4TREE the houry/monthy version of 3PG. The 3PG runs on a monthly basis, the Nenus will run in hourly, daily, five-day and monthly, but the main focus will be in run on daily basis. The Nenus will be allowed to use weather information more flexible and can run with just monthly maximum and minimum temperatures, rainfall and radiation but can by taking advantage from use other variables available such as wind and relative humidity. Will correction technique of the radiation based on the topography from the routine used in 4TREE. You will have a more detailed water and multilayer balance. Nenus is an ongoing project. It is being developed in C + +. At this stage already has an graphics interface, runs on a monthly basis in the traditional way and partially on a daily basis and has an embedded database for registration of parameters.

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# Photosynthetic parameters and *Eucalyptus* clones growth under different climatic conditions in Brazil

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The *Eucalyptus* cultivated area in the world increased rapidly over the last two decades, exceeding 19 million hectares (IGLESIAS TRABADO et. al, 2009). *Eucalyptus* forest plantation in Brazil increased its annual average increment from 10-20 m<sup>3</sup> ha<sup>-1</sup> yr<sup>-1</sup> in the 1960s to 40-50 m<sup>3</sup> ha<sup>-1</sup> yr<sup>-1</sup> nowadays (Stape, 2004), due to: i) improvement in genetics and forest management and ii) weather conditions that allow the fast growth of forest species. As plantations have been moving to dry sites causing less productive land availability it is necessary to evaluate the *Eucalyptus* ecophysiological responses to different water regimes. An understanding about carbon allocation acquisition by the plants and the physiological responses to changes in environmental variables are essential to estimate forest growth, as well as a more precise selection of species to be planted in areas with adverse conditions, then a study about the physiological behavior and growth of different *Eucalyptus* clones under different climatic conditions was done.

Four clones of *Eucalyptus grandis x urophylla* named A, B, C and D were planted in two different locations: a wet climate area, Eunápolis/BA (16°22 '40''S, 39°34'48''W), with average annual rainfall of 1200 mm and average annual temperature of 23°C, and a dry climate area in Salto da Divisa/ MG (16°0 '10''S, 39°56'49''W), with average annual rainfall of 900 mm and average annual temperature of 22°C. The photosynthetic measurements occurred in 2010, when the forests were aged 22 months. Physiological measurements were performed through an equipment IRGA model Li-Cor 6400 (USA).

In Eunápolis (wet climate) the total precipitation was 1270 mm in 2010, and the rainfall was well distributed throughout the year. In Salto da Divisa (dry climate) the total precipitation was 936 mm, and June, July and August were the driest months of the year (Figure 1).

The volume growth of wet climate area was 67% higher than the dry climate one. This difference shows the importance of water to increase forest productivity. In a study with a *Eucalyptus grandis* x *urophylla* clone in northeastern Bahia, state of

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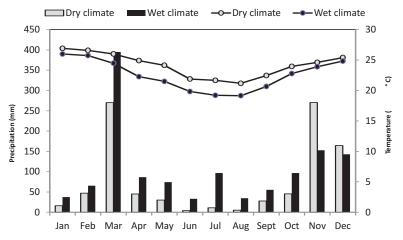


Figure 1. Precipitation and temperature throughout the year for both areas of study.

Brazil, the irrigation increased the biomass ranged from 15 to 17% (Stape et. al, 2008). The photosynthetic rate average along the day was much higher (13.5 mol CO2 m-2 s-1) in all genetic material planted in the wet climate area when compared to the dry climate area (3.4 mol CO2 m-2 s-1). Vapor pressure deficit (VPD) average on wet climate area was 1,7kPa, and maximum of 2,6kPa. The plants presented a linear decline of the stomatal conductance due to the VPD increase. On the dry climate area, the VPD average was 2,5kPa and maximum of 3kPa, and plants have kept their stomata almost closed due to the low soil moisture and high VPD (Figure 2).

The transpiration and stomatal conductance values were significantly higher on wet climate considering all the evaluated clones, especially for clone D, which had a different behavior from other genetic material on both areas. This clone, when compared to the other materials, showed a lower volume growth in this area with transpiration and stomatal conductance values below the other clones averages and a higher volume growth in the dry climate area (Table 1), presenting to be more adapted to drier conditions.

Clone	Wet climateTranspirationStomatalVolume(mmol.m <sup>-2</sup> .s <sup>-1</sup> )Conductance(m³/ha)(mol.m <sup>-2</sup> .s <sup>-1</sup> )			Dry climate           Transpiration         Stomatal         Volume           (mmol.m <sup>-2</sup> .s <sup>-1</sup> )         Conductance         (m³/ha)           (mol.m <sup>-2</sup> .s <sup>-1</sup> )         (mol.m <sup>-2</sup> .s <sup>-1</sup> )			
А	4,18 aA	0,25 aA	107,4 abA	0,98 aB	0,036 aB	39,7 abB	
В	3,73 aA	0,22 aA	126,7 Aa	0,38 bB	0,015 bB	34,4 abB	
С	3,55 aA	0,22 aA	118,0 aA	0,74 aB	0,020 bB	29,9 bB	
D	2,49 bA	0,11 bA	95,62 bA	0,73 aB	0,022 bB	42,61 aB	

**Table 1.** Transpiration, Stomatal Conductance and Volume averages of the clones A,B, C and D in the highest and lowest rainfall area.

\* Values followed by different letters at P = 0,05. Capital letters compare different areas, and lower case letters compare different clones.

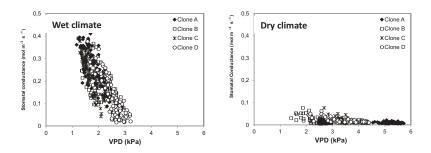


Figure 2. Stomatal conductance and VPD on wet area (A) and dry area (B).

# Relating growth and physiological responses of an Eucalyptus clonal plantation to soil water availability in northeastern Brazil

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The steady increase in world demand for wood products has leading to an expansion in planted area of fast-growing Eucalyptus species wide world. This expansion, generally extending plantations to dry and less productive sites, carries the need to access the potential productivity on new areas and increase or maintain production levels on existing planted areas, considering the long-term environment sustainability (Stape et al., 2010). Understanding the physiological processes that underlie and determine the way trees respond to environmental conditions is fundamental to our ability to anticipate and predict the responses of forests to particular situation or events (Landsberg & Sands, 2011). Ecophysiological approaches, such as efficiency of resources use in forests, are important to understand relationships on the soil-plant-atmosphere system (Binkley et al., 2004). In this context, the comprehension about the physiological processes that control resource use efficiencies by Eucalyptus is valuable in order to establish site-specific forest management strategies, such as silvicultural practices and genetic materials indications.

This study was carried out aiming to evaluate Eucalyptus growth and physiological responses to different water regimes, where the soil water availability was handled by three treatments: a) Control: plots with water availability controlled by rainfed b) Reduced: plots with 1/3 of precipitation removed c) Irrigated: plots with water supplied by irrigation, about two times the average potential evapotranspiration. Irrigation started on June 2010, when the experiment was 9 months old.

The study site is located at Eunapolis city, Bahia state, Brazil ( $16^{\circ}20'46''S$ ,  $39^{\circ}35'7''W$ ) with an average rainfall of 1.200 mm yr-1 and a mean annual temperature of 23° C. The slopes are gentle (< 3%) with well drained soils, 25-55% clay and low to moderate organic C (1.0 - 2.5 kg C m-<sup>2</sup>), classified as Ultisol.

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The experiment area was installed in September 2009, with clonal Eucalyptus in 3.0 x 3.0 m spacing, using a randomized four blocks design. All plots received the same soil preparation and amount of fertilizer.

Trees weekly increment on diameter at breast height (DBH), leaf area index (LAI) and soil water content (SWC) until 1.0 m depth (at each 0. 1 m) were measured along the evaluated period. Also, photosynthetic measurements were performed through an equipment IRGA model Li-Cor 6400 (USA), in September 2010, when the forests were 12 months old.Climate data was obtained by an automatic weather station, located near (< 1km) to the study site.

During the evaluated period (September 2009 - May 2011) the total precipitation was 2.271 mm (ranging from 13 to 395 mm per month) and the total water supplied by irrigation was 1.422 mm. Monthly rainfall, irrigation and soil water content (SWC) along the period are presented in Figure 1. There was no strong dry period observed.

The SWC at 1.0 m depth remained high through the period (June 2010 to May 2011) on irrigated plots, varying from 175 to 189 mm, due to water supplied by irrigation. Control treatment SWC varied from 114 to 151 mm and reduced treatment variation was 124 to 140 mm, indicating that reducing 1/3 of rainfall may be not enough for creating a effective soil water deficit, considering the south Bahia

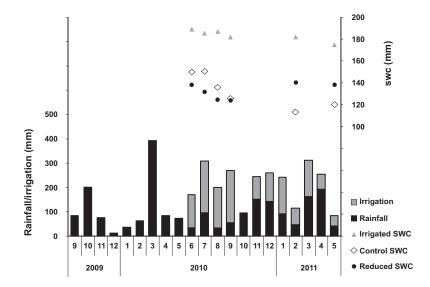
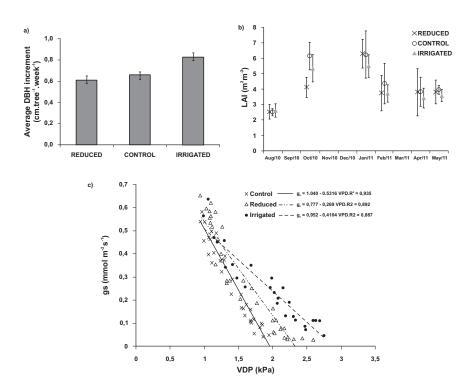


Figure 1. Soil water content (SWC) measurements on the three treatments and monthly rainfall and irrigation (only for the irrigated treatment), showing variations along the evaluated period.

regional climate pattern, where rainfall is well distributed along the year.

Several studies demonstrated the high influence of soil water availability on forest growth (Almeida et al., 2004, Stape et al., 2010). In this study, the influence of SWC on stand volume is also observed (figure 2a). DBH growth were higher on irrigated plots, averaging 0.96 cm tree-1 week-1, whereas control and reduced plots did not differ to each other, (0.70 and 0.61 cm tree-1 week-1, respectively). Nevertheless, differences between SWC on three treatments evaluated did not affect significantly values of LAI, as shown on figure 2b.

There is a strong correlation between LAI and forest growth (Smethurst et al., 2003). Thus, the higher growth rates on irrigated plots and no difference of LAI values among treatments (figure 2a) evidenced a better resources use efficiency by irrigated treatment. Trees from irrigated treatment had different physiological responses to VPD values (figure 2c), suggesting that trees in high SWC conditions



**Figure 2.** (a) Average weekly DBH increment on trees for the treatments and (b) LAI variation for the treatments along the period. (c) Stomatal conductance (gs) responses to vapor pressure deficit (VPD), for the treatments. All equations were higher significantly (p-value < 0,001).

decrease stomata conductance more slowly withVPD increasing, elevating transpiration and photosynthesis rates.

We can conclude that reducing 1/3 of precipitation did not produce a limitation on soil water availability which affects stand productivity, due to regional climate pattern. High values of SWC increase trees growth on irrigated plot, although, LAI values was not affected by SWC, suggesting a increase on resources use efficiency. Stomatal conductance responses to VPD were significantly influenced by SWC.

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### Distribution of throughfall in an *Eucalyptus dunnii* plantation, Rosário do Sul, RS, Brazil

Catarine Consensa

The water in quantity may be renewed by the hydrological cycle. Thus, the inputs and outputs of water from one watershed are closely linked with processes hydrological such as rainfall, surfacerunoff and subsurface groundwater flow, and losses through evaporation and transpiration (VIEIRA, 1982). Forests have a close relationship with the hydrological cycle of a watershed, interfering with the movement of water in its various compartments. The main influences of the forest is in receipt of canony rainfall, when it first gives the splitting of water, where a part is temporarily retained by the canopy and then is evaporated, a process called interception. The other part of the rain reaches the forest floor in the form of droplets, called internal precipitation and also by stemflow (ARCOVA et al., 2003). The same author, mention the throughfall is precipitation that reaches the forest floor, including drops that pass directly through the openings between the canopy and drops spillover canopy. The recently forestation in the Pampa Biome and the transformations in the landscape by agricultural activities, change areas until then it was occupied for natural pasture and extensive livestock. The transformation isn't just visual, but also implies changes in the environment and social environmental changes. Second Tucci and Clarke (1997), changes in natural and artificial vegetation watershed hydrological influence their behavior, producing a wide variety of impacts on the environment and the availability of water resources. However, there is interest in studying the behavior of global rainfall and throughfall for studies of rainfall interception canopy in a Eucalyptus dunnii forest in a watershed belong to the Pampa Biome located at Rosário do Sul county, RS, Brazil. This study was conducted aiming to value the disposition of collectors throughfall in Eucalyptus dunnii forest, in their respective positions: between the line of planting, under canopy and between the canopy. Thus, the variation of the throughfall in planting Eucalyptus dunnii using 25 collectors and 60 using data collectors used in the present study. The climate for the study region is classified as Temperate Tropical, presenting a range of variation of precipitation between 1500 to 1600 mm year<sup>1</sup>, the average annual temperature is around 18 <sup>o</sup> C with amplitudes averaging 12 ° C and 23 ° C (Caye and Eckert, 1995). According to Streck et al. (2008),

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both basins, are located on Argissolo Bruno Acinzentado Alítico. To evaluate global rainfall, two gauges were used (d = 20 cm). To evaluate global rainfall (GR), 2 rain gauges (d = 20 cm) were installed, throughfall (T) was evaluated based on 60 rain gauges systematically installed inside the studied area to compare the data collectors in relation to the 25 that were previously used in the area. During the period (January to July, 2011), every fifteen days samples were collected. The measurement of precipitation was performed using throughfall collectors developed and tested by the group GERHI (Gestão de Recursos Hídricos), opening collection of 100 mm in diameter. Data were tabulated and processed by the software Excel 2007, and analysis was performed in two ways: descriptive statistics - data organization (arithmetic mean, standard deviation, coefficient of variation) and regression analysis simple (correlation between global precipitation, throughfall). Observe with the analysis preliminary of the data that the coefficients of variation didn't change significantly after the installation of new collectors for internal characterization of rainfall in the planting of *Eucalyptus dunnii*., in other words, the sample collectors 25 represents very satisfactorily the throughfall in the forest. the standard deviation of the samples using 25 and 60 collectors remained in the range of 8,71 to 27,73 and 10,27 to 27,74, respectively. The averages observed to variation coefficient decreased from 25,28 to 24,67 with the increasing number of collectors (from 25 to 60), so the change wasn't significant. Therefore, preliminary, analysis of the data that it measures the variation coefficients didn't change significantly with increasing number of collectors installed in the area.

Key-words: throughfall, eucalyptus.

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### Gains with supplementary fertilization and weed control on the *Eucalyptus* yield

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### Background

The mean annual increment (MAI) of Brazilian *Eucalyptus* went from 36,7 m<sup>3</sup>ha<sup>-1</sup> yr<sup>-1</sup> in 2005 to 41,3 m<sup>3</sup>ha<sup>-1</sup> yr<sup>-1</sup> in 2010 (Abraf, 2010). This significant gain in yield is closely related to the genetic improvement, forest management, mainly due to weed control and fertilization.

The application of fertilizers is the most effective technique to accelerate growth and increase the yield of planted forests in the humid tropics. The biggest gains, in general, are related to P application, followed by K. There are however, situations in which the responses to other nutrients such as Ca, S and B, is also considerable (Gonçalves et al. 2008; Barros, et al. 2004; Barros & Comerford, 2002).

Weed control is a crucial factor in the minimum cultivation system. It has long been appreciated that weeds can reduce the availability of light to tree and compete for water or nutrients (Gonçalves et al. 2008).

The main goal of this study was to determine the effects on yield control of weeds management and / or additional fertilization in relation to management.

### Methods

The assay was established in Caravelas, Bahia State (Brazil) in February 2007, spaced 3,0 x 3,0 meters under Yellow Ultisol sand clay loam, without cohesion. It was used a randomized block design, with two clones (*E. grandis x E. urophylla* – clone A; Hybrid *E.grandis* - clone B), three treatments and four replications. Each experimental plot was consisted of 80 plants, being 20 plants measured. The treatments are: "Control" (commercial planting and manuring conventionally used by the company - weed control before planting, in three, six and/or 12 months after planting, when necessary), "Weed Management" (management of weeds and ants fighting, when necessary, further held from 12 months of age - these activities occur at 19, 25, and 32 months after planting), "Fertilization and Weed" (additional

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weed management and supplemental fertilization). Treatments Control and Weed Management received the fertilization A, while the treatment Fertilization and Weed received B fertilization, in installments over the cycle (Table 1).

The forest inventory (height and diameter at breast height - DBH) occurred in the second, third, fourth and fifth years old. These data were used to calculate the individual volume with bark (IVB) and the equation  $IVB = 0,000112544*DBH^{1,920560695}*Heigth^{0,71692354}*EXP(-0,274948148/DBH)$ , where m<sup>3</sup> VIB, DBH is in cm, and height is in m; and determine the average annual increment (MAI).

The statistical analysis was made by applying analysis of variance considering as factors of variation the clones, treatments and time of measurement, being the MAI response variable. The study was supplemented with 5% Tukey test to compare means between treatments, using the software STATISTICA 9.0 (Statsoft, 2010)

### **Results and Discussion**

Throughout all evaluations the clone A exceeded the clone B. Considering the fifth year after planting, on average, MAI of clone A was 55% higher than B, approximately, and the respective yields equal to 46,86 and 27,44 m<sup>3</sup> ha<sup>-1</sup> yr<sup>-1</sup> (Figure 1a) . In clone B was not found statistically significant differences among the treatments (Figure 1b). However, to clone A the best management practices was the Fertilization and Weed, followed by Weed Management and Control for last, with 51,83, 46,93, 41,70 m<sup>3</sup> ha<sup>-1</sup> yr<sup>-1</sup>, respectively.

Kg	Ν	$P_2O_5$	K <sub>2</sub> O	S	В	Zn	Cu	Mn	Мо
Fertilization A*	103,0	142,5	65,0	67,2	5,0	0,8	-	-	-
Fertilization B**	173,0	253,5	243,0	159,6	16,0	16,2	2,2	22,0	0,2

Application time: \*planting; e three; six; and 12 months after planting; \*\* planting; e three; six; 12; 19; 25; and 32 months after planting. All treatments receive liming (1.000 kg ha<sup>-1</sup>)

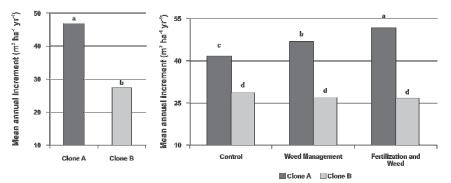
		Clone A			Clone B	
Parameters	Control	Weed	Fertilization	Control	Weed	Fertilization
		Management	and Weed		Management	and Weed
b1	41,27	43,94	51,08	28,77	26,92	26,88
b 2	3,47	3,83	3,44	4,36	4,52	4,42
b3	2,27	2,50	1,99	2,89	3,37	3,03
r	0,85	0,65	0,84	0,67	0,59	0,67
r²	0,92	0,81	0,91	0,82	0,77	0,82

\*Model: MAI = b1/(1+exp(b2-b3\*x)); MAI = mean annual increment; x = time, in years.

Different genetic materials can present differences in the efficiency of nutrient acquisition from the soil and/or in the utilization of nutrients absorbed in the production of trunk dry matter or of another product that is taken from the forest (Barros et al., 2004).

Evaluating the clone A is possible to infer that the weeds control or additional fertilization can increase productivity by 10%, or 5,00 m<sup>3</sup> ha<sup>-1</sup> yr<sup>-1</sup>. The combination of these two practices has increased yield by 25%, equivalent to 10,00 m<sup>3</sup> ha<sup>-1</sup>year<sup>-1</sup>, observing the Fertilizer and Weed treatment in relation to Control (Figure 1b).

The Figure 2 represents the MAI throughout the years for the two clones and the Table 2, shows the parameters of the equations, for which has obtained good adjustment of the data. The coefficient of determination (r2) range from 0,77 to



**Figure 1.** Mean annual increment in 5<sup>th</sup> year; a) Clones b) Interaction between clone and treatment. Means followed by same latter among treatments do not differ significantly (Tukey test, P < 0, 05).

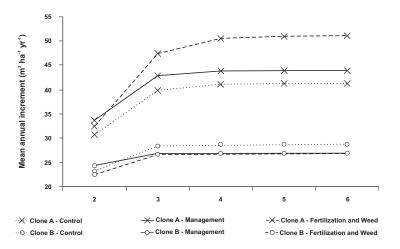


Figure 2. Mean annual incremente (MAI) of clone A and B; treatments: Control; Weed Management; Fertilization and Weed.

0,92. The growth curve of clone A is steeper than the clone B (Figure 2). From the third year there is a tendency to stabilize the MAI, except for the clone A and Fertilization and Weed treatments, which is delayed until the fourth year.

A plausible explanation for the null response to treatment in clone B refers to its inadaptability to this region. Physiological disorder symptoms were observed, as intense defoliation forming a sub-canopy, bark release, stem and branch lesions, loss of apical dominance by death of apical meristems, leaves curling, dieback, adventitious sprouts on the trunk and branches and plant death in highly susceptible materials.

### Concllusion

- Clone A MAI was 55% higher than clone B.
- Clone B is unadapted to the region where it was planted and developed symptoms of physiological disorder.
- Additional weed management or fertilization amendments were not effective in clone with physiological disorder.
- Concern convention management, additional weed control and/or extra fertilization must be considerate to improve *Eucalyptus* yield.
- The economy investment on weed management or fertilizer amendments must be guarantee by using a physiological adaptability clone.

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# Assessing the relationship between stem production and leaf area index (LAI) at canopy closure for *Eucalyptus* clones in Brazil

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### Introduction

Leaf area index (LAI) is defined as the total one-sided area of photosynthetic leaf tissue per unit ground surface area (Jockheere et al., 2004 apud Watson, 1947). LAI is closely related with biomass productivity and to several ecophysiological processes that drives the growth of plants, such as photosynthesis and transpiration, and is an essential variable for hydrological and growth models (Stape et al. 2004, Almeida et al. 2007, Hubbard et al. 2010).

### **Material and Methods**

We evaluated the LAI of 2 seed-origin (# 1 and 2) and 13 clone materials (# 4 to 16) of *Eucalyptus* selected in different regions of Brazil and planted together in Sao Paulo State to compare the ecophysiological behavior among genetic materials.

The fifteen genetic materials were planted in October 2009, on 12 x 16 trees plots (3 m x 2 m spacing) in 10 blocks distributed across a gradient of productivity in a 200 ha landscape in Itatinga, Brazil. At 1 year after planting, DBH and total height were measured for all genetic materials and plots, 8 trees of each material were harvested and allometric equations were generated to estimate stem, branches and foliage biomass. In order to estimate leaf area index (LAI) we applied the foliage biomass equation to the surveyed plots and multiplied by an average specific leaf area (SLA; m<sup>2</sup> leaf kg<sup>-1</sup> leaf) to obtain the canopy leaf area from foliage biomass. We used an average value for SLA of the 11.2 m<sup>2</sup> kg<sup>-1</sup> found by Marrichi (2009) for different clones of *Eucalyptus* planted in the same site.

### Results

Across the 10 repetitions, LAI ranged from 0.75 m<sup>2</sup> m<sup>-2</sup> to 3.52 m<sup>2</sup> m<sup>-2</sup>, and stem biomass ranged from 0.49 Mg ha<sup>-1</sup> to 4.14 Mg ha<sup>-1</sup>. For each clone, higher LAI values lead to higher stem biomass (Figure 1). However, there were a large variability on Stem production/LAI among clones (Figure 1), indicating that the selected clones and seeds will allow an adequate comparison between the light use efficiency among these genetic materials. These are partial results and the correction of

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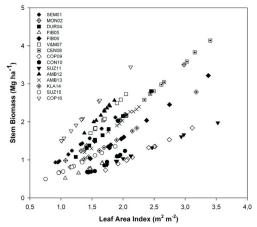


Figure 1. Relationship between stem biomass and LAI for seed- and clone-origin Eucalyptus.

using SLA for each genetic material will be incorporated to more accurately estimate LAI.

### Conclusion

The Eucalyptus clones select in Brazil for wood production show in a young age (canopy closure at 1 year-old) different ecophysiological patterns regarding stem production associated with leaf area index. The understanding of such patterns in the short- (canopy closure) and long-term (full rotation) will provide better information for both process-based modeling and tree breeding programs.

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### Seasonal specific stream flow in *Eucalyptus* catchments in tropical region

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### Introduction

Hydrological role of forests, especially in *Eucalyptus* plantation areas has attracted considerable attention from the community over the last decades (Andréassian, 2004), due to the high annual expansion rates (about 2.5 million ha) (FAO, 2006). In Brazil, *Eucalyptus* plantations covers about 4.5 million ha and they have followed the same trend of wood demand increment. Afforestation effects over rainless periods are becoming a greater social and environmental concern in these plantations (Iroumé et al., 2005).

Flow duration curves are useful to analyze how stream flow is affected by catchment management. It could shows different dynamics, depending on your inclination. A flatter curve indicated that stream has reduced the greatest potential underground water. However, when the flow curves is steepy, it can be inferred that there is greater potential for maximum flows. (Pinto et al., 2003). In this paper, we analyze the effect on flow water rates over two years in the dry and rainy season, in two catchments covered by *Eucalyptus* plantation.

### **Material and Methods**

Experimental catchments used in this study are part from the Program for Monitoring and Modeling Catchment (PROMAB). It is a cooperative program from IPEF (Institute of Forestry Research and Studies) since 1987, coordinated by the Laboratory of Forest Hydrology, Department of Forest Sciences (ESALQ/USP). The program has 17 experimental catchments, of wich ten catchments are covered with *Eucalyptus* plantations, three catchments with Pine plantations and four catchments with native forest.

The catchment one and two, comprises 424 ha and 1563 ha, located (23°55' S 48°22' W, 21° 35' S 47° 33' W) in São Paulo state in southeastern Brazil at average elevation of 705m and 638 m, respectively. In each area there is a V-notch weir

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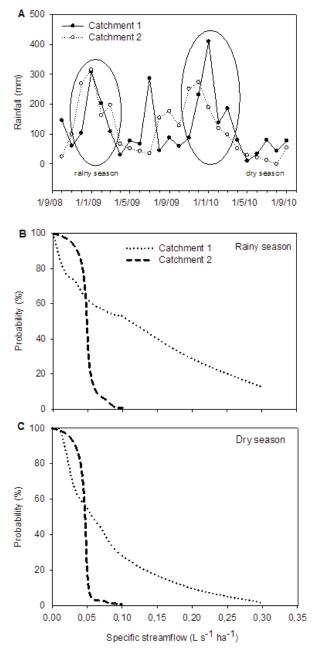


Figure 1. Specific streamflow curves over two years in *Eucalyptus* experimental catchmets.

constructed at the outlet of the catchment and the streamflow has been measured continuously every 15 minute using a datalogger. Rainfall data have been recorded at the same method by an automatic station that was installed near the V-notch weir. In order to compare the different areas, instantaneous flow rates (litres per second) were divided by catchment area and plotted over time to give the probability of streamflow water.

#### **Results and discussion**

Catchments curves represent the probability that stream flow equals or exceeds selected levels. Rainy and dry season's curves for the two years in *Eucalyptus* afforested areas are shown in Figure 1A. Rainy season (from October to March) and dry season (from April to September) presented similar trend in both areas. The average rainfall in the study period for catchment one was 1481 mm and 1413 mm for the catchment two (Figure 1A) and the mean annual temperature was 20 °C in both catchments.

Average specific stream flow in catchments ranged from 0.047 Ls<sup>-1</sup>ha<sup>-1</sup> to 0.054 Ls<sup>-1</sup>ha<sup>-1</sup>. Over the 2 years, the largest occurrences of specific stream flow ranged from 0.1 Ls<sup>-1</sup>ha<sup>-1</sup> to 0.3 Ls<sup>-1</sup>ha<sup>-1</sup> in the catchment one. In rainy season the average interval probability of this specific stream flow corresponded to 29% and in dry season to 11% (Figure 1B). However, in the catchment two, the largest occurrences ranged from 0.04 Ls<sup>-1</sup>ha<sup>-1</sup> to 0.06 Ls<sup>-1</sup>ha<sup>-1</sup> with probability in the rainy season varying from 57% to 51% of the dry season (Figure 1C). In catchment two there was no significant variability of the specific streamflow among the seasons.

We could conclude that catchment one requires a different forest management in terms of *Eucalyptus* plantations planning (spatial and temporal land use, genetic material, spacing, rotation) mainly due to significant reduction of specific stream flow water in the driest season of the year.

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# Growth dominance and the effect of dominant trees to stand level productivity of an *Eucalyptus grandis* plantation in Brazil

Otávio Campoe<sup>1,6</sup>, José Luiz Stape<sup>2</sup>, Jean Paul Laclau<sup>3, 4</sup>, Yann Nouvellon<sup>3, 5</sup>

### Introduction

On intensively managed *Eucalyptus* plantations the competition among trees for natural resources (water, nutrient and light) increases dramatically after canopy closure, which happens between 12 and 24 months-old, depending on initial spacing and site productivity. As competition for resources begins the stratification of the trees by size starts leading to classes of dominance, resulting in suppressed and dominant trees (Binkley 2004). The emergence of patterns of dominance has known negative impact on stand productivity (Boyden et al. 2008, Stape et al. 2010).

We evaluated patterns of dominance on the growth of a commercial *Eucalyptus grandis* plantation, from seeds origin, from 6 to 7 years old in Brazil (São Paulo State), on 12 plots distributed across a 90 ha landscape with a significant productivity gradient. We also analyzed the relative contribution of dominant trees to total stem production to gain insights into the effect of heterogeneity of tree sizes intostand-level productivity.

# **Materials and Methods**

# Study site

The study site was located in the Southeast of Brazil, in São Paulo State, at 22°58'04''S, 48°43'40'' W, in a typical operational plantation of *E. grandis*. The mean annual rainfall was 1391mm and mean annual temperature was 19.2°C. The mean annual air relative humidity was 77%, with minimum values during winter (close to 45%).

Soils are very deep Oxisols developed on cretaceous sandstone in the upper part of the study site (750 m above sea level) with low clay content (20%) and basaltic material at the lowest elevation (725 m above sea level) with high clay content (40%)

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The study site was planted in December 2002 with *Eucalyptus. grandis* (W. Hill ex Maiden) seedlings from a 2<sup>nd</sup> generation seed orchard (Coff'sHarbour – Australia provenance) following minimum cultivation techniques of site preparation (Gonçalves et al. 2004), in a 3.75 m x 1.60 m spacing (1666 trees per hectare).Glyphosate was used (4 L ha<sup>-1</sup>) to eliminate competing vegetation prior to site preparation until canopy closure (approximately 18 months). Leaf cutting ants (*Atta* sp.and *Acromyrmex* sp.) were controlled using sulfluramide-based baits whenever necessary. Fertilizers and lime were applied from planting up to the 3<sup>rd</sup> year, totalizing 62 kg N ha<sup>-1</sup>, 26 kg P ha<sup>-1</sup>, 97 kg K ha<sup>-1</sup>, 300 kg Ca ha<sup>-1</sup> and 144 kg Mg ha<sup>-1</sup>.

#### Forest growth

The diameters (*D*, 1.3m above ground level) were measured on all trees in the 90 ha stand (H"145,000 measured trees) during February of 2008. This census was used to select 12 plots to span the range of productivity in the stand. Each plot contained 6 rows of 14 trees/row (84 trees,  $504m^2$ ), with a range of basal area from 23 m<sup>2</sup> ha<sup>-1</sup> to 32 m<sup>2</sup> ha<sup>-1</sup> at six years old.

Tree growth of the 12 plots was assessed by measuring *D* and total height of all trees at the beginning (September 2008) and at the end (September 2009) of the study period.

Stem biomass was estimated by allometric equations fitted using PROC NLP of SAS (SAS Institute, Cary, NC, USA) and maximum likelihood estimations (Saint-André et al. 2005).

#### **Growth Dominance**

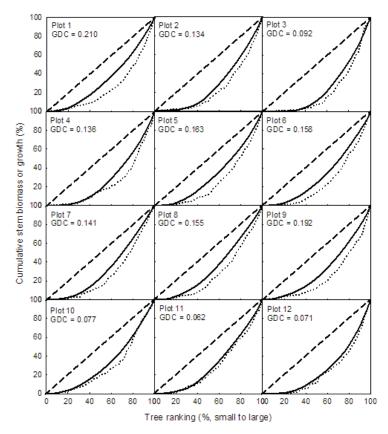
The growth dominance was determined by ranking the trees in ascending order, evaluating the cumulative increase in stem biomass or stem growth as a function of cumulative stock of stem biomass. Patterns of growth dominance can be calculated as a single coefficient ranging from 0 to1, similar to the Gini coefficient (Binkley et al. 2006). Patterns of positive dominance occur when larger (dominant) trees of a stand represent a proportionally greater growth than smaller (non-dominant) trees.

#### Results

The survival for the 12 plots ranged from 82 to 95%. The stock of stem biomass at the beginning of the study presented a variation of 55.1% (116 to 180 Mg ha<sup>-1</sup>).

The stem growth over the year ranged from 12 Mg ha<sup>-1</sup>yr<sup>-1</sup> to 24 Mg ha<sup>-1</sup>yr<sup>-1</sup>. All plots along the gradient of productivity showed positive growth dominance coefficient, on average 0.133 (CV=36%).

Along the gradient of productivity, the contribution of dominant trees for the stem biomass and stem growth was similar. Ranking the trees in each plot in ascending order in relation to the stem biomass, the 20% larger trees showed on average 43% of stem biomass of the forest (ranging from 35% to 55%) and 55% of the stem growth (ranging from 44% to 69%), showing disproportional contribution of dominant trees to forest productivity. For all plots, the stem growth curve is always below the stem stock curve, indicating that the dominant trees are becoming even more dominant at the end of the 7 year-rotation (Figure 1).

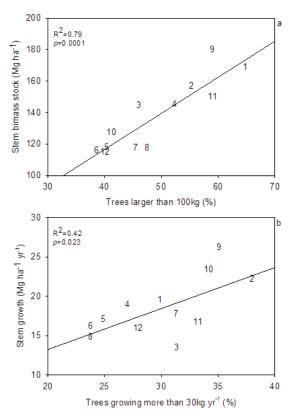


**Figure 1.** Ascending tree stem biomass ranking in relation to stem biomass (solid line) and stem growth (dotted line) from 6 to 7 years showing that larger trees contribute disproportionately to both stock and growth of stem. The dashed linerepresents the1:1 line.

Despite the 12 plots with different levels of productivity present similar contributions of dominant trees for stock and growth of stem, the increase of productivity at stand level is related to the proportion of dominant or suppressed trees of the stand. The larger the tree (high biomass), the greater the production of wood, therefore stands with greater proportion of dominant trees have a larger stock and are more productive.

#### Conclusion

Larger trees (dominant on the stand) grow faster than suppressed trees, displaying a disproportional contribution to stand level stem biomass stock and growth. This finding has major impacts on forest management, especially for fertilization regime, re-planting and thinning.



**Figure 2.** Stem biomass stock as a function of the percentage of dominant trees (larger than 100 kg of stem tree<sup>-1</sup>, a) and stem growth as a function of the percentage of trees with high stem productivity (stem production larger than 30 kg tree<sup>-1</sup> year<sup>-1</sup>, b).

Analysis of growth dominance patterns and evaluation of the contribution of dominant trees to the productivity of the stand are important to understand forest plantation dynamics and provide useful information for management decision making.

Dominance patterns rise due to variability in silvicultural activities leading to uneven availability of natural resources and seedling quality. Specifically for this seed origin forest, it is important to consider the genetic variability among trees.

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POSTER SESSION 5. DISEASES AND PESTS

# Assay on distribution, preferences and performances of Phoracantha recurva and Phoracantha semipunctata (Coleoptera: Cerambycidae) in various Eucalyptus species in Tunisia

Samir Dhahri<sup>1</sup>, Mohamed Lahbib BenJamaa<sup>1</sup>, Faouzia Charfi-cheikhrouha<sup>2</sup> and Francois Lieutier<sup>3</sup>

### Introduction

Following their host tree, the beetles *Phoracantha semipunctata* and *Phoracantha recurva* have been introduced into Tunisia and cause damage to the *Eucalyptus* species. More recently introduced, *P. recurva* is extending at the expense of *P. semipunctata* but the relative distribution of the two species is unknown in Tunisia. Very little is known also in this country about the preferences and performances of the insects in their different host species under different climates. We present here a study built to clarify those aspects, a first step towards understanding the mechanisms of host resistance.

### **Materials and Methods**

The experiments were carried out in 10 Eucalyptus plantations located in various bioclimatic areas. Four localities were situated in the arid area (ar), one in the semi-arid area (sar), two in the sub-humid area (shu), and three in the humid area (hu). Beginning of July 2009 in each locality and for each selected *Eucalyptus* species, three trees (10-15 year-old in hu, shu, sar; 20-25 year-old in ar) were striped from their bark on a 10 cm high belt, at 20 cm above ground, to attract beetles. The selected *Eucalyptus* species were *E. camaldulensis* (hu, shu, sar), *E. gomphocephala* (hu, shu, sar, ar), *E. leucoxylon* (sar), *E. occidentalis* (sar, ar), *E. astringens* (hu), *E. cineria* (shu), *E. saligna* (shu), *E. microtheca* (ar). In September, all experimental trees were harvested, measured and their stem was cut into 1 m long logs which were brought to the laboratory to follow beetle emergence. After emergence had stopped, logs were quantified in each log. They were attack density per m<sup>2</sup> of bark, number of initiated larval galleries per attack, larval survival (number of old larval galleries / number of initiated larval galleries), emergence

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rate (number of emerging beetles / number of old larval galleries), density of emerging beetles, brood productivity (density of emerging beetles / attack density), percentage of *P. recurva* among emerging beetles, and sex-ratio of each emerging beetle species. Results from logs of a same tree were gathered to obtain data at the whole tree level, or separated into four groups distinguishing four equally long quarters in the stem of the tree: basal quarter (zone 1), upper basal quarter (zone 2), below top quarter (zone 3) and top quarter (zone 4). Comparisons between means were performed by GLM procedure, followed by Scheffe tests. Pearson's and Spearman's correlations were calculated. All statistical treatments were performed with the SAS software.

#### **Results and Interpretation**

#### Insect localization and preferences

*P. recurva* was more frequent than *P. semipunctata* but the two species were present in all investigated bioclimatic areas and, very likely, in all localities in these areas. *E. gomphocephala* and *E. camaldulensis* were attacked in all localities where they were present, *E. gomphocephala* equally by both insect species, *E. camaldulensis* more frequently by *P. recurva. E. occidentalis* was attacked by both species in all localities of the arid area, while *E. microtheca*, present in all localities in that area, was never attacked.

#### **Correlations between variables**

All parameters related to brood production (attack density, number of initiated larval galleries per attack, larval survival, density of emerging beetles, emergence rate, and brood productivity) were positively correlated to each others. The percentage of *P. recurva* among emerging beetles was positively correlated to the attack density and the number of initiated larval galleries, suggesting that high densities and high fecundity were mainly due to this species. Negative correlations were obtained between larval survival and sex-ratio of both species, suggesting that larval mortality mainly affected future females. No insect parameter was related to tree size.

#### Effects of localities and tree species on insect variables

The locality effect, tested in each tree species, showed that several insect variables differed among localities but without any link with the location of the

localities in particular bioclimatic areas. Moreover, insect parameters sometimes differed between localities belonging to a same bioclimatic area, as for *E. gomphocephala*. The tree species effect, tested in each locality, showed that *E. gomphocephala* differed from *E. occidentalis* in the arid area regarding the attack density, the number of initiated larval galleries per attack, and the density of emerging beetles. However, the direction of the differences depended on the locality.

# Variations in insect variables according to the stem zone of the tree

In *E. gomphocephala, E. camaldulensis, E. leucoxylon* and *E. occidentalis,* the basal zones of the trees supported higher attack densities and higher densities of emerging beetles than the top zones. However, in *E. camaldulensis* and *E. gomphocephala*, the larval survival was higher in the top than in the basal zones, probably because of stronger intra-specific competition between larvae in the basal zones, as consequence of higher attack densities. Consequently, brood productivity was higher in the top zones, at least in *E. camaldulensis*.

# Conclusions

Both *Phoracantha* species are very likely present in all *Eucalyptus* plantations in Tunisia and information has been obtained regarding their relative abundance. Useful information has also been obtained about their preferences among tree species and their performances in brood production according to tree zones. However, no reliable results were obtained regarding effects of bioclimatic zones or tree species on these parameters, essentially because of variations and lack of tree species replicates among localities. Future experiments in more controlled conditions, using logs from various tree species installed in several localities in each bioclimatic area should bring information on these aspects.

# Approaches to predicting current and future distributions of *Puccinia psidii* in South America under climate change scenarios

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#### Background

Puccinia psidii is the cause of Eucalyptus/guava/myrtle rust disease of many host species in the Myrtaceae family, including guava (Psidium spp.), eucalypt (Eucalyptus spp.), rose apple (Syzygium jambos), and ohia (Metrosideros polymorpha) (Farr and Rossman 2010). First reported in 1884 on guava in Brazil (Maclachlan 1938), the rust has since been detected in other South American countries (Argentina, Colombia, Paraguay, Uruguay and Venezuela), Central America (Costa Rica and Panama), Mexico, the Caribbean (Cuba, Dominica, Dominican Republic, Jamaica, Puerto Rico, Trinidad and Tobago, Virgin Islands), USA (Florida, California, and Hawaii), and most recently Japan and Australia (Graça et al. 2010). Of present concern is the recent introduction of the rust pathogen to Hawaii, where it infects an endemic tree species known as ohia, the dominant tree species in Hawaii's remnant native forests. The introduction of additional rust strains could further threaten forests in Hawaii (Loope and La Rosa 2008). *Eucalyptus* rust poses serious threats to several hosts in the Myrtaceae including Eucalyptus, a genus native to Australia, which is planted extensively in numerous tropical and subtropical countries (Graça et al. 2010). Despite the potential threats to numerous forest ecosystems worldwide and the expanding geographic range of this disease, little is known about the potential distribution of this pathogen under changing climates.

Bioclimatic modeling methods to predict present and future suitable climate spaces for many tree species have already been developed (Rehfeldt et al. 2006). Similar approaches can be used to predict areas where the pathogen is climatically well-adapted for comparison with areas of host (Myrtaceae) adaptation and maladaptation. The objective of this study is to predict current and future distributions of P. psidii under climate-change scenarios using bioclimatic modeling. The infor-

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mation generated from this study will be used to evaluate pathways of pathogen spread and conduct risk analysis.

# Methods

We used a maximum entropy approach with 169 geo-referenced isolates of P. psidii from Brazil, Uruguay, and Paraguay. MaxEnt (Maximum Entropy Species Distribution Modeling) version 3.3.3e (Phillips et al. 2004) was used to predict the current distribution of P. psidii using high-resolution climate surfaces of 19 bioclimatic variables (i.e., annual mean temperature, annual precipitation, precipitation coldest quarter, etc.). These data were obtained from WorldClim (worldclim.org) and are based on interpolations of observed data from 1950-2000 (Hijmans et al. 2005). The current prediction model was then projected onto statistically downscaled (delta method) future climate surfaces for the 2050s (years 2040-2069) using the A1B SRES (Special Report on Emission Scenarios) scenario and CCCMA-CGCM 3.1 (Canadian Centre for Climate Modeling and Analysis – third generation coupled global climate model) global climate model (Ramirez and Jarvis 2010).

#### **Results and Discussion**

Results from these preliminary models for the year 2050 suggest decreasing suitability of P. psidii along the central Brazilian coast and stable to increasing suitability for southern Brazil, and Paraguay (Figs. 1 and 2). Recent analyses on the genetic diversity of P. psidii suggest several genetically distinct groups/races of this rust (Graça et al. 2010). This approach can be used to model each of these races to see if they favor disparate environmental conditions. Additional points from areas with known populations of rust (i.e., United States, Caribbean, Mexico, Costa Rica, and Colombia) can also be included to predict worldwide *P. psidii* distribution.

Predictions of the present and future distribution of *P. psidii* can help guide forest managers to implement appropriate forest practices to manage Eucalyptus rust according to current and future climates. This study is of great relevance to about one-half of the forested land area of the tropics and sub-tropics, where the 4,500 species of Myrtaceae grow naturally and/or are actively cultivated. This area includes all countries that have significant investments in Eucalyptus forestry. Thus, information from this study can help identify areas at risk for *P. psidii* establishment, based on climatic envelopes. This information can be used to help prevent introductions of *P. psidii* to global populations of Myrtaceae within regions that are at risk for *P. psidii* establishment.



**Figure 1.** Predicted current (based on years 1950-2000) suitable climate space for *Puccinia psidii* in South America based on 169 occurrences. The dark-gray shade represents areas with suitable climate for *P. psidii*, and darker gray to black shades represent areas with increased climate suitability. This model prediction used MaxEnt and 19 bioclimatic surfaces from worldclim.org.



**Figure 2.** Model projection of suitable climate space for *Puccinia psidii* for the 2050s (based on years 2040-2069). The dark-gray shade represents areas with suitable climate for *P. psidii*, and darker gray to black shades represent areas with increased climate suitability. This model prediction used MaxEnt and 19 bioclimatic surfaces from worldclim.org. This prediction is based on the CCCMA-CGCM 3.1 global circulation model and the A1B SRES scenario.

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# Comparing field screening of *Eucalyptus* for rust resistance with artificial infection in controlled conditions

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#### Introduction

*Eucalyptus* rust is a disease caused by the fungus *Puccinia psidii* and their infestation occurs during planting until about 12 months. The symptoms of this disease occur in the tissues of young leaves and stems. As a result, the affected tissues dry up and die (Krugner et al., 2003).

This disease has caused significant losses in productivity of *Eucalyptus* plantations in Brazil, reaching up to 25% at harvest age (Masson, 2009).

The most efficient method of controlling this disease is genetic improvement through selection of resistant materials (Krugner et al., 2003).

Usually the analysis is done in the laboratory, where the fungus is inoculated under controlled conditions of reproduction. However, we have observed differences in results of these tests with the results obtained in field trials. So, the objective of this study was to compare the results of artificial infection with the field test and propose a complementary evaluation to classify the clones in their susceptibility to rust.

### Methods

The work was divided into both steps: assessment of tolerance to rust in i) controlled environment (1) and ii) field (2).

The controlled environment consisted of chambers located in the Federal University of Viçosa (UFV), where the plants were kept in a mist chamber for 24 hours in the dark at 25 ° C and subsequently kept at 22 ° C with a photoperiod of 12 h (Ruiz et al., 1989) and luminous intensity of 40 imoles photons.m<sup>-2</sup>. s<sup>-1</sup>.

The second stage was carried out in the field in Mogi Guaçu, in a planted forest in State of São Paulo in southeastern Brazil. This region has the CWA-type climate (Köeppen), with 70-75% of the rainfall occurring in the summer (October to March). The Mogi Guaçu region has a mean annual temperature of 21 ° C, annual rainfall of 1,300 mm, and the predominant soils are Oxisoils.

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Twelve clones were evaluated in both steps. In step 1, 20 seedlings of each clone were planted in pots of 2 liter and inoculated 30 days after transplantation. The evaluation was performed 12 days after inoculation classifying tolerant clones (S1 and S0) and susceptible (S2 and S3) (Junghans et al., 2003).

In field step the same clones were planted in single tree plot design, with 10 repetitions. The evaluation was performed 6 months after planting, at the time of occurrence of primary disease in the test region (Furtado, 2010). The evaluation was performed according to the scale proposed by Takahashi (2002), where the footnote below 0.5 represents a resistant clone, between 0.5 and 1.5 a tolerant clone and 1.5 a susceptible clone.

In both stages there was a descriptive statistics, ranking the clones according to the methods proposed in the two conditions.

#### Results

In controlled conditions six clones were classified as susceptible, while only one material was framed in this phenotype in the field evaluation. This result was similar to that found by Pinto (2010), comparing planting in a field with a controlled condition showed that the first observed about 0.4% of the materials susceptible to rust and in controlled test this value rose to 9.3%. The author concluded that the optimal conditions for infection and more homogeneous dispersion of the pathogen may have led to these results. Dianese (1986) evaluating 27 provenances of 12 species in a field screening evaluation in Bahia state, Brazil, found a difference in the rust resistance of the same materials planted in Minas Gerais state, because of differences in behavior of the pathogenic populations. Dianese (1984) found a large difference in resistance between species, as well as Xavier et al. (2007) showed that there are differences within the same species for rust resistance.

These variations, both in relation to pathogen and the genetic resistance differences, make the laboratory tests only one phase of the determination of this characteristic and turn necessary to carry out field tests in uncontrolled conditions to assess the specific pathogen-environment interaction..



Figure 1. Scale to evaluate controlled conditions (left) and to evaluate field trials (right).

	Condition							
Clone	Cont	rolled	Fie	eld				
	Scale	Fenotype	Scale	Fenotype				
1	\$3	Susceptible	2,20	Susceptible				
2	SOHR/S1	Resistant	0,30	Resistant				
3	SOHR/S1	Resistant	0,22	Resistant				
4	S3	Susceptible	0,20	Resistant				
5	S1	Resistant	0,20	Resistant				
6	S2	Susceptible	0,20	Resistant				
7	S3	Susceptible	0,10	Resistant				
8	S1	Resistant	0,00	Resistant				
9	S3	Susceptible	0,00	Resistant				
10	S0/S1	Resistant	0,00	Resistant				
11	SOHR	Resistant	0,00	Resistant				
12	S2	Susceptible	0,00	Resistant				

Table 1. Results in controlled	and field conditions	, in grades and fenotype according
to the authors cited.		

#### Conclusion

- There is a difference in the behavior of genetic materials for resistance to rust between the tests performed under controlled conditions and field;

- Because of this we suggest the implementation of the field phase to validate the laboratory results, reducing the risk of eliminating an elite clone by poor characterization of susceptibility to rust.

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# Variability of the fungus *Puccinia psidii* Winter in commercial plantations of *Eucalyptus* sp.

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*Puccinia psidii* Winter is a very unusual rust, with an extremely wide host-range within the Myrtaceae. Economically speaking, the most important hosts are *Eucalyptus* species, grown on a very large scale on plantations in Brazil. The adequate resolution of population genetic variability in P. psidii affecting eucalyptus, through the use of RAPD markers (Random Amplified Polymorphic DNA) based on PCR (Polymerase Chain Reaction), is required, to better understand the distribution of the fungus on the tree itself and between the different clones. This would be of great assistance in developing and appropriately using the already existant control measures.

Analysis was based on samples of 56 pustules fungosities collected from six different clones located in Jacareí, São Paulo State, Brazil, in a breeding-plantation belonging to the company Fibria SA. Heights for collection varied for a more comprehensive sampling, with a view to detecting any possible genetic differences, according to location.

According to dendrogram analysis of the pooled data, the initial formation of two groups, with considerable distances between individual populations of group 1, and short distances between those of group 2, are possible indications of a speciation process, thereby revealing the possible formation of a new race. Analysis of separate data revealed high polymorphism, with no significant differences between fungi and collection points, a clear indication of the potential for spread and virulence of this pathogen. These results open perspectives conducive to the development of specific markers for different races of rust, thus additional support in the choice of new strategies for breeding resistant plants.

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POSTER SESSION 6. BREEDING

# Early growth performance and wood properties of *Eucalyptus* inter specific hybrids at Coastal and Inland sites in Southern India

Kamalakannan R., Suraj P.G., Arutselvan T., Mohan Varghese

# Background

Eucalyptus camaldulensis is the major source of raw material for pulp and paper industry in India. The average pulp yield of currently planted clones is 47%. Capacity expansion in India in recent years has brought new research needs and challenges to meet the huge demand for wood raw material (Kamalakannan, 2008). The demand for quality planting stock with higher pulp yield has become a priority to improve the profitability of the business. Eucalypt hybrids have been deployed to enhance pulp yield in several countries (Vigneron 2001). Though India is a major eucalypt planting country there are not many hybrids planted on a big scale. Hence an attempt was made to develop hybrids to increase eucalypt pulp yield in India. Interspecific hybrids of *E. camaldulensis* with *E. urophylla* and *E. pellita* were developed and tested in two different agroclimatic regions in southern India. Hybrids of E. grandis with E. urophylla, E. tereticornis and E. camaldulensis are now managed profitability in large plantations around the world. Nikles (1992) reviewed the success stories of operational interspecific hybrids in Brazil, China, Congo and South Africa. This study reports the early growth performance and pulp yield of inter-specific hybrids, E. camaldulensis x E. urophylla and E. camaldulensis x E. pellita in coastal and inland sites in southern India.

# **Materials and Methods**

16 interspecific hybrid families were developed by crossing *E. camaldulensis* clones with pollen collected from selected trees of *E. urophylla* and *E. pellita*. The progeny were tested in a coastal( $15\dot{U}16' N$ ,  $80\dot{U}00' E$ ) and inland site( $11^{2}19 ' N$ ,  $77^{2}56' E$ ) in southern India. RCB design was used with 9 tree blocks in 3 replications at a spacing of 3 x 1.5 m. Open pollinated seedlings of *E. camaldulensis* and *E. urophylla* were used as control. Hybrids survival, height, diameter at breast height and gall pest infestation was assessed at the age of 18 months.

**Wood basic density**: was measured using 5 mm increment cores by water displacement method (Kube and Raymond 2002).

Pulp Yield and Lignin: were estimated from powdered core samples by NIR

ITC R&D Centre, India

spectroscopy using a Bruker Multi Purpose Analyzer (MPA) (Bruker Optics, Ettlingen, Germany) with the integrating sphere accessory over the range 12,800-3,600 cm<sup>-1</sup> at 2 cm<sup>-1</sup> resolution. Wood meal (powdered core samples) was placed in quartz vials for analysis. The average value of three measurements was used for each sample. Spectral processing and calibrations were performed using the Bruker QUANT software package within OPUS version 6 (Bruker Optik, 2006). NIRS calibration models developed for *E. camaldulensis* (Ramadevi et al., 2009) was used to estimate kraft pulp yield (KPY) of hybrids.

#### **Results and Discussion**

Survival was high in the 16 inter-specific hybrid families with more than 96% trees surviving at both trial locations. Morphological variation in hybrids was assessed using characters like leaf shape, stem colour, branching characters. Paternal (*E.urophylla*) characters were expressed in 34% of hybrids and 36% of the hybrids showed maternal (*E. camaladulenis*) expression for the morphological traits. The remaining 30% showed intermediate characters of both parents. The overall growth was better in coastal than in inland trial at 18 months. At both trial sites open pollinated seed of *E. camaldulensis* performed better than that of the paternal parent (*E. urophylla*). Hybrids pulp yield values ranged from 45.3 to 51.4% at the inland and from 46 to 53.2% at the coastal sites (Table 1). The average wood density in the inland site was 512 kg/m<sup>3</sup> compared to 499 kg/m<sup>3</sup> at the coastal site (Figure 1). Wood density of hybrids was lower than that of *E. camaldulensis* and in general intermediate to both parents. There was no significant genetic correlation between wood density and growth traits (R<sup>2</sup> = 0.001).

	ing in content in mana and coastar trials.								
S.N	o Family ID		Inland trial				Coastal trial		
		Ht(m)	Dbh(cm)	PY(%)	Lignin(%)	Ht(m)	Dbh(cm)	PY(%)	Lignin(%)
1	39	6.1	5.3	48.6	25.0	6.7	6.9	51.2	23.0
2	47	5.9	5.4	50.6	22.6	5.4	6.0	51.0	21.0
3	42	5.8	5.1	50.3	22.5	6.0	6.7	51.9	21.3
4	45	4.6	3.9	50.5	23.9	3.4	3.2	50.4	23.9
5	E. urophylla	4.3	3.8	51.2	23.2	4.6	4.2	52.1	21.0
6	E.camaldulensis	5.7	4.5	48.2	25.2	5.4	6.0	49.4	24.3
Trial	Max	7.5	8.8	51.4	28.2	8.0	9.8	53.2	27.3
Trial	Min	2.1	1.3	45.3	21.8	2.5	2.1	46	21.2
Trial	Mean	5.2	4.5	48.2	22.3	5.4	5.5	49.2	22.5

**Table 1.** Interspecific hybrids and parental species growth performances, PY andlignin content in Inand and Coastal trials.

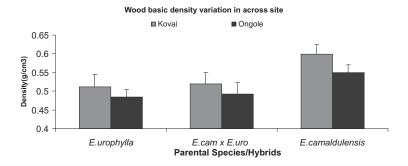


Figure 1. Interspecific hybrids and parental species wood density variation in Inland and Coastal trials.

# Conclusion

Intespecific hybrids of *E. camaldulensis* and *E. urophylla* can improve pulp and wood yield over the currently planted *E. camaldulensis* in southern India. . Suitable parents need to be screened from large family based trials of both species to develop hybrids suited to the coastal and inland sites.

**Key words**: *E. camaldulensis, E. urophylla*, inter specific hybrids, growth, wood traits.

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# A study on superior eucalypt species and provenances introduction for China's Central Sub-tropical area (Hunan Province)

Lin Mujiu

**Abstract** To select superior eucalypt species, 18 eucalypt species and their 69 provenances have been introduced into China's central subtropical area —-Hunan province. Data from multiple testing sites have been jointly analysed. When the species were evaluated by index composited by: survive rate0stem volume0wood property and ration of DBH(in cm) to Ht (in m). The result shows: E. dunnii, E. benthamii and E.dorrigoensis are belong to the best species. They can adapt to Hunan's high temperature in summer and low temperature in winter. And, They have fast growth, good wood properties and good disease resistance. The best provenances of E. dunnii and E. dorrigoensis were originally from : 152°082 ^ÿ152°392 E028°^ÿ30°052 S and best E. Benthamii was from: 150 °23'E, 33049'S. Species that have shown potential in warmer areas in Hunan province are: E amplifolia, E. saligna and *E. Deanei*. There cold tolerance are a little lower than the 3 species above. But other important traits are all good. Species that have been proved can't tolerant Hunan's cold winter include: E. grisea, E. longirostrata, E. major, E. propingua, E. punctata, E. thozetiana, E. andrewsii, E. elata and E. fastigata. E. smithii and E. nitens have been proven to have good cold tolerance, but they can't adapt to the high temperature and high humidity summer in Hunan. Not suggested for large planting. E. macarthurii has poor stem form and be deemed unsuitable as timber plantation species. Hence, we conclude: The areas we should focus on to select superior eucalypt species is where *E. dunnii* natural distributed in. The section of Eucalyptus genus that we should focus on are: Maidenaria, Transversaria and Exsertaria. Keyword: Eucalypt, Species, Provenances, Selection, central subtropical area in China

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# Genetic variability in *Eucalyptus* clones for rust *Puccinia psidii* resistance

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### Introduction

*Eucalyptus* is a culture of great economic, environmental, and social importance to Brazil. There are currently about 4.2 million hectares planted for pulpwood, charcoal, lumber mill, reconstituted panels, poles, fence posts, construction and railroad ties (FONSECA et al., 2010).

The increase of silviculture by the areas into the warmer and humid regions, the plantings of susceptible species of disease or of non genetic diverse clonal materials and recurrent repetition of planting of the same genotype on the same area have created favorable conditions for disease occurrence. Among them, the rust caused by *Puccinia psidii* fungus is one of the most limiting diseases to the establishment of new *Eucalyptus* plantations (FERREIRA, 1989; TAKAHASHI, 2002).

The disease control has been accomplished for several procedures, such as: use of fungicides and the use of resistant plants. The use of resistant plants is more advisable for several reasons, such as, low cost, convenience and lower environmental impact due to the decrease of fungicide uses (ALFENAS et al. 2004; LAU et al., 2007).

Thus, the selection of clonal genetic material for rust resistance is very important, to recommend different clones for many different soil and climatic regions through Brazilian environments.

The study aimed to estimate the genetic parameters of *Eucalyptus* clones for rust resistance caused by *Puccinia psidii* fungus.

# Methods

The experiment was carried out into the Itatinga Experimental Station/USP,

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Itatinga, SP, Brazil. The clones were provided by the: Eucatex company, AcelorMittal company and Itatinga Experimental Station. The experiment was set up in randomized blocks with 10 *Eucalyptus* clones, five plants per plot and replicated in nine blocks, totaling 450 plants. The characters were: plant height and rust desease at six months old.

To determine the levels of clone resistance against the fungus at six months old were used a scale adapted from Aparecido et al. (2003). From those results, the data were transformed for  $\sqrt{x+0.5}$  and the genetic parameters were estimated using the Selegen software, by the statistical model: y = Xr + Zg + Wp + e.

#### **Results and Discussion**

Evaluation by six months old *Eucalyptus* clones showed an average of 2.06 m of plant height.

The results of analysis of variance presented significant statistical values at 1% F test probability for rust resistance among clones. These differences showed there is high genetic variability for the studied trait, and the clones are potential for selection (Table 1).

The coefficient of error variation  $(CV_e)$  for rust resistance, after data transformations, showed values of 12.48% (Table 2) within the patterns of eucalypt.

The heritability coefficients showed relatively high values for rust resistance of *Eucalyptus* clones. The heritability of genotype means  $(h^2_{mc})$  was 0.98 for heritability of broad sense for individual levels was 0.71. It is important to emphasize that the variation within clones (0.0266) is due to the environment variation.

The coefficient of genotypic variation ( $CV_{gi}$ ) for rust was 33.63% showing potential for selection. The accuracy value of 0.99 ( $A_{cclon}$ ) is appropriate according to Resende (2007) showing a high precision for selection of genotypes.

### Conclusions

The clones have shown potentialities for selection for *Puccinia psidii* resistance for Itatinga/SP region.

Sources of variation	DF	SS	MS	F	
Fixed Effect	8	0.0619	0.0077	0.6638	
Clone	9	6.9634	0.7737	66.3519*	
Error	72	0.8396	0.0117	-	
Within	-	-	0.0266	-	

Table 1. Analysis of variance for resistance to Puccinia psidii in clones of Eucalyptus.

\* Significant at 1% probability (Pimentel-Gomes, 1978).

Genetic parameters	Rust
h² <sub>g</sub>	0.719829 +- 0.1171
h² <sub>aj</sub>	0.760815
C <sup>2</sup> <sub>parc</sub>	0.053872
h <sup>2</sup> <sub>mc</sub>	0.984929
A <sub>cclon</sub>	0.992436
CV <sub>gi%</sub>	33.634
CV <sub>e%</sub>	12.482
CV <sub>r</sub>	2.6917
Mean	0.8651

**Table 2.** Estimation of heritability  $(h^2)$  and coefficients of variation (CV) in clones of *Eucalyptus* under attack of *Puccinia psidii* at six months old in the field.

 $h_g^2$  = broad sense individual heritability;  $h_{mc}^2$  = heritability of genotype means;  $A_{cclon}$  = accuracy of selection of genotype;  $CV_{gW}^2$  = coefficient of genotypic variation;  $CV_{gW}^2$  = coefficient of relative variation.

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# Genetic variability in eucalypt clones for frost tolerance

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### Introduction

The genus *Eucalyptus* is cultivated in almost all regions of Brazil. Today with advancement of forestry technology is possible to obtain high eucalypt growth rates from 35 to 55m<sup>3</sup>.ha<sup>-1</sup>.ano<sup>-1</sup> (FOELKEL, 2007).

The selection of clones with good silvicultural and technological characteristics is the target of *Eucalyptus* breeding programs. In those programs the genetic clonal materials are evaluated in several stages before final selection and after for recommendation into commercial plantations (MORI and MORAES, 2010).

An important region of eucalypt silviculture is the Southern of Brazil. Through there, the selection of frost tolerance clones is of essential importance to maintain high silviculture yield in that region.

The present study aimed to estimate the genetic variability of eucalypt clones in frost environment.

#### Methods

The experiment was carried out at Palmasola S. A. forest company, located in Santa Catarina State, Brazil, where the frost average is about 20 times a year. The clonal genetic materials were obtained from Itatinga Experimental Station/ SP, Brazil. The experiment was arranged in randomized blocks design, with 29 *Eucalyptus* clones (*E. saligna* and *E. torelliana* species, *and* hybrids of *E. urophylla* x *E. grandis*, *E. grandis* x *E. camaldulensis*, and spontaneous hybrid *of E. dunnii*), with six plants / plot in six replications, totaling 1.044 plants. The evaluated characters were: diameter at breast height and plant height at 12, 24, and 30 months old, wood volume and survival at 30 months old. The data were analyzed by Selegen software using the statistical model: y = Xr + Za + Wp + e (RESENDE, 2006).

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# **Results And Discussion**

At 30 months old evaluation, the *Eucalyptus* clones reached average of 10.35m in plant height and 11.62cm in dbh. The results of analysis of variance have shown significant values at 5% probability by F test for plant height and dbh characteristics by the all tested ages.

As can be observed at table 1 there were differences into the broad sense individual heritability  $(h_g^2)$  and heritability of genotype means  $(h_{mc}^2)$  into the three studied ages, ranging from 0.11 to 0.42 and from 0.67 to 0.93, respectively for dbh.

The coefficients of error variation (CV<sub>o</sub>) for dbh were from 10.8 to 11.8, and for

**Table 1.** Genetic parameters of *Eucalyptus* clones in Palmasola, SC, Brazil, for diameter at breast height (dbh), plant height (ph) characters at 12, 24, and 30 months old and wood volume (wv) character at 30 months old.

Genetic	12 months		24 m	24 months		30 monts	
Parameters	dbh	ph	dbh	ph	dbh	ph	wv
h²,	0.1098	0.2214	0.3416	0.1669	0.4280	0.3103	0.3591
Ŭ	+- 0.0294	+- 0.0418	+- 0.0543	+- 0.0380	+- 0.0624	+- 0.0531	+- 0.0571
h² <sub>aj</sub>	0.140	0.259	0.400	0.238	0.490	0.412	0.416
C <sup>2</sup> <sub>parc</sub>	0.218	0.146	0.147	0.299	0.127	0.247	0.136
h <sup>2</sup> mc	0.666	0.840	0.898	0.720	0.927	0.852	0.907
A <sub>cclon</sub>	0.816	0.916	0.947	0.848	0.962	0.923	0.952
CV <sub>Pi%</sub>	7.36	7.56	13.49	13.60	15.78	14.00	32.78
CV <sub>e%</sub>	12.76	8.07	11.13	20.76	10.84	14.24	25.69
CV,	0.576	0.937	1.211	0.655	1.456	0.983	1.275

**Table 2.** Genetic parameters for survival in clones of *Eucalyptus* in frost conditions at30 months old.

Genetic para	meters		Survival			
h²,	h²_					
h² aj			0.209			
C <sup>2</sup> <sub>parc</sub>			0.131			
h <sup>2</sup> <sub>mc</sub>			0.816			
Accion			0.903			
CV <sub>gi%</sub>			18.41			
CV <sub>e%</sub>		21.39				
CV			0.861			
Mear	1	0.843				
Sources of variation	DF	SS	MS	F		
Fixed Effect	5	0.3410	0.0682	2.0934		
Clone	28	4.9699	0.1775	5.4489*		
Error	140	4.5604	0.0326	-		
Within	-	-	0.0911	-		

 $h_g^2$  = broad sense individual heritability;  $h_{mc}^2$  = heritability of genotype means;  $A_{cclon}$  = accuracy of selection of genotype;  $V_{g\%}^{*}$  = coefficient of genotypic variation;  $CV_{e\%}^{*}$  = coefficient of error variation;  $CV_r$  = coefficient of relative variation. \* Significant at 5% probability.

plant height were from 8.1 to 25.7, showing they are appropriated to *Eucalyptus* patterns for field conditions.

The coefficient of error variation  $(CV_e)$  for survival in frost conditions, showed values of 21.39% (Table 2) presenting significant statistical F test values for 1% probability at 30 months old.

The coefficient of heritability mean of genotypes  $(h_{mc}^2)$  has shown a value of 0.82, high for survival of *Eucalyptus* clones after frost event. While for broad sense heritability of plant level  $(h_m^2)$  was 0.18.

The coefficient of genotypic variation ( $CV_{gi}$ ) for survival was 18.41%, showing potential for selection. The accuracy value of 0.90 ( $A_{cclon}$ ) is suitable according to Resende and Barbosa (2005), showing a good accuracy for selection of genotypes in commercial planting.

The best clone for frost event were *E. dunnii* hybrids and the worst ones were of *E. torelliana* species.

### Conclusions

The studied clones have presented potentialities to be cultivated at Palmasola / SC region because some of them are frost tolerant. They presented genetic variability for frost event.

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# Comparison of methods for controlled pollination in hybrids *Eucalyptus globulus* Labill.

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### Introduction

In Eucalyptus tree improvement and controlled pollination programs, production of hybrids with a component of *E. globulus* is of great importance (Eldridge *et al.* 1993). This is explained by the species' relevance in the pulp and paper industry, due to the higher yield and quality of pulp from its hybrids were pure Eucalypt species normally cannot attain (Potts and Dungey, 2001). With this objective, in

February 2010 in Hernandarias, Department of Alto Paraná (Paraguay), controlled crosses were made using two species as mothers, namely E. grandis and *E. urophylla* crossed to pollen of *E. globulus*.

# Methods

We compared three methods of controlled pollination in order to determine which of them would get the largest number of hybrid progenies. The methods for comparison were the conventional method (C), One Stop Pollination (OSP) (Harbard *et al.* 1999; Harbard *et al.* 2000) and Artificially Induced Protoginy (AIP) (Assis *et al.* 2005). In all methods, bags were placed for eight days isolating treated flowers. Pollination was carried out in nine mothers (six *E. grandis* - three *E. urophylla*), 276 flowers with C method, 219 with OSP method and 2832 with AIP method (Table 1).

# **Results and Discussion**

The harvest of capsules was better with the conventional method, while the largest number of seeds per flower pollinated was obtained with AIP. The number of hybrid plants was similar between C and OSP, while AIP had the lowest value, 90.3% less (Table 2). Extrapolating the results to a hundred flowers pollinated there were no significant differences in the number of contaminated plants between C and OSP (2.2 plants/100 flowers 'Contaminate'), while the largest number of hybrid plants were obtained with the OSP treatment. The highest contaminated treatment was AIP (96.9% higher than C and OSP), Figure 1. In breeding programs

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Treatments	Flowers pollinated	Numbers of mother pollinated	Capsules (%)	Seed/flowers ± std error
Conventional	276	5	69	2,0 ± 2,7
AIP	219	6	58	2,4 ± 2,9
OSP	2832	9	48	1,7 ± 1,6
Total	3327	20		

 Table 1. Germination rate of the capsules and seeds of flowers pollinated.

Table 2. Percentages of plants obtained by treatment.

Treatments	Flowers pollinated	Numbers of mother pollinated	Normal Hybrid (%)	Not normal Hybrid* (%)	Contaminate** (%)
Conventional	276	5	73,6	23,8	2,6
AIP	219	6	7,1	0,6	92,3
OSP	2832	9	73,7	24,1	2,1
Total	3327	20			

\* Not normal hybrid: abnormal seedlings, \*\*Contaminate: pure species, not a hybrid.

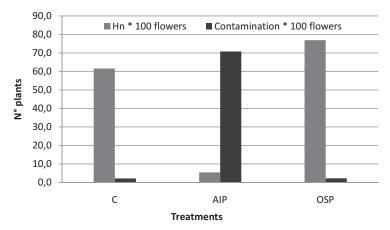


Figure 1. Number of plants per treatment per 100 pollinated flowers.

where there is no known ability to self-fertilization of mothers is better to use the OSP or C method to ensure the greatest number of hybrid plants by crossing.

These results correspond with the findings of Cassim et al. (2005); however they did not measure contamination rates, and with Dickinson et al. 2010. In a similar study, Horsley et al. (2010) also found good seed yield using AIP method, but higher contamination rates; and the authors also suggested the use of OSP method for hybrid production because of its lower contamination.

#### Conclusions

Therefore, these results suggest that for hybrid breeding programs where selffertilization ability of each mother is not known, it is recommended to use the OSP or C method to ensure higher production of hybrid plants per crossing.

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## Effect of environmental conditions of mother plants in rooting of *Eucalyptus globulus* Labill. mini cuttings

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Many years of research have failed to provide technological solutions to overcome recalcitrance to rooting in hardwood temperate species. A complementary solution is to directly select for propagation characteristics in the main or elite breeding populations and improve the cloning potential of desirable genotypes. In fact, improved *Eucalyptus globulus* genotypes are generally very sensitive to the environmental conditions during propagation and require a very accurate control. A better knowledge about the best environmental conditions is a very important issue to increase rooting efficiency in commercial mini cutting production units. The effectiveness of this environmental control can be improved by the use of mathematical models to predict the internal greenhouse conditions for a set of external conditions using different set points of the available equipment.

The main goals of the present study were to evaluate the influence of mother plants environmental conditions in the rooting of three clones of *E. globulus* (AL1, AL2 and AL3) propagated through mini cuttings.

The study was conducted in a "Venlo" greenhouse covered with glass, equipped for the vegetative propagation of *Eucalyptus globulus* Labill. through mini cuttings, located in Viveiros do Furadouro, Altri Group, in Óbidos, Portugal. The mother plant unit has 86.000 mother plants in 672 m<sup>2</sup>, managed under a hydroponic system using drip irrigation and perlite as substrate. This greenhouse has independent and automatic environmental control and includes equipments such as heating, pad cooling, thermal/shade screens, natural and dynamic ventilation. In these conditions it is possible to have year round production. The rooting unit has 360 m<sup>2</sup> and independent automatic environmental control. The rooting substrate mixture was composed of peat (25%) and vermiculite (75%). The data about environmental

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conditions and rooting was compiled in weekly intervals. To identify the time period with stronger influence in rooting ability, we studied the week of minicuting preparation, the week before, two weeks before, three weeks before and four weeks before and the possible relations between these weeks.

Each week was characterized by climate parameters such as temperature, humidity, vapour pressure deficit (VPD) and solar radiation. Each climate parameter was subdivided in several variables totaling 44 climate variables for each week that was characterized. To determine the optimum conditions for the vegetative propagation of three clones, a multivariate analysis was performed for the key components. For the more explanatory variables a regression analysis was carried out.

The more explanatory environmental variables of the mother plants unit, affecting rooting ability, was the temperature and VPD average of the four weeks preceding mini cutting preparation. Different climate effects were observed for the clones under study and they could be grouped in two sets, one set with the AL1 and AL3 clones which were influenced by temperature and VPD and the other set with AL2 for which no evidence of climate influence was detected. When average temperature was analyzed, a maximum rooting score of 76,3% was obtained for 21,4°C for AL1 and 64,2% for AL3 at 23,9°C. The vapor pressure deficit also influenced rooting in AL1 and AL3 and for a VPD of 0, 85 kPa, a rooting percentage of 85,5% was obtained for AL1 and 66,0% for GM258. The interaction between the optimum environmental conditions for vegetative propagation (both at the mother plant unit and rooting unit) and the ability to predict climate conditions inside the greenhouse, by climate modulation, open the way to test and simulate different control strategies of the available equipment, allowing for maximum rooting efficiency with lower energy consumption.

### Development of species-specific markers to five *Eucalyptus* species

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#### Background

The genetic improvement of eucalyptus is time-consuming mainly because of its long time to reach reproductive maturity along with its mixed mating system. Each cycle of Eucalyptus improvement program, depending of species, takes 5 to 20 years and it should have as few errors as possible [1]. Eucalyptus breeders frequently face difficulties to identify closely related species and their hybrids which share common morphological traits, some of them expressed only later in the breeding cycle [1]. In this context, molecular markers can provide information to detect pure species and interspecific hybrids.

A strategy to identify species specific molecular markers is to use RAPD (Random Amplified Polymorphic DNA) marker combined with BSA (Bulked Segregant Analysis) [2]. RAPD technology supplies a band pattern conclusive and easy to analysis [3], despite its low reproducibility. Because of this, RAPD markers are converted into SCAR (Sequence Characterized Amplified Region) markers.

This work aimed the development of species-specific SCAR markers to five species of commercial *Eucalyptus* (*E. tereticornis, E.grandis, E. brassiana, E. saligna* e *E. urophylla*).

#### Methods

We used individuals of five pure species of *Eucalyptus* (*E. tereticornis*, *E.grandis*, *E. brassiana*, *E. saligna* e *E. urophylla*) originated from Suzano Papel & Celulose Company.

DNA extraction was performed, and its concentration was quantified using spectrophotometer (Thermo Scientific NanoDrop<sup>™</sup> 1000), further, subjected to electrophoresis in 1% agarose gel and stained with ethidium bromide.

Ten individuals were randomly selected among normal and abnormal groups.

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DNA from ten normal individuals was mixed, creating the normal bulk, and DNA from abnormal individuals were mixed, creating the abnormal bulk. The bulks were amplified with 112 random primers (kits AD, H, J, K, M, R, Q, X, Y e W – Operon Technologies, Inc.), and the amplicons were submitted to electrophoresis in 1% agarose gel stained with ethidium bromide.

The amplicons of most informative polymorphic RAPD were extracted from agarose gel, and DNA fragments were purified using the Illustra GFX PCR DNA (GE Healthcare). Purified fragments were cloned into vector pGEM-T Easy (Promega) and inserted into UltraMAX DH5±-FT competent Escherichia coli cells (Life Technologies). DNA of bac colonies with insert was sequenced by ABI3100 Genetic Analyzer (Applied Biosystems). SCAR primers were designed based upon the abovementioned sequencing and tested in 30 individuals for validation.

#### **Results and Discussion**

We detected 187 candidates polymorphic bands, of which 44 have proved being reliable after validation carried out by genotyping individually all plants that composed the bulks. As expected, although having polymorphism between species, none of the polymorphic markers were totally exclusive to one species and absent in the others.

However, combining sets of two or more polymorphic markers with contrasting frequencies among species, it was possible to discriminate all species with high confidence.

We designed 8 SCAR primers from polymorphic markers sequenced: SA1 and SA2, which bands appeared only in E. saligna; UX and UH, which bands appeared only in E. urophylla; GA and GX, which bands appeared only in *E. grandis*; BH, which

RAPD marker appeared only in E. brassiana; and W, which bands were absent only in *E. brassiana*. All these markers were absent in E. tereticornis. Results of SCAR validation are demonstrated in Table 1.

It is clear that only one marker is not efficient to identification of one species among these five eucalypt species. However, markers combinations allow the identification of *E. saligna*, *E. tereticornis* individuals among all others species, and species identification of a species compared each other.

The SA2 marker alone allows the E. saligna identification with 89% of efficiency among the five species. E. tereticornis demonstrated a pattern of band absence of SA2, UX, GA and BH markers, thus the absence of bands in the combination of this primers allows the discrimination of E. tereticornis from other species of this study. In addition, markers combinations increased the efficiency in distinguish these five species two by two (Table 2). For example, when SA2 was combined with UX, it increases the efficiency to distinguish *E. saligna* from *E. brassiana*; when combined with UX and BH, the efficiency to distinguish *E. saligna* from *E. urophylla* is higher; and the same happens in the combination with GA and BH, to distinguish *E. saligna* from *E. tereticornis*.

Primers SCAR	Specificity	Amplicon size	E. saligna	E. urophyllla	E. grandis	E. brassiana	E. tereticornis
SA1	E. saligna	400	100%	100%	100%	100%	100%
SA2	E. saligna	750	89%	0%	1%	0	%0%
UH	E. urophyllla	600	100%	100%	90%	80%	90%
UX	E. urophyllla	700	0%	55%	1%	33%	0%
GA	E. grandis	600	20%	100%	100%	40%	0%
GX	E. grandis	700	40%	60%	100%	100%	80%
BH	E. brassiana	700	20%	0%	100%	40%	0%
W	E. brassiana	1500	66%	100%	100%	66%	100%

Table 1. Specificity, amplicon size and band frequency of SCAR markers.

**Table 2.** SCAR markers combinations can be used to distinction of species two by two according to markers frequency.

Species	SCAR markers							
	SA1	SA2	UH	UX	GA	GX	BH	W
E. saligna vs E. urophylla		х		х			Х	
E. saligna vs E. grandis		х						
E. saligna vs E. brassiana		Х		Х				
E. saligna vs E. tereticornis		Х			Х		Х	
E. urophylla vs E. grandis				Х			Х	
E. urophylla vs E. tereticornis				Х	Х			
E. grandis vs E. tereticornis					Х		Х	
E. brassiana vs E. tereticornis				Х	Х		Х	

#### Conclusions

These results indicate that the development of SCAR markers from intensive RAPD marker screening can be an inexpensive way to discriminate different species and possible hybrids. However, it is necessary a combination of two or more primers for greater efficiency for distinguishing species.

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### Establishment of barbatimão (Stryphnodendron adstringens mart) progeny trial

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#### Introduction

The Cerrado is a tremendous renewable source of medicinal plants, food, fiber, wood, and energy (charcoal). It has been studied for the last century, under many different aspects. Goodland & Ferri (1979) described that the reasonable use of those resources does not occur, perhaps due to the lack of studies on appropriate management techniques of that biome.

Because of the lack of information to establish integrated management plan for conservation purposes focusing on the extraction of some plant species for medicinal purposes in Cerrado vegetation, the main objective of this paper is to get information on genetic variability of barbatimão (Stryphnodendron adstringens MART.), a species widespread well known as a important useful species.

#### Methods

The progeny seeds were collected from five different locations, being three in Botucatu municipality (Botucatu Forest State; Rubião Junior District), one in Itu municipality, and in the Avaré municipality (Andrade and Silva Forest State).

#### **Quantitative Genetic Parameters Estimation**

To estimate quantitative genetic parameters, the studied characters were plant height, canopy diameter, and survival.

The characters were studied in individual levels into the analysis of variance and estimated by Selegen software (Resende, 2002). The half-sibs were set up as single tree plots, with fifteen replications.

To study survival of plants, five neighbor blocks were joined resulting in an analysis with three blocks and five plants per plot to estimate the survival percentage in plot level, since the small development of seedlings have still been caused competition between treatments. The statistical analysis was performed with data transformed by log (x +10) to get normal distribution on data. The

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heritabilities in narrow sense at individual level, among average of progenies, and within progenies were estimated.

In June of 2009 was set up a progeny trial. Although the seedling presented small canopy, after a year we observed that the root systems were well formed. The literature is poor about production of seedlings of Cerrado species. Usually they present slow canopy growth, therefore with vigorous root systems.

#### **Results And Discussion**

After a year, the growth was almost zero of their seedling canopies of the experiment. The mortality that has observed in the nursery, continued by field, due to through the 2009, the environmental conditions was hard. The overall survival rate dropped to 23%. To analyze the data was necessary to use SELEGEN software by the REM / BLUP methodology for unbalanced experimental errors.

Although earlier to estimate genetic parameters especially because of low rate of development of plants data was analyzed and present in Table 1. We observed a strong block effect showing that environmental variation was large, and experimental error also large. Sharp environmental heterogeneity effects are very common in poor soils such as also occur in Cerrado.

In table 2 are presented low heritability among progenies, within progenies, and at plant individual levels to three studied characters, with the best estimate was canopy diameter at progeny level (0.278) with 0.527 of accuracy, slightly lower than the recommended (0.60). By the results, we suggest that will be better to wait more several years to have better evaluation of plant growth. The species seems to develop its underground parts before developing their aerial part. The same behavior we observed in nursery. Observation on field have shown that first plants develop their diameter at soil level and only after that initiate aerial development It seems that only in that stage plants show their real growth potential. This study has not been done yet, so it was not possible to establish a comparison with literature.

Table 1. Analysis of variance of data from canopy diameter, plant height, and survival
of transformed data for log x +10 for Stryphnodendron adstringens progenies with one
year old.

Parameter	ter Canopy diameter		er P	Plant Height			Survival Log (x+10)		
FV	df	SM	F	SM	F	df	SM	F	
Block effect	14	189.41	5.27**	200.75	7.98**	2	0.4209	5.50**	
Progenie	64	49.77	1.38 ns	27.44	1.09ns	64	0.0770	1.01ns	
Residue	896	35.95	-	25.17	-	128	0.0765	-	

**Table 2.** Estimates of heritability at plant individual level (h<sup>2</sup>a), the progeny mean level (h<sup>2</sup>mp) within progenies (h<sup>2</sup>ad), accuracy in the selection of progenies (Ac), coefficient of genetic variation at the individual level ( $CV_g$ %), at progenies ( $CV_g$ %), coefficient of experimental variation ( $CV_e$ %), coefficient of relative genetic variation ( $CV_r$ %), coefficient of relative genetic variation ( $CV_r$ %), of characters and average of canopy diameter, plant height, and survival for transformed data (log x +10).

• •			0		vival (x+10)
0.100	+-0.119	0.024	+-0.058	0.010	+-0.040
0.527		0.288		0.085	
0.077 15.477		0.018 9.280		0.007 1.898	
7.738 48.326		4.640 59.777		0.949 19.225	
0.160 12.407		0.078 8.393		0.049 1.439	
	0.100 0.278 0.527 0.077 15.477 7.738 48.326	0.278 0.527 0.077 15.477 7.738 48.326 0.160	(cm)         (c           0.100         +-0.119         0.024           0.278         0.083           0.527         0.288           0.077         0.018           15.477         9.280           7.738         4.640           48.326         59.777           0.160         0.078	(cm)         (cm)           0.100         +-0.119         0.024         +-0.058           0.278         0.083         0.527         0.288           0.077         0.018         15.477         9.280           7.738         4.640         48.326         59.777           0.160         0.078         0.078	(cm)         (cm)         Log           0.100         +-0.119         0.024         +-0.058         0.010           0.278         0.083         0.007           0.527         0.288         0.085           0.077         0.018         0.007           15.477         9.280         1.898           7.738         4.640         0.949           48.326         59.777         19.225           0.160         0.078         0.049

#### Conclusion

The current stage of trial development with one year old has presented low heritabilities for surviving, canopy diameter, and plant height.

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## *Eucalyptus benthamii*: considerations on the genetic improvement program carried out by Embrapa and its partners in Brazil

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*Eucalyptus benthamii* has been indicated for planting in the Southern Region of Brazil at altitudes ranging from 800-1,400 m, which are normally prone to frosts of up to -6°C. Technological assessments of the wood (density, calorific power, lignin and carbohydrates contents) have showed a particular aptitude of this species for energetic purposes. It has been also observed a continuous expansion of the planted area during the last five years and recent estimates show that approximately 15,000 ha are currently occupied by this species.

The genetic improvement program carried out by Embrapa has completed 23 years, and the first experimental field (0.5 ha) was established in Colombo-PR (25°19'S; 49°09'W; 941 m) in 1988. Successive thinnings allowed transforming that area in a "Selected Mother Trees" category of Seed Collection Area, containing 120 plants at present. A second seed production field, originated from the latter, is located in Ponta Grossa-PR (25°09'S; 50°04'W; 880 m). This Clonal Seed Production Area (1.09 ha, 160 plants) was formed by planting rooted stems of 16 clear-cut trees after selection at the age of eight years for growth rate, stem form and sanity.

As the original population (Wentworth Falls-NSW, Australia: 33°48'S; 150°24'E; 150 m) exhibits a narrow genetic basis (7-10 trees), Embrapa has decided to make efforts to enlarge it. To do so, 30 open pollinated progenies from Kedumba Valley-NSW (33°49'S; 150°23'E; 140 m), six from Bents Basin-NSW (33°52'S; 150°38'E; 40 m) and also seedlots in bulk from Crossley SSO (33°28'S; 145°00'E; 90 m) and SSO Barclays Deniliquin (35°01'S; 145°13'E; 100 m) were imported from CSIRO in 2005, contributing to achieve an appropriate genetic basis available for the long-term institutional multi-generation recurrent selection program focused in better adaptation, growth rate and wood quality.

In order to evaluate the performance of these new introductions, two progeny tests were established in State of Paraná in 2007, at the localities of Ponta Grossa (25°10'S; 50°03'W; 890 m) and Guarapuava (25°40'S; 52°06'W; 880 m). The assessment for DBH in Ponta Grossa (32 months) showed the following statistical-

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genetic parameters: mean, 12.51 cm; narrow sense coefficient of heritability (h2a), 0.47; among progenies coefficient of heritability (h2mp), 0.84; individual genetic coefficient of variation (CVgi), 9.86%; among progenies genetic coefficient of variation (CVgp), 4.93%. The assessment for Cylindrical Volume in Guarapuava (40 months) showed the following statistical-genetic parameters: mean, 0.56 m3; h2a, 0.31; h2mp, 0.81; CVgi, 13.05%; CVgp, 6.52%. These results are very promising to attend the purposes highlighted above and also oriented the initial thinnings by the elimination of the trees with lesser individual additive values, including those ones forked, broken and diseased.

Concerning the hybridization strategy, two approaches are being adopted. One of them includes the spontaneous hybrid *E. benthamii* x *E. dunnii*, which has been studied since 2006, showing a high heterosis for growth rate in comparison with the parent species. Besides, the observed maintenance of the cold tolerance of the maternal species, *E. benthamii*, has been of great importance. At the moment, nine genotypes are under inspection by means of a clonal test established in Ponta Porã-MS (22°33'S; 55°39'W; 632 m), in 2010. The other approach is to get hybrids by using controlled pollination. The first crosses between *E. benthamii* (as male parent) with *E. grandis* and *E. "urograndis*" were performed in 2008, originating hundreds of seeds, whose respective plants are under evaluation in a field trial established in Guarapuava in 2010.

Despite of the progress on the knowledge of this species, some aspects must be emphasized in future researches. For instance, during the last two years (2010 and 2011), the winter was notoriously cold in the States of Paraná and Santa Catarina. In some sites the temperatures have reached very low values of up to -12°C, causing the complete loss of aerial parts of the plants of young plantations of up to eight months of age. The immediate consequence was the delay on their growth and, in some degree, the reduction of the stands due to the death of less tolerant plants, indicating that more efforts are necessary on this topic in the next steps of the breeding program.

The use of genome-wide selection (GWS) method in this species, in association with NIRS technology, is other important and pioneer initiative of the Program, the first example of the simultaneous use of the traditional and innovative techniques in charge of a public company in Brazil. The basic idea is the development of models for ultra-early selection of the offspring for adaptation, growth and physical and chemical properties of the wood, saving time and money in the recurrent selection and also bringing benefits for the identification of elite clones in the next future.

Keywords: adaptation, progeny test, hybridization, genome-wide selection.

### In vitro and ex vitro adventitious rooting of Eucalyptus benthamii

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The micropropagation allows to obtain large numbers of plants from a few explants in a short time and small area [1,2], with proven applications in the forestry sector, especially by minor genetic variations in relation to the original explant [2]. Due *Eucalyptus benthamii* be recent in forestry plantations, there are limitations on seed and seedling production and the absence of a multiplication protocol has limited advances on breeding programs of this species. We aimed to assess the adventitious rooting (in vitro and ex vitro) and acclimatization for the formation of a clonal micro-garden of Eucalyptus benthamii. Elongated shoots in vitro (BP101 and BP118 clones) with 1.5 cm of length were transferred to glass flask (7 x 6 cm) containing 40 mL of WPM culture medium [3], supplemented with 0.2 mg.L-1 NAA (á-naphthaleneacetic acid), 0.2 mg.L-1 IBA (indole-3-butyric acid) and 20 g.L-1 glucose. After in vitro rooting, the micro-plantlets were transferred for plastic pots (11 x 11 cm) with 200 cm3 substrate composed by medium vermiculite and decomposed pine bark (2:1, v/v). The *ex vitro* rooting was carried by direct transfer of *in vitro* elongated shoots for conical plastic tubes (55 cm3) with substrate composed by medium vermiculite and decomposed pine bark (2:1, v/v). Plant growth regulator for induce adventitious rooting was not used. The micro-plantlets rooted (both in vitro and ex vitro) were transferred to greenhouse with 50% shade during 21 days for acclimatization and, after, were transferred for full sun area in order to rustification (i.e. hardening) by 30 days. The experiment was completely randomized, and six replicates containing 12 shoots were used. Data were submitted to Hartley test (P<0.05) and analysis of variance (P<0.01 and P<0.05). The data were compared by Tukey test (P<0.05). The *in vitro* rooting of BP101 and BP118 clones (60 to 80%) was successful at 21 days in WPM culture medium supplemented with 0.2 mg.L-1 NAA, 0.2 mg.L-1 IBA and 20 g.L-1 glucose. Histological analysis of adventitious root revealed connection with the stem vascular cambium, without the occurrence of

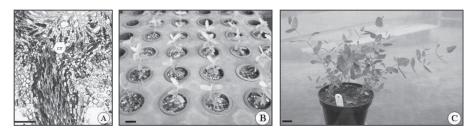
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**Figure 1.** Adventitious rooting of *Eucalyptus benthamii* micro-cuttings. (A) transversal cut of stem showing normal vascular connection of *in vitro* adventitious root with the vascular cambium (vc), bar: 100im; (B) *ex vitro* rooted micro-plantlets in conical recipient containing medium vermiculite and decomposed pine bark (2:1, v/v) at 30 days, bar: 2.0cm; (C) micro-stumps (*in vitro* and *ex vitro* rooting) for supply of new shoots at 240 days, bar: 2.0cm.

indirect organogenesis (i.e. callus) (Figure 1A). The BP101 clone presented 40% and BP118 clone presented 32% of micro-plantlets acclimatized, at 30 days. During the *ex vitro* rooting (percentage > 80%), the rooting of clones was higher than *in vitro* rooting (Figure 1B). The BP101 clone presented 65% and the BP118 clone 87% of acclimatization. The *ex vitro* rooting was less onerous and favored the survival of rooted micro-plantlets, resulting high percentage of acclimatization. The micro-plantlets of BP101 and BP118 clones were used to form a clonal micro-garden of *Eucalyptus benthamii* conducted in semi-hydroponic system to future investigations of *ex vitro* rooting from micro-cutting technique (Figure 1C).

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# Seed Source Variation in Growth Performance of Eucalyptus species Provenance cum Progeny Trials in South India

R. Seenivasan , P.Chezhian, G. Venkatesan and V. Prasath

In India at present paper industries are using *Eucalyptus tereticornis* and *E.camaldulensis* clones and seedlings for large scale plantation program. The average productivity of India is 20m<sup>3</sup>ha<sup>-1</sup>year<sup>-1</sup> and pulp yield is ranging from 42 to 45 %. Last fifteen years, Indian paper industries are planting the same clones for plantation program and the yield was reduced drastically and more pest and disease problem. To conquer this crisis, TNPL has started to develop new inter-specific hybrid clones for high pulp yield and insect pest tolerant and established provenance trail at different agro climatic region for long term breeding and in response to climate change.

Results of provenance cum progeny trials of *Eucalyptus urophylla, E. pellita E. grandis* and *E. occidentalis* laid out in 2010 at three sites viz. TNPL Campus (Karur, Tamil Nadu), Chinnamanali (Namakkal, Tamil Nadu) and Kurukathi (Tirupur, Tamil Nadu) located in sub tropical region of India are reported. Fourteen provenances

Seedlot No	Species	Provenance	No of parent trees	Altitude (M)	Latitude (°N)	Longitude (°E)
20687 (S1)	E.urophylla	SSO PHILIPPINES	32	950	14.35	122.00
20686 (S2)	E.urophylla	SPA PHILIPPINES	47	1000	14.31	120.50
20685 (S3)	E.urophylla	SPA PHILIPPINES	20	960	13.78	123.50
20684 (S4)	E.urophylla	SPA PHILIPPINES	54	950	14.21	124.50
20682 (S5)	E.urophylla	SSO SOUTH AFRICA 1 <sup>st</sup> gen	50	945	13.65	123.70
20681 (S6)	E.urophylla	SSO Thailand	70	970	13.45	100.19
21083 (S7)	E.pellita	SSO China	10	154	39.55	116.25
21019 (S8)	E.pellita	Conn logging area south Cardwell, QLD	10	40	18.26	146.08
20946 (S9)	E.pellita	SSO QLD 2 <sup>nd</sup> gen	47	0	0	0
20659 (S10)	E.pellita	MERU WP, PNG	10	40	8.27	141.28
20668 (S11)	E.grandis	COPPERLODE, QLD	8	425	16.58	145.45
18702 (S12)	E.grandis	BELLTHORPE, QLD	15	200	26.52	152.45
21111 (S13)	E. occidentalis	SSO South Western VI	C 63	180	37.58	145.54
13640 ( S14)	E. occidentalis	BREMER BAY	10	60	34.24	119.22
S15 (Local)	E.urophylla	Dandli , Karnataka, In	idia 1	472	15.26	74.61
S16 (Local)	E. pellita	Dandli , Karnataka, In	dia 1	472	15.26	74.61
S17 (Local)	E.grandis	Ooty, Tamil Nadu, Indi	a 1	2240	11.40	76.70

Table 1. Details of seed source and their geographical locations.

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		, , ,	,
Seed lot	Survival %	Height (cm)	Collar dia (cm)
S1	84.1	45.0	2.50
S2	40.7	16.0	1.20
S3*	NG	NG	NG
S4	46.7	15.0	1.20
S5	83.9	31.0	1.90
S6	91.6	53.0	2.60
S7	40.6	10.0	0.90
\$8	98.1	27.0	2.08
S9	93.9	29.6	2.10
S10	71.8	24.4	1.86
S11	97.2	20.0	1.50
S12	81.6	36.6	2.16
\$13	90.6	26.6	2.10
S14	92.7	26.5	2.05
Average	77.96	27.75	1.86
Max	98.10	53.00	2.60
Min	40.60	10.00	0.90

#### Table 2. Performance of seed sources at nursery stage (3 months).

\*Not Germinated

Table 3. Growth performance of various seed sources after field planting.

Seed lot	CG (cm)	DBH (cm)	Ht (m)
S1	14.5	9.4	3.7
S2	12.9	7.6	3.8
S3*	-	-	-
S4	13.1	8.6	4.1
S5	11.0	7.6	3.4
S6	12.5	8.0	3.1
S7	11.7	6.4	3.0
S8	11.4	6.6	2.9
S9	13.8	8.9	3.9
S10	9.9	5.9	3.0
S11	8.0	5.2	3.5
S12	8.2	5.3	3.7
S13	6.7	4.0	3.6
S14	6.9	4.2	3.4
S15	8.5	5.6	3.5
S16	14.0	8.6	3.0
S17	9.5	5.9	3.2
Mean	10.8	6.7	3.4
Max	14.5	9.4	4.1
Min	6.7	4.0	2.9
SD	2.6	0.9	1.6

\* Not included

representing 428 families from Australia, Papua New Guinea (PNG), China, Philippnes and Thailand viz. Bellthorpe, QLD; Copperlode, QLD; SSO South western VIC; Victoria, Bremer Bay, WA; Meru WP, PNG; SSO QLD 2<sup>nd</sup> gen, QLD; Conn logging area south Cardwell, QLD; SSO China; SSO STH Africa 1<sup>st</sup> Generation, South Africa; SPA Philippines, Philippines; SSO Philippines, Philippines; SSO Thailand, Thailand were evaluated from nursery stage to field performance (age 12 months). As a local seed source open-pollinated seeds collected from selected trees of *E.urophylla* SPA, *E.pellita* SPA, *E.grandis* SPA and GA 283(*Eucalyptus urograndis*) were used to serve as check material (control). Significant differences between the provenances and families at age of 3 months and eight month were observed for height, collar diameter and Girth at breast height. The fist year result shows that, each two seed lot of *E. urophylla* and *E. pellita* performed well in all three locations. These selected CPTs are multiplied through mini-cutting technique for deployment of clonal forestry and inter and inter species hybridization program.

**Key words**: *Eucalyptus*, Clonal forestry, Inter and intra species, Genetic diversity, Provenance

## Genetic variation in growth, cold tolerance and coppicing in *Eucalyptus dunnii* trials in Hunan

Li Bohai<sup>1</sup>, Luo Jianzhong<sup>2</sup> and Roger Arnold<sup>2,</sup> LiZhihui<sup>3</sup>

#### Summary

Trials of Eucalyptus dunnii families, with 84 open-pollinated families representing 14 natural stand seed sources from Australia and one seed orchard source, were established at two sites in central and southern Hunan province in 2004. These were assessed at ages two and a half to three and a half years (around half the average rotation length of eucalypt plantations in China) for tree growth and stem straightness and cold tolerance was assessed at one of the sites. At the other site, all trees were cut back to stumps at approximately age four years, following ice storms that lead to stem breakage of almost all trees. Subsequently, coppicing traits of number of sprouts per stump and DBH and height of the largest sprout were assessed at 14 months after felling. Significant differences were observed between seed sources for almost all traits, and average individual tree volume of the best seed source at each site was more than 60% above that of the poorest seed source at the same site. The magnitude of variation between families within seed sources was generally greater and more often significant than the variation between seed sources - especially for average tree volume. The magnitude of the variations observed in stem form, though significant, were minor in magnitude. Significant seed source differences were also found for cold tolerance at one site and for all three coppice traits assessed at the other sites. Estimates of withinprovenance individual tree heritability for individual tree volume ranged from 0.00 to 0.21±0.12, for stem straightness from 0.00 to 0.10±0.09, for coppice traits at one site from  $0.14\pm0.11$ , for number of sprouts per stump up to  $0.42\pm0.17$ , for DBH of the largest coppice on each stump, and at the other site heritability for cold tolerance was 0.11±0.10.

**Key words:** *Eucalyptus dunnii*, growth rate, heritability, genetic variation, genetic correlation, selection, coppice

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POSTER SESSION 7. MIXED PLANTATIONS AND AGROFORESTRY

# Preliminary results on litter decomposition and soil microbial activity in *Eucalyptus grandis* and *Acacia mangium* plantations. Consistency with the Home Field Advantage theory?

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**Keywords:** litter decomposition, home-field advantage, microbial soil respiration, nutrient cycling, *Eucalyptus grandis*, *Acacia mangium*, mixed-species plantations.

#### Introduction

Litter decomposition is a major flux of the biological cycle in forest ecosystems. It is a main driver of stand production on highly-weathered tropical soils (Laclau et al, 2010) through the release of nutrients available for tree uptake. Litter decomposition is both driven by decomposer organisms, the composition (bacteria, fungi, invertebrates) and functioning of which vary with environmental conditions, and by litter chemical composition (Corbeels *et al.*, 2003). In a mixed-species plantation with nitrogen fixing species and eucalyptus, Xiang and Bauhus (2007) observed that litter decomposition may be increased by the amount of N available to decomposers. However, plant species may create specific conditions that enhanced decomposition of their own litter in accordance to the Home-Field Advantage theory (Vivanco and Austin, 2008).

The objective of the study was 1) to test if the decomposition of *Acacia mangium* and *Eucalyptus grandis* leaf and fine roots met HFA hypothesis, and 2) to assess how soil microbial activity varied between *E. grandis* and *A. mangium* stands.

#### Materials and methods

The study was conducted 15 km from Itatinga, state of São Paulo (22°58'S, 48°43'W). The soils were Ferralsols (FAO classiûcation) developed on cretaceous sandstone, Marília formation, Bauru group. A complete randomized block design with three replicates was set up within a trial comparing pure *A. mangium* and *E.* 

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*grandis* stands and mixtures with various densities of both species. The trees were planted at a spacing of 3 m x 3 m. This trial was established in 2003 on an area managed with *Eucalyptus* for 70 years. The trial was harvested in May 2009 and planted again in November 2009.

A first experiment aimed at quantifying the kinetic of litter decomposition of *E. grandis* and *A. mangium*. We prepared litter-bags (2 mm rigid nylon mesh) of 15 cm x 15 cm and 20 cm x 30 cm with 7 g of fine roots (diameter < 2 mm) and 10 g of senesced leaves, respectively. These materials were collected on 6-year-old stands of both species. The litterbags were placed in October 2010 in the pure *E. grandis* (E) and pure *A.mangium* (A) plots, and additionally in first rotation plots of *A. mangium* (A1R) planted in November 2009 after the harvesting of an *E. grandis* stand. These last plots were located along the western border of the trial. The spacing of the trees was 3 m x 3 m. The treatments are given in table 1.

Eight litterbags of both leaf species were placed on the soil in each plot below 3 trees with the same basal area as the mean of the plot. For both species the litterbags with fine roots were buried at 5 cm in the soil, nearby the litterbags with leaves. The litterbags were distributed to cover the row and inter-row spatial variability. Plastic boards separating litterbags of both species were installed down 15 cm to prevent cross infection by fungus hyphae. It was placed 432 litterbags with leaves (2 species x 8 positions x 3 trees x 3 plots x 3 blocks) and 216 litterbags with fine roots (4 positions instead of 8 positions). The litterbags were collected every 3 months during 24 months for leaves and during 12 months for fine roots. The litterbags collected at each date were chosen at random, with a same position for all treatments at a given date of collection. The litter bags were brought to laboratory after field collection. Remaining leaves and fine roots were dried at 65°C during 4 and 7 days, respectively. Dry weights were assessed after ash content correction. Differences between treatments were assessed

The second experiment was conducted in the E and A plots and in a mixture in a proportion of 1:1 between *E. grandis* and *A. mangium* (AE – soil collected at the base

	Plots					
Types of litter	Acacia	Acacia first rotation	Eucalyptus			
Acacia leaves	ALA	ALA1R	ALE			
Eucalyptus leaves	ELA	ELA1R	ELE			
Acacia roots	ARA	ARA1R	ARE			
Eucalyptus roots	ERA	ERA1R	ERE			

Table 1. Treatments in the litter bags decomposition experiment.

of *A*.mangium trees, and EA – soil collected at the base of *E*. grandis trees). Litter samples were taken inside eight 0.5 m × 0.5 m squares at the base of 9 trees in each plot. After oven-drying at 45 °C until constant mass, the material was ground (<0.5 mm) for determination of N and C concentrations by LECO. Soil samples in the 0-10cm layer were collected at the base of 9 trees in each plot at 14, 19, and 25 months after planting. Decomposer community activity was assessed by basal respiration of the microorganisms (Alef, 1995) in 100 g of field-moist soil samples in hermetically closed vials containing 20 mL of 0.5 N NaOH as  $CO_2$  trap. The moisture was adjusted to 60% of soil water holding capacity and the samples were incubated for 21 days. The NaOH solution was replaced every two days and the  $CO_2$  released by microbial activity in the period was assessed by titration of the remaining NaOH with a standardized 0.5 N HCl solution.

The data sets were submitted to two-way (experiment 1) and one-way (experiment 2) ANOVA and means comparison by Duncan's test (P <0.05).

#### **Results and discussion**

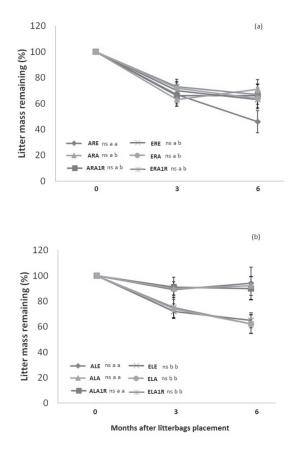
#### Leaves and fine root decomposition

Mass loss rates were higher during the first three months both for fine roots and leaves (Fig. 1). This phase was likely to correspond to decomposition of the most labile compounds and leaching of soluble materials (Mello *et al.*, 2007; Castellanos-Barliza and Leon Pelaez, 2011).

We did not observe any significant differences among treatments in fine root decomposition, except with ARE after 6 months. Mean percentage of remaining mass were of 46% and 64% for ARE and the average of the other treatments, respectively (Figure 1a). This higher decomposition rate could be due to the chemical properties (*e.g.* cellulose, lignin, polyphenols) of the Acacia fine roots, facilitating the action of microorganisms able to decompose Eucalyptus roots that are more lignified (ongoing Van Soest analyses) and present lower N concentration (Bouillet *et al.*, 2008). Significant differences in leaves decomposition rates were observed at both dates between Acacia and Eucalyptus, with mean percentage of remaining mass of 90 % and 92 %, and 73% and 62%, at 3 and 6 months respectively. By contrast we found no significant differences between plot types for a given leaf species. These results suggested that the first stages of leave decomposition were more driven by litter quality than habitat (St. John *et al.*, 2011).

#### Decomposer community activity

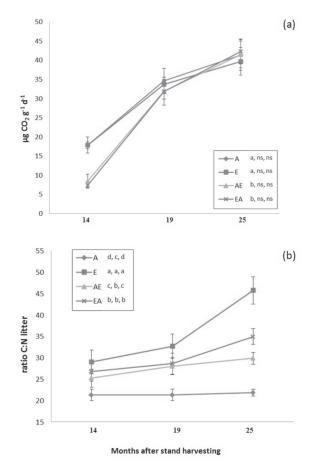
We observed an increase with time in  $CO_2$  emissions whatever the treatments (Fig. 2a). Soil microbial respiration was significantly lower at 14 months after stand harvesting in the mixture than in the monocultures. No more significant differences among treatments were observed thereafter. The flux of  $CO_2$  measured at 14 months could be mostly due to the decomposition of organic matter incorporated in the soil before stand harvesting. The following increase in soil microbial respiration could be linked to the decomposition of high amounts of harvest residues incorporated in the soil after 18 months. The litter remaining on



**Figure 1.** Percentage of remaining mass of fine roots (a) and leaves (b) after 3 and 6 months of decomposition in litter bags. Standard deviations are given (n= 3). Significant differences for a given date are indicated by different letters (p<0.05). The treatments are defined in table 1.

the soil exhibited at 14 months C/N ratio of 22 to 28 according to the treatments. A dramatic increase in C/N ratio was observed for Eucalyptus in the following 6 months (Fig 2b). These results suggested 1) a first stage in litter decomposition (> 12 months) with low rates of mass loss, in accordance which was observed in the first experiment, and 2) a possible following limitation in Eucalyptus leaves decomposition due to lower available N to decomposers.

Fig 2.  $CO_2$  flux emitted by microbial soil respiration (a) and C:N litter (b) in monoculture of *A. mangium* (A) and *E. grandis* (E) and in mixture (AE e EA) at 14, 19 and 24 months after stand harvesting. Significant differences for a given date are indicated by different letters (p<0.05).



**Figure 2.**  $CO_2$  flux emitted by microbial soil respiration (a) and C:N litter (b) in monoculture of *A. mangium* (A) and *E. grandis* (E) and in mixture (AE e EA) at 14, 19 and 24 months after stand harvesting. Significant differences for a given date are indicated by different letters (p<0.05).

#### Conclusion

These results showed that the HFA theory was not met in the first 6 months of leaves and fine roots decomposition, both for Acacia and Eucalyptus. The biochemical properties of the material were likely to be the main drivers of mass loss during this first phase of litter decomposition.

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# Leaf area index and eucalypt clone coppice growth in agroforestry systems in the cerrado region in Brazil

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#### Introduction

Eucalypt is widely planted in Brazil and most plantations are established in a density of about 9 to 12 m<sup>2</sup>/plant with a rotation age of 5 to 7 years. Recently, eucalypt has also been planted in wider spacing, by farmers and forest companies, to allow the establishment of agricultural crops or pasture between the tree planting lines. In such a system, the adequate management of growth resources such as nutrients, water and light is primordial to obtain high productivity of all the components of the consortium (Dubè *et al.*, 2002; Oliveira Neto *et al.*, 2010).

The understanding of eucalypt forest stand crown dynamics in agroforestry systems is helpful to evaluate the growth potential of the crops or pasture of the consortium. Most canopy studies, in special related to the solar radiation transmittance and leaf area index, are undertaken for the first rotation (Andrade *et al.*, 2002; Chaves *et al.*, 2007; Oliveira *et al.*, 2007). However, eucalypt has the ability to regenerate by coppice and the management of a second or more rotations should be considered. Coppice sprouts grow very fast at early stages (Cacau *et al.*, 2008; Oliveira *et al.*, 2008) and may intercept solar radiation which can be detrimental to crop and or pasture growth to be established in consortium with eucalypt coppice in the second rotation. Total coppice leaf area per stump of an *Eucalyptus camaldulensis* hybrid clone, at ages of 12 and 15 months, were, respectively, 17 % and 24 % greater than for 24 months intact plants (Oliveira, 2006).

#### Material and methods

In this study, leaf area index (LAI) of a stand of eucalypt clone (*Eucalyptus camaldulensis* hybrid) coppice was evaluated in an agroforestry system (10 x 4 m spacing), in the "cerrado" region, Brazil (17º36' S, 46 º42' W and altitude of 550 m).

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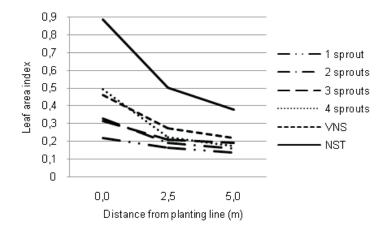
The soils present low nutrients availability and there is a very strong water deficit during six months of the year. When the eucalypt plants were established, rice was sowed in the first rainy season, soybean in the second one, and in the third rainy season *Brachiaria brizantha* was sowed for cattle grazing up to the tree harvesting age (ten years). After eucalypt harvesting, the coppice management experiment was set in a completely randomized design with the following management methods: no sprouts thinning, sprouts thinning nine months after harvesting leaving one, two, three or four sprouts per stump, and sprouts thinning 12 months after harvesting, leaving a variable number of sprouts (one to five dominant sprouts). Sprouts diameter (*dbh*), total height (*Ht*), and leaf area index (*LAI*) were measured at the age of 12 months. The *LAI* was measured between plants in the planting line and at 2.5 and 5.0 m from the planting line, 0.5 m above ground level, with the LI-2050 sensor and LI-2000 datalogger.

#### **Results and discussion**

Leaf area index of 12 months old clone eucalypt coppice in a 10 x 4 m stand was higher between plant lines averaging 0.45 and decreased sharply between planting lines for all coppice management studied (Figure 1). Similar trend was observed for 12 and 15 months old coppice of another clone established in 9 x 3 m spacing in the same region (Oliveira, 2006). The no sprout thinning treatment presented the highest leaf area index, reaching very high value (0.89) between plants in the planting line, which promoted LAI increase between rows. Higher LAI means lower photosynthetic active radiation (PAR) transmittance for the crops of the consortium, indicating that this treatment should not be used in SAF's with eucalypt coppice. When the sprouts were thinned to a variable number (1-5 sprouts per stump) or, thinned to four sprouts per stump, LAI was intermediate, being lower than 0.5 in the planting line. Sprout thinning to one, two or three sprouts per stump presented the lowest LAI values: <0.33 in the planting line and < 0.25 between rows. If the investor has greater interest on crops and pasture, thinning to less than three sprouts per stump is recommended once PAR transmittance will be maintained sufficiently high to allow higher yield. Also, the sprouts diameter will be even higher.

#### Conclusions

It is recommended to apply coppice thinning to leave up to three sprouts per stump in an agroforestry systems to allow enough solar radiation transmittance for the crops and pasture established between tree planting lines.



VNS - Variable number of sprouts. NST - No sprouts thinning

**Figure 1.** Leaf area index of 12 months old eucalypt clone coppice under different sprout thinning regimes, in an agroforestry system (spacing of 10 x 4 m), in Vazante, MG, Brazil.

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# Amounts of structural and non structural carbon and nitrogen in *Eucalyptus,* grown in monoculture and in association with *Acacia* as nitrogen fixing species

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The introduction of nitrogen-fixing species (NFS) in traditional single-species plantations is thought to increase tree growth and biomass productivity of commercial species such as *Eucalyptus*. NFS may increase soil nitrogen availability and avoid potential environmental hazards and economic costs inherent to the application of nitrogen fertilizers, offering opportunities for a sustainable management of intensively-managed forest plantations. While it has been demonstrated that the establishment of NFS in forest plantations significantly increased the levels of carbon, nitrogen and phosphorus in the soil, less is known about the internal cycles of these elements at the tree level. This is a prerequisite for a better understanding of the growth response of trees in mixed-species plantation. We aim to know if the introduction of Acacia mangium in Congolese Eucalyptus plantations influences allocation patterns of structural and non structural carbon and nitrogen in the different compartments of eucalypts. The exhaustive distribution of non structural and structural compounds has been investigated in single-species plantations and in mixed-species plantations. The purposes of the experiment were: (1) to quantify differences in carbon and nitrogen storage among different organs of Eucalyplys urophylla x grandis, (2) to compare reserve distribution in eucalypts grown in pure or in mixed stands, and (3) to analyze seasonal variations of storage compounds. The different components of the internal cycle of carbon and nitrogen (absorption, allocation and remobilization) and their contributions to the whole plant budget (storage, growth, metabolism) has been studied by destructive samplings operated on a selection of non-N-fixing trees in pure and mixed stands at two determinant periods of the year (active growth at the middle of the rainy season and end of the dry season). Roots, leaves, branches, stems and stumps has been harvested, and for each organ, structural compounds (structural proteins, celluloses, lignins...) and non structural compounds (soluble proteins, soluble sugars, starch, amino acids and nitrates) have been analyzed by biochemical assays. Taking into account the biomass of each tree compartment, the amounts of carbon and nitrogen will be estimated for the entire trees using allometric relationships.

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POSTER SESSION 8. WOOD TECHNOLOGY

# Near infrared spectroscopy (NIRS) as a tool to discriminate the source *Eucalyptus* species of solid wood samples

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Keywords: classification; wood; NIRS; PLS-DA;

#### Introduction

In 2010, 4.754.334 ha of *Eucalyptus* were planted in Brazil, representing 68% of the total area of cultivated forests in the country. Most wood is used for pulp and paper manufacturing, followed by charcoal as an energy source for steel production.

In this last segment, Brazil stands out as the largest worldwide producer, with a consolidated manufacturing technology. Most *Eucalyptus* species planted in Brazil belong to the same phylogenetic subgenus and section, making their wood phenotypically very similar. Typical ways to classify wood by color, density and anatomical traits have not been sufficiently discriminatory to identify the source species. Innovative methods used for botanical identification such as the use of DNA markers or chemical fingerprint via stable isotopes are not readily applicable to wood samples in the field. Rapid methods to identify the source species of wood samples under field conditions would be extremely useful for quality control in industrial processes as well as preliminary decision-making in inspection of certified wood trading. Near infrared spectroscopy (NIRS) is a non-destructive analytical technique, widely used to estimate chemical and physical wood properties which may also allow the discrimination of wood and charcoal. It is fast, simple and does not require complex pre-treatments. In this work we were interested in assessing the performance of NIR spectroscopy associated with multivariate statistics to discriminate the wood of the three most widely planted *Eucalyptus* species and a hybrid in Brazil.

#### Materials and methods

Solid wood samples of three species [Eucalyptus grandis E. Mill ex Maiden (3

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years), *E. pellita* F. Muell (3 years) and *E. urophylla* S.T. Blake (3 years)] and the hybrid *E. urophylla x E. grandis* (7 years) were obtained from CENIBRA (Celulose Nipo-Brasileira SA) and V&M Florestal, both located in Minas Gerais, Brazil. One hundred and fifty samples of wood with dimensions of 2x2x2 cm were taken from three trees per species and the hybrid. Free of defects specimens were air-dried and sanded with a 100 grit sandpaper. Three reflectance spectra were obtained directly on the polished surface of each sample, using a fiber-optic probe coupled to a FT-NIR spectrometer (Bruker, model Tensor 37, Germany) equipped with the OPUS software (version 6.5). The spectra were collected at 4 cm<sup>-1</sup> intervals over 4000 to 9000 cm<sup>-1</sup> and spectra obtained as the average of 16 scans. The instrument reference was a mirror standard provided by the equipment manufacturer. All measurements were made in a controlled humidity environment (<60%) and average temperature of 23 °C.

#### Data analysis and development of discrimination methods

For model development and validation the 600 samples were divided into calibration and test sets respectively.

The calibration set consisted of 400 samples. It was used for the optimization of the method, *i.e.*, to choose the best pre-processing of spectra, to set the selection of the spectral range that provides the lowest estimation error of the classes and to select the number of latent variables by a cross-validation procedure. The performance of the discrimination method was validated in a test set of 200 samples. The classification method was developed based on the model of partial least squares for discriminant analysis (PLS-DA). Four different methods of classification were used to discriminate each species or hybrid. Methods were developed using the OPUS/QUANT software, which has a routine that allows to test different pre-processing and spectral region automatically. Initially, a preliminary optimization was carried out using all the calibration spectra. Then, the spectra average was calculated and a new optimization was performed. Finally, the test samples were analyzed and the validation parameters of each method were calculated.

#### Results

From the preliminary optimization pre-processing for the methods evaluated for model development, the first derivative was the one that provided the best result for species and hybrid discrimination simultaneously. From this result, the spectra derivative and their average were calculated. At the end of the second stage of optimization, the best models still required the straight line subtraction preprocessing.

In addition, the following spectral intervals were selected: 4600 to 9012 cm-1 for *E. pellita*, *E. grandis*, *E. urophylla* and from 5448 to 9012 cm-1 for the hybrid. The estimate for class numbers by the PLS-DA presented low mean errors in the test set (RMSEP). The RMSEP obtained were 0.09, 0.08, 0.08 e 0.08, for the discrimination of the species *E. pellita*, *E. grandis*, *E. urophylla* and hybrido, respectively.

Shows the dispersion obtained for the discrimination of the hybrid specie for the others. It can be observed that a clear separation between the samples and the discrimination threshold was obtained, which confirm the performance of the method.

Similar results were observed for the other species. So a clear discrimination was obtained and the identification of the source species based on the wood samples can be performed without error, at a 95% confidence level.

#### Conclusions

The classification methods developed using NIRS proved to be effective to discriminate the source species from solid wood samples spectra. These results indicate that this method could be expanded, with similar performance, to a wider range of species. The discrimination of wood derived from phylogenetically more distant species such as those of *E. camaldulensis, E. globulus, E. dunnii* and *E. pilularis* that belong to separate sections or even genera such as *Corymbia citriodora* should be even more effective. Furthermore NIRS should also be efficient to discriminate the species source of charcoal in the field. All these applications are currently envisaged by our group in the context of the NEXTREE research project.

#### Acknowledgments

FAP-DF Project grant NEXTREE; CNPq

# Study of industrially produced chip dimensions using descriptive statistics

João Paulo Grande<sup>1</sup>, Fábio Monteiro Leite de Souza<sup>2</sup>, Cristiano Bueno de Moraes<sup>3</sup>, Cláudio Angeli Sansígolo<sup>4</sup>

#### Introduction

The chip quality has become a very important factor in pulp/paper mill due to rising raw material costs (ABTCP, 1999).

Smook (2002) reported that chip quality is measured by size uniformity and contaminant absence. All chips that are 10 to 30 millimeters long and 3 to 6 millimeters thick usually have good quality.

The chip size uniformity is essential for uniform pulp quality, because the larger dimension variation is, the more demanding the chemical treatment effectiveness gets (INGRUBER et al., 1985).

Broderick et al. (1998) studied the statistical distribution of chip size fractions separated by a screening classifier. The authors concluded that some statistical variables such as standard deviation, skewness and kurtosis showed some significant effects on paper strength.

This study aimed to examine industrial chip sizes and fractions obtained by manual sorting, using data descriptive statistics.

#### **Material and Methods**

20 kilograms of classified chips were used in this study. They were collected in a mill located in the town of Lençois Paulista, state of São Paulo, Brazil. The chips were produced by seven-year-old *Eucalyptus grandis* bolts.

Initially, the chips were air-dried, then ¼ of the chip sample was separated for the control treatment, and the remaining chips were stripped off all knots. The chip sample without knots was manually classified into six different dimension fractions, and each fraction was used as a reference to classify all chips.

Each treatment had 300 chips that were separated by the quartering method

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and measured (length, width and thickness) by hand using a caliper. The chip length was the distance between the cutting blades of the chipper, the width was the distance between the two sides of the length, and the thickness was the distance between the two sides of the width. The descriptive statistical analysis of data was performed using the R program.

### **Results and Discussion**

Table I presents a summary of the descriptive statistical analysis of the dimension with average value, median, variation coefficient, skewness and kurtosis of the fractions and the control.

The average dimension values (length, width and thickness) decreased from fraction 01 to fraction 06. Within each fraction, the dimension means decreased in the following order: length, width and thickness.

The positive skewness, observed in all fractions and control chips in the three dimensions, revealed the occurrence of a larger distance and a lower frequency of larger chips when compared to smaller ones in the median. Figure 01 illustrates this behavior.

The chip dimension distribution was also observed by Twaddle (1997), where there was smoothed distribution skewness in which the tail extended toward greater size chips.

Negative kurtosis values of fractions 02, 03 and control, indicated a leveling of frequency distribution. Fraction 02 was the only one that presented two dimensions

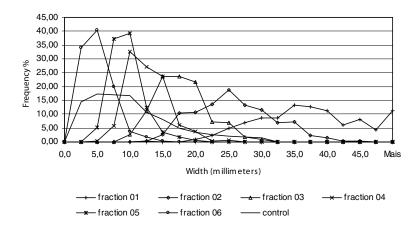


Figure 1. Chip width relative frequency distribution of fractions and control.

Treatment	Distribution statistics	Length	Width	Thickness
Fraction 01	Average, mm*	36.96	36.63	4.96
(big)	Median, mm*	34.50	36.00	4.50
	Variation coefficient	30.14	23.89	45.11
	Kurtosis	18.82	0.36	6.32
	Skewness	3.28	0.43	2.06
Fraction 02	Average, mm*	29.83	24.76	3.80
(médium 1)	Median, mm*	29.20	24.35	3.80
	Variation coefficient	17.80	23.84	28.48
	Kurtosis	4.57	-0.34	-0.29
	Skewness	1.62	0.34	0.21
Fraction 03	Average, mm*	26.54	16.87	3.14
(medium 2)	Median, mm*	26.00	16.50	3.00
	Variation coefficient	17.50	23.64	33.12
	Kurtosis	2.04	0.21	-0.02
	Skewness	0.94	0.53	0.47
Fraction 04	Average, mm*	24.86	11.69	2.77
(medium 3)	Median, mm*	24.50	11.00	2.80
	Variation coefficient	19.95	26.75	32.81
	Kurtosis	3.83	1.02	1.00
	Skewness	0.84	0.86	0.71
Fraction 05	Average, mm*	22.68	8.28	2.08
(medium 4)	Median, mm*	23.00	8.00	2.00
	Variation coefficient	21.37	29.54	36.83
	Kurtosis	0.99	2.42	1.94
	Skewness	0.21	1.24	0.98
Fraction 06	Average, mm*	18.72	3.87	1.18
(pins)	Median, mm*	19.20	3.80	1.10
	Variation coefficient	33.52	59.15	62.79
	Kurtosis	0.94	1.90	3.37
	Skewness	0.47	1.10	1.32
Control	Average, mm*	23.42	9.08	2.13
	Median, mm*	22.70	7.85	1.95
	Variation coefficient	32.65	69.68	55.56
	Kurtosis	26.69	0.73	-0.11
	Skewness	3.29	1.05	0.61

Table 1. Summary of the descriptive statistical analysis of chip dimensions.

mm\* = Millimeters

(width and thickness) with negative kurtosis and low variation coefficients; therefore, it was the most homogeneous.

Fractions 01, 06 and control presented high variation coefficient in three dimensions, while the others had lower values. In these fractions, the variation coefficient increased in the following order: length, width and thickness of the chips.

The variation in the control treatment can be attributed to the absence of classification. Even though fraction 01 and 06 were classified, they also showed

high variations. The control treatment and these two fractions were influenced by the chipper adjustment; however, fractions 01 and 06 were susceptible to efficiency variations of the screening process because some chips eventually passed by the screening and increased the variability of the first and last fractions and did not have the maximum or the minimum established size.

The variation coefficient of the dimensions increased in the following order: length, width and thickness. This behavior can be attributed to the chipper greater control over length and bigger shear rupture in the longitudinal section. Thus, chip width and thickness are the most affected dimensions by chipper breaks, transport and screening.

Hartle (1986) Hartle (1996) reports 20% variation coefficient for chip length. He comments that chippers with greater variations that the ones studied here are working below ideal conditions.

### Conclusions

The chips used in experiment had high variation and skewness dimension distribution;

Kurtosis helped to identify more homogeneous chip fraction;

Thickness and width are the highest variations of chip dimensions in all fractions and treatment control.

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## Evaluation of wood surface roughness of eucalyptus species submitted to cutterhead rotation

Camila Santos Doubek Lopes, Adriana Maria Nolasco, Mário Tomazello Filho

### Introduction

This work deals with the evaluation of surface roughness of *Eucalyptus grandis, E. dunnii and E. urophylla*, peripherally milled in 7 nominal feed rates (3, 4, 6, 8, 11, 15 and 22 m / min), to contribute to the identification of species most suitable for use in solid wood products.

In Brazil, there are few studies on the quality of the machined surface of eucalyptus wood for different machining processes. The optimization of the machining of wood results in benefits such as reduced energy costs demanded by the machine tools, increased use of wood, increased tool life and productivity, decreased cost of machining and the final product (Smith, 2002).

### Methods

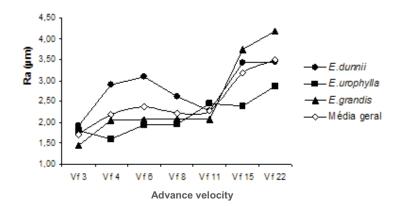
Specimens were prepared from the wood of E. grandis, E. urophylla and E. dunnii 3 trees per species, 18, from EECF ESALQ / USP, municipality of Anhembi, São Paulo, Brazil, located 47'de latitude 22 ° S, longitude 48 ° 09 'W longitude, 500 m altitude with gently rolling topography, soil podzolic sandy and deep, CWA climate (hot and rainy summer and moderately cold and dry winter), average annual temperature of 21 ° C and annual rainfall of 1,350 mm. Each tree was cut two logs baseline (4 m in length), which were deployed in tangential boards and air-dried until it reaches equilibrium moisture content of 12%. So were cut 48 specimens per species in the dimensions of 15 x 3 x 115 cm (width, thickness and length). In each specimen were performed three measurements with the roughness. The peripheral milling machining consisted of specimens of wood with a vertical axis milling machine (router), so discordant in 7 nominal feed rates (3, 4, 6, 8, 11, 15 and 22 m / min) in the direction parallel to the fibers (direction 90-0) in line with the normal planing, milling and sawing circular (Gonçalves, 1993). The axis of rotation of the router molding machine was measured with tachometer manual for this particular situation, with five measurements for application in the calculation of the average 5,814 rpm. In evaluating the surface roughness of the wood was applied methodology

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Velocities (m/min)		Mean values o	of <i>Ra</i> (µm)	
	E. dunnii	E. urophylla	E. grandis	Average
Basic density (g/cm <sup>3</sup> )	0,61	0,69	0,58	-
Vf 3	1,92	1,80	1,44	1,72
Tukey	Ab	Ab	Ab	С
CV(%)	27,21	21,22	24,01	24,15
Vf 4	2,90	1,61	2,04	2,19
Tukey	Aa	BB	Bb	b
CV(%)	27,41	27,25	26,96	27,20
Vf 6	3,09	1,93	2,08	2,37
Tukey	Aa	BB	Bb	b
CV(%)	26,76	24,52	25,17	25,48
Vf 8	2,61	1,96	2,09	2,22
Tukey	Aab	Ab	Ab	b
CV(%)	28,96	22,20	28,67	26,61
Vf 11	2,29	2,45	2,08	2,27
Tukey	Ab	AA	Ab	b
CV(%)	25,11	35,26	36,34	32,24
Vf 15	3,43	2,38	3,76	3,19
Tukey	Aa	Bab	Aa	а
CV(%)	34,79	23,35	29,82	29,32
Vf 22	3,46	2,87	4,19	3,50
Tukey	Aba	BA	Aa	а
CV(%)	29,30	23,05	23,30	-
Overall average	2,81	2,14	2,53	-
Tukey	А	С	В	-
Mean CV (%)	27,75	28,50	25,26	-

**Table 1.** Average values of Ra for the 7 forward velocities Vf, for *Eucalyptus dunni, E. urophylla and E. grandis* wood.

Means followed by same letter - uppercase and lowercase on the line in the column - do not differ statistically by Tukey test with a significance of 5% (p < 0.05).



**Figure 1.** Average values of Ra for each forward velocity for *E. dunnii, E. urophylla* and *E. grandis* wood.

Gonçalves (1993), which is probing the mechanical equipment by Taylor Hobson roughness model Surtronic 3 +, with rod measuring probe tip with diamond, and conical-spherical nose radius of 2 micrometers. We conducted exploratory analysis of data obtained at each speed (test of homogeneity of variance outliers, scale and size of the sample) and analysis of variance with comparison of the interaction of factors and variables (Tukey's test, significance level 0.05) considering the wood of three species of eucalyptus and 7 different velocities (3x7 factorial trial).

### **Results and Discussion**

The test results of applying the 7 speed improvement over the quality of the machined surface of the Wood specimens of three species of eucalyptus are presented in Table 1 and Figure 1.

### Conclusion

The surface roughness of the wood increased with increasing feed per tooth (fz) and speed (Vf). The wood *E.urophylla* showed the best results for the quality of the machined surface and the species indicated for use in products with higher added value. The wood of *E. dunnii* showed the worst performance.

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# Variation in pulp yield and paper properties of ITC Bhadrachalam clones of Eucalyptus

H. D. Kulkarni

### Abstract

Eucalyptus is a major species in India for pulp and paper production. Through tree breeding programme, ITC has developed eucalyptus clones and in the process more than 225 clones are evaluated for pulp and paper properties. Both physical and chemical wood properties have considerable influence on pulp and paper properties. The high pulp yield of 50 to 53% is recorded for *E. tereticornis* clones 3, 6, 7, 10, 27, 158, 273, 274, 279, 286, 288, 411, 412, 2014, and its hybrids 2050, 2121, 2129, 2135, 2140, 2143, 2156, 2294, 2306, 2315 and 2401 as against 44% from the wood derived from unimproved seed source plantation. Yield increase from 48.4 to 51% for clone 3 and 47.4 to 49.8% for clone 7 from year one to year four indicated influence of age on pulp yield.

The holo-cellulose content varied from 54.3 to 70% with a mean value of 63.5%. Lignin (range 13.9 to 35.7%) and pentosans (9 to 17.2%) content showed high variation with mean values of 29.3 and 13.8% respectively. The strength properties indicated by strength index value varied considerably from 20 to 85 with a mean value of 41.

Seventeen clones of *E.grandis* from 643 to 659 numbers showed high pulp yield of 50 to 53% but did not perform well at Bhadrachalam location. These *E.grandis* clones however, are performing well at Hindustan News Print Limited (HNL), Kottayam, Kerala. Pulp yield of 48 to 50% for Urograndis, 45 to 50.4% for *E. urophylla*, 51.7% for *E. torelliana* and 46% for *E. alba* clones was recorded.

Significant increase in pulp yield from 46 to 50.8% and strength index from 33 to 87.8 is shown by the hybrid progeny clones 2120, 2121 and 2156 over their parent clone 6, 10 and 27.

As the pulp yield derived from wood has multiple effect on costs for growing, harvesting, transport and processing hence, clones with high pulp yield, higher strength index and high productivity are chosen for promoting farm forestry pulpwood plantations on large scale for meeting the raw material demand of the mill.

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### Introduction

Eucalyptus wood has been an important raw material for pulp production. Its contribution to the world market of pulp is continuously growing. It is a very good wood source for the paper mills as the yield of many eucalyptus species is high and pulps are very easy to bleach to high brightness. Fiber of eucalyptus is highly valued in the world market as the fibers are small particularly in diameter and have relatively thick wall for their size. This fiber property leads to less flocculation and good sheet formation (Dean, 1995). The small fiber enhances opacity due to extra surface area for bonding; add to visual appearance and better performance during processing and in end product (Tewari, 1992). The wall thickness imparts increased porosity and better drying on higher machine speed. Further, the stiff fibers of eucalyptus give high bulk, more open sheet and good strength in wet state. Higher bulk gives better printability on coated board and good runnability.

Growing eucalyptus plantations for pulp and paper / paperboard was the best option for the paper mill of ITC at Bhadrachalam. The company started a Tree Improvement Programme for eucalyptus in the year 1989, selected more than 1200 plus trees and tested for productivity and adaptability (Kulkarni and Lal 1995). Nearly 107 promising clones were short listed for mass propagation for raising clonal plantations on commercial scale to meet the raw material demand on continuous and sustainable basis. The productivity of clonal plantation improved to 25 MT/Ha/Yr from 6 MT/Ha/Yr from that of seed route plantations. These clones were later subjected to pulp and paper properties study to up scale the plantation programme with high pulp yielding clones (Kulkarni, 2001, 2004, 2008; Venkatesh and Kulkarni, 2002).

Baldois (1992) graded hardwood pulps as low, moderate and good categories based on pulp productivity, pulp yield, active alkali requirement, tear and tensile index parameters. In the present study also an attempt is made to grade 225 clones for pulp and paper properties based on proximate chemical analysis and strength properties.

### **Material and Methods**

Samples for pulp and paper properties evaluation were collected from trees grown in clonal testing areas in Khammam, West Godavari and Prakasham districts of Andhra Pradesh. These trees were felled, billeted, debarked and chipped in a disc chipper. The analysis was carried out in the Central Laboratory of the mill according to TAPPI standard method (TAPPI, 1954). The chips (size - 8 to15 mm width, 20 to 30 mm length and 2 to 6 mm thickness) with moisture content of 12 to 18% were kraft cooked in Rotary Tumbling type digester of 25 L capacity. The chips were charged with 17% active alkali at temperature 165 °C with time to temperature and time at temperature was 105 and 90 respectively. The bath ratio maintained at 1:2.8. The data on ash (T-211 of TAPPI), A-B extractives (T-204 of TAPPI), lignin (TAPPI 1943; T-222 of TAPPI), holo-cellulose (TAPPI-1954; T9 m54 of TAPPI), pentosans, screened pulp yield, rejects, brightness (unbleached), solids, organics and inorganics was recorded in percentage. The unbleached viscosity (UBV) in cps and residual active alkali (RAA) was recorded in gpl. The bleached pulp was refined to 40° SR. Standard hand sheets were prepared and tested for physical strength properties. The strength properties such as bulk in cc/gm and breaking length in meters was also recoded while, kappa, burst and tear factors were recorded directly as numbers. The strength index was worked out by using the following formula.

Strength Index = Breaking length / 100 + Burst factor + Tear factor - 100

### **Results and Discussions**

The results of proximate chemical analysis and strength properties for 213 *E. tereticornis* clones revealed wide variation (Table 1). The ash content ranged from 0.37 to 4.2% with a mean of 1.3%. Clone 319 showed minimum ash content while, clone 2231 gave 4.2%. The alcohol – benzene (A-B) extractive content ranged from 1.24 (for clone 2294) to 8.4% (for clone 266) with a mean of 3.3%. Presence of more

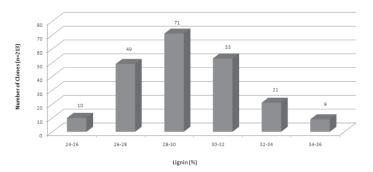
Parameter	Unit	Min	Max	Mean
Ash	%	0.37	4.20	1.3
A-B extractives	%	1.24	8.40	3.3
Lignin	%	24.7	35.7	29.4
Holo-cellulose	%	54.30	70.00	63.7
Pentosans	%	9.00	17.20	13.9
Screen pulp yield	%	44.00	53.00	47.9
Rejects	%	0.23	4.10	1.2
Карра	No.	19.00	29.70	22.0
UBV	Cps	11.90	22.80	14.8
Brightness	%	24.40	39.00	32.8
R.A.A.	Gpl	3.10	13.30	6.9
Solids	%	11.00	19.20	14.4
Organics	%	51.50	62.00	56.8
In-organics	%	38.00	48.50	43.0
Bulk	cc/gm	1.34	1.97	1.7
Burst factor		26.00	58.00	34.9
Tear factor		44.00	78.00	54.3
Breaking length	Μ	3900	8000	5194
Strength index		20.00	85.00	41.0

 Table 1. Variation in pulp & paper properties for E. tereticornis clones.

extractive content tends to increase the consumption of chemicals during pulping and reduce pulp yield.

In respect of lignin, clone 2396 showed lowest lignin (24.7%) while, clone 2324 had (35.7%) highest lignin content. Out of 213 clones analyzed, 71 clones fall under 28 to 30% class intervals while, 9 clones in 34 – 36% and 10 clones in 24 to 26% class intervals (Fig. 1). The unbleached brightness of pulp varied from 24.4 to 39% and the average brightness was observed at 32.8%. More lignin content means high chemical consumption in cooking and bleaching of wood. Moreover, higher lignin has significant impact on refining and paper properties. Lignin is hydrophobic and contains chromophoric groups therefore, pulp has poor swelling characteristics (Singh and Rawat, 2000) hence, low fiber bonding results in low strength properties. Kappa number, a measure of residual lignin, varied from 19.1 to 29.7 with average value of 22 indicating variation in lignin content in the 213 clones analyzed in the study. Bleaching chemicals are required more to bleach higher amount of lignin. Sharma *et.al.*, (1987) opined that to produce easily bleachable grade pulp of satisfactory brightness, more lignin has to be removed by increasing active alkali charge and pulp yield and kappa number decreased with increased chemical charge.

For holo-cellulose content, the readings varied from 54.3 to 70% with a mean value of 63.7%. Out of 213 clones, nearly142 clones occurred in 61 to 66% class interval followed by 41 clones in 66 to 70% class interval (Fig. 2). The lowest holo-cellulose content of 51-56% was shown by clone 411 and 417 which is not a desirable property. The highest holo-cellulose content of 70% was shown by clone 343. More cellulose content means better the property for paper making. The



Lignin content in E. tereticornis clones

Figure 1. Lignin content in E. tereticornis clones.

pentosans percentage varied from 9 to 17.2 with a mean value of 13.9.

The important parameter for pulp properties is the screened yield. Eucalyptus requires less chemicals (17%) to obtain 46 to 48.8% pulp yield at Kappa No. 22 to 20. The screened pulp yield for 213 clones ranged from 44 to 53% with a mean value of 47.8%. Highest number of clones (51 clones) gave 49% pulp yield (Fig. 3). The best performance at 53% was shown by clone 2129 while the least performance at 44% of pulp yield was shown by 147, 319, 433, 2069, 2261, 2262, 2264 clones.

The clone 2008 showed higher per cent of rejects at 4.10% while, lowest value of less than 0.5% was recorded in 11 clones. The average value of 1.2% was recorded for 99 clones out of 213 clones. Dadswell and Wardrop (1959) linked rejects with that of density and opined that with increase in density of wood pulp, rejects also increase as well as tear and tensile indices decline. Unbleached viscosity (UBV) varied (range 11.9 to 22.8 cps) considerably with a mean value of 14.8 cps. Similar

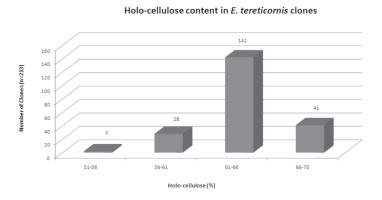
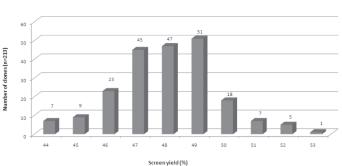


Figure 2. Holo-cellulose content in E. tereticornis clones.



Pulp screen yield for E. tereticornis clones

Figure 3. Pulp screen yield for E. tereticornis clones.

trend was also observed for other parameters like R.A.A, solids, organics and inorganics.

The eucalyptus clones showed higher bulk of 1.7 cc/gm (range 1.34 to 1.97). Stiff fibers of eucalyptus give more bulk due to which more open sheet and good strength in the wet state is obtained where eucalyptus pulp show distinct advantage compared to other hardwoods. The bulkier sheets with increased porosity give better drying on higher machine speed reducing the drying costs considerably. Therefore, higher bulk gives better printability on coated board with good runnability. Sharma and Bhandari (1983) reported in eucalyptus that pulp with higher bulk formed bulkier sheets than those with less bulk. In the present study also clones with high bulk values gave bulkier sheets. Bulk and stiffness are critical to paperboards manufacture and is an important property of eucalyptus clones which is employed for production of paperboards in ITCs mill at Bhadrachalam. However, during a trial run with only eucalyptus wood it was observed that there is high amount of fluff generation and fines are on higher side (4.5 to 4.7%) which creates problems at paper machine level. Blending eucalyptus fiber with other pulp wood species appears to be an answer to this problem.

Strength properties in respect of burst and tear factor and breaking length variation was significant and indicated in the form of strength index which ranged from 20 to 85 with a mean at 41. The maximum breaking length of 8000 m was recorded for clone 2385 while; the average breaking length was at 5194 m. There are 40 clones showing strength index above 50 which are preferred for paperboard making (Fig 4). A strong and significant trend of low pulp yield and strength index with high content of lignin, rejects, kappa number and AB extractives is observed with respect to 7 clones of *E. tereticornis* (Table 2).

Results of proximate chemical analysis and strength properties (Table 3 and Fig. 5 to 8) indicate that the screened pulp yield was higher (49 to 52.8%) for clones of *E. grandis, E. urophylla, E. torelliana, E. globulus* and Urograndis hybrids while, it

Clone A- No.	B extractiv (%)	es Lignin (%)	Holo-cellulose (%)	Kappa (No)	Rejects (%)	Screen pulp yield (%)	Strength Index
147	3.3	32.2	62.3	23.6	1.4	44	20
319	4.1	29.2	65.2	21.3	0.53	44	29.6
433	4.5	27.1	64.5	21.4	1.38	44	39.2
2069	2.86	31.3	61.4	25	2.21	44	29.9
2261	2.48	31.2	62.4	23.9	3.82	44	29.5
2262	3.2	28.3	67	24.8	0.94	44	42.3
2264	3.8	30.1	62.1	22.3	1.5	44	51.5

 Table 2. Low pulp yielding E. tereticornis clones with other properties.

Parameter	Unit						Mean						
		U 2253	U 2254	U (BCM)	U 283 (HPL)	E.u 347	E.u 348	E.t 455	E.g 643 (HNL)	E.g (OOTY)	E.c 678	E.gl (Ooty)	E.a
Ash	%	0.98	1.18	1.59	1.1	0.98	1.96	1.89	1.79	2.87	0.98	0.74	0.4
A-B extractives	%	4.1	2.69	3.1	3.2	6.2	4.9	4.0	3.79	3.11	4.6	3.7	2.5
Lignin	%	28.5	26.90	28.9	31.0	26.5	31.3	26.1	28.90	29.6	32.9	26.1	27.9
Holo-cellulose	%	66.4	67.70	62.8	64.4	64.2	60.7	64.0	64.10	59.8	60.8	68.2	60.3
Pentosans	%	15.6	16.40	18.7	16.6	13.8	11.9	16.5	15.79	11.6	15.9	16.47	14.1
Screen pulp yield	%	49.4	50.18	49.0	47.9	45.0	50.4	51.7	52.80	51	45.67	49	46.0
Rejects	%	0.7	1.59	1.6	0.9	0.7	1.6	0.3	1.16	1.1	0.54	1	1.4
Карра	No.	22.8	21.10	22.0	23.0	24.6	24.0	22.0	22.20	21.2	21.9	22	21.0
UBV	%	19.2	14.20	15.1	17.4	15.7	14.8	17.2	20.66	18.5	13	15	14.5
Brightness	%	34.0	30.00	32.1	35.0	34.0	36.0	34.0	36.50	36.1	26.9	35	36.0
R.A.A.	Gpl	7.6	7.10	6.8	9.2	8.8	7.9	8.6	9.40	9.8	4	9.5	8.6
Solids	%	13.1	12.50	13.5	14.2	13.0	12.2	13.7	14.10	13.9	17.2	20	18.4
Organics	%	60.5	55.40	56.0	61.2	61.9	64.5	63.7	54.60	53.5	56.8	55	53.0
In-organics	%	39.5	44.60	44.0	38.8	38.1	35.5	36.3	45.40	46.5	43.2	45	47.0
Bulk	cc/gm	1.45	1.68	1.8	1.6	1.7	1.7	1.61	1.62	1.5	1.8	1.4	1.6
Burst factor	-	44	37	40	41	35	31	38	44.1	49	29	43	38.0
Tear factor	-	63	63	55	66	51	55	54	72	65	42	58	48.0
Breaking length	М	5980	5690	5680	5542	4900	4800	5250	5621	6420	3900	6970	4940.0
Strength index	-	66.8	56.9	51.8	62.42	35	34	44.5	72.31	78.2	10	70.7	35.4

Table 3. Pulp & paper and strength properties for clonal eucalyptus species.

Strength index for E. tereticornis clones

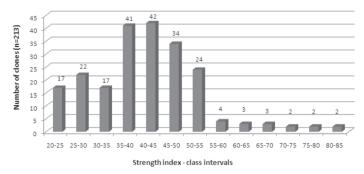


Figure 4. Strength index for E. tereticornis clones.

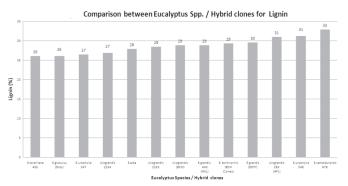


Figure 5. Comparison between eucalyptus Spp. / hybrid clones for lignin.

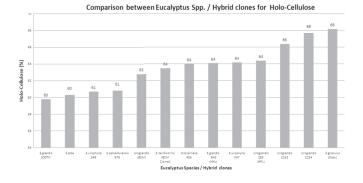


Figure 6. Comparison between eucalyptus Spp. / hybrid clones for holo-cellulose.

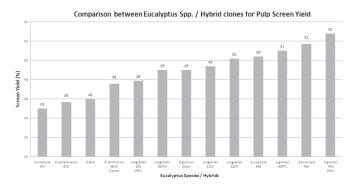


Figure 7. Comparison between eucalyptus Spp. / hybrid clones for Pulp screen yield.

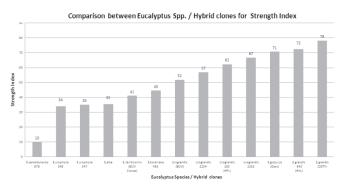


Figure 8. Comparison between eucalyptus Spp. / hybrid clones for strength index.

was on lower side (46%) for *E. alba* and *E. camaldulensis*. The strength index was high (above 50) for *E. grandis, E. globulus* and Urograndis hybrid whereas, *E. alba*, E. camaldulensis, E. torelliana and E. urophylla showed considerably lower strength index. Breaking length was invariably high for *E.grandis, E. globulus, E. urophylla* and Urograndis hybrids indicating that the fibers are longer and stronger in comparison to E. tereticornis, E. camaldulensis and E. alba. Apart from the above, wide variation in lignin content is also recorded between species while, other parameters remained the same with less variation. The above results clearly show that best fiber for paper making cans be derived from species such as *E. globules*, E. grandis, E. urophylla, E. torelliana and Urograndis and other hybrids than pure species E. tereeticornis, E. camaldulensis and E. alba. Seventeen clones of E.grandis from 643 to 659 numbers showed high pulp yield of 50 to 53% but did not perform well at Bhadrachalam location. These *E.grandis* clones however, are performing well at Hindustan News Print (HNL), Kottayam, Kerala. E. globules (blue gum) is not adaptable to Bhadrachalam site as it requires higher elevation and temperate climate. The clones of above species gave excellent properties with respect to pulp and paper properties and similar results are recorded by Tewari (1992) for eucalyptus kraft pulps which give a unique combination of strength, bulk and opacity. This combination together with excellent sheet formation caused by extremely small fibers makes eucalyptus pulp as an ideal raw material for fine papers.

From the data of Table 4 indicate that the proximate chemical compositions and strength properties of eucalyptus are highly influenced by age. The lignin per cent increased with age from 23.4 to 27 and 28.4 to 31.3 for clone 3 and 7 respectively. Similar trend was also recorded for screened pulp yield 48.4 to 50.4% for clone 3 and 47.44 to 49.8% for clone 7. Strength index increase from 30.33 to 46.38 for clone 3 and 37.8 to 46 for clone 7 suggests that the fiber strength is less due to young age while, required strength in fiber is attained at 4 years. Other parameters did not drastically vary.

The hybridization between best clones 6, 10 and 27 was carried out in order to obtain hybrids with superior pulp and paper properties. Perusal of Table 5 reveals that hybrids clones 2120, 2121 and 2156 have shown improvement with regard to screen yield and strength properties while, marginal improvement in other parameters is also seen. Many hybrids have outperformed in the field as well as with pulp and paper properties and are being planted on large scale in the catchment area of the company.

Parameter	Unit		Clo	ne 3			Clor	1e 7	
		1 Yr	2 Yr	3 Yr	4 Yr	1 Yr	2 Yr	3 Yr	4 Yr
Ash	%	1.03	1	1.74	1.04	1.31	1.11	1.28	0.92
A-B extractives	%	3.48	2.35	2.99	3.71	4.33	2.98	3.2	3.67
Lignin	%	23.4	24.6	24.1	27	28.4	30.4	31.4	31.3
Holo-cellulose	%	65.1	65.5	62.3	61.2	63	64.9	63.3	64.2
Pentosans	%	14.39	14.57	14.54	15.54	15.28	16.44	15.7	16.9
Screen pulp yield	%	48.4	49.3	50.4	51	47.44	47.6	48.4	49.8
Rejects	%	1.1	1.12	1.46	1.87	0.59	0.69	0.86	1.11
UBV	Cps	13.7	14.5	14.9	16	14.1	14.2	15.5	14.9
Brightness	%	35.3	35.2	35.3	35.2	28.4	31.4	33.5	35.9
R.A.A.	Gpl	7	5.3	7	5.6	6.3	7	7.6	8.1
Solids	%	13.7	12.6	13.8	12.4	12.5	13.6	12.6	13.5
Organics	%	54.6	55.4	56.2	56.8	54	55.6	56.8	57.9
In-organics	%	45.4	44.6	43.8	43.2	46	44.4	43.2	42.1
Bulk	cc/gm	1.7	1.7	1.7	1.8	1.7	1.7	1.63	1.71
Burst factor		29.5	30.5	35	37	30	31	33.2	35.6
Tear factor		54.5	54.5	53	55	53	55	54	57.6
Breaking length	Μ	4633	4661	5378	5438	5480	4986	5203	5280
Strength index		30.33	31.61	41.78	46.38	37.8	35.86	39.2	46

Table 4. Age-wise analysis of clone 3 and 7 for pulp and paper properties.

 Table 5. Comparison between parent and hybrid progeny for improvement of pulp & paper traits in ITC Bhadrachalam clones of eucalyptus.

Properties	Unit	(a	Parent (at 6 yrs age)			Hybrid clone (at 3 yrs age)			
		6	10	27	2120	2121	2156		
					10x6	10x27	10x27		
	Pro	ximate cher	nical pr	operties					
Lignin	%	30.4	31.1	28.2	32.8	29.6	26.7		
Holo-cellulose	%	64.2	63	68.5	66.7	67.1	70.1		
Pentosans	%	11.4	9.5	16.1	11.8	13.9	14.1		
Screened pulp yield	%	48.9	49.5	46	50.2	50.8	50.6		
		Strength	propert	ies					
Bulk	cc/gr	1.5	1.5	1.9	1.7	1.5	1.6		
Burst factor	-	39.2	39.8	31.2	43	43.8	40		
Tear factor	-	66	64	57.5	78	80	62		
Breaking length	Μ	5520	5350	4425	6000	6400	5400		
Strength index	-	60.4	57.3	33	81	87.8	56		

Rao, *et.al.*, (1999) rated 5 Bhadrachalam clones of eucalyptus (clone 3, 4, 6, 7 and 10) as high pulp yielding clones, with high holo-cellulose content (66.64 to 71.44%), lignin of 24.92 to 30.17% and pulp yield of 48.3 to 53.3%.

To better identify the clones with best overall properties they are ranked from best to worst in each category in the present study and if the clones repeat in top 25 in the list, they are cleared for large scale multiplication and planting under farm forestry programme promoted by the company. Out of 213 clones following clones are short listed based on highest screened pulp yield and strength index - clone 3, 6, 7, 10, 27, 158, 273, 274, 279, 286, 288, 411, 412, 2014, and hybrids 2050, 2121, 2129, 2135, 2140, 2143, 2156, 2294, 2306, 2315 and 2401. Nearly 8 clones from the above group have shown highest strength index (clone 10, 274, 288, 2129, 2140, 2143, 2306 and 2401). These are being cleared for large scale planting under farm forestry.

Eucalyptus clones with high productivity, best pulp and paper properties even if recommended for planting may not serve any purpose if they are affected by gall disease caused by *Leptocybe invasa* insect. Clones resistant to gall (Kulkarni, 2010) along with pulp and paper properties are required to be promoted in the plantation programme. Hence, clones 1, 7, 320, 411, 413, 513, 612, 2008, 2145, 2253, 2254, 2306 now with moderately high pulp yield and strength properties are being recommended for planting on large scale.

Supply of fiber is the key issue facing the pulp and paper industry in India. Raw material and processing costs raise serious concerns over competitiveness of the industry. While considering the entire production chain from wood procurement to paper products, with optimum wood density m (550 to 600 kg/cum), higher pulp yield allows reduction in wood requirement and savings on wood cost. The unit cost of this extra pulp is the variable cost of bleaching and drying. Wood with high pulpwood yield will have a lower chemical demand. Higher pulp yield with improved pulp strength has cost productivity. Improved pulp yield reduces fixed costs (eq. capital cost per tonne of production). It is estimated that less alkali consumption, less use of bleaching chemicals, less power consumption, less wood requirement, more digester productivity, more steam generation from lignin leads to sizable cost savings. The economics of harvesting, transport and processing are greatly improved when the pulp yield is high. Pulp yield has a multiplier effect on costs for growing, harvesting, transport and processing (Dean, 1995). Therefore, breeding programme should consider cost of pulp production and accordingly plan clonal development and deployment.

### Conclusion

Wood raw material quality sets the quality of pulp and paper. Within the 225 eucalyptus Bhadrachalam clones analyzed for proximate chemical analysis and strength properties, there are wide differences in pulp and paper making properties. Pulp yield and strength properties are the important characteristics which are financially rewarding and advantageous to the paper mills. These clones are ranked

according to the pulp yield and strength properties and top 25 clones are recommended for large scale multiplication and planting under farm forestry programme of the company.

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## Comparative study of the dendrometric parameters of *Eucalyptus urophylla* S.T.Blake and the hybrid *E. urophylla* x *E. grandis* in commercial (3x3 m spacing per tree) and silvopasture (9x2 m spacing per tree) stands

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### Introduction

In Brazil, as in several other countries, significant advances in intensive clonal silviculture have been achieved. Significant differences have been observed between cloned species and hybrids of *Eucalyptus spp*. regarding growth, development and wood quality. At the same time, the concept of Agroforestry Systems (AFS) has spread throughout the country in the past years. Among those systems, silvopasture, combining pasture, livestock and cultivation of wood in the same area, emerged as the form most adequate for coping with the limited resources available in Brazil.

The region of Mato Grosso do Sul is traditionally focused on the production of meat and milk. The vast areas just used for grazing and the influence of wood prices has lately attracted the interest of local wood producers and companies. Silvopasture systems could lead to a substantial increase of income and promote the industrialization of the region by providing affordable resources in greater quantity and diversity. Also, an increase of direct and indirect employment through an increase in local productivity could be expected. Silvopasture systems are also known to ameliorate soil properties (SILVA, 2003).

The study's objectives are to analyze dendrometric parameters of *Eucalyptus urophylla* S.T.Blake and a hybrid of *Eucalyptus urophylla* x *Eucalyptus grandis*, both planted in conventional spacing (3x3 m per tree) and silvopasture spacing (9x2 m per tree).

### **Materials and Methods**

The experimental site at Boa Aguada farm, owned by the Mutum Group (Mutum Reforestation), is located in the municipal district Ribas do Rio Pardo in the southeastern region of the brazilian federal state of Mato Grosso do Sul (20º 26'

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35" S and 53º 45' 33" N) at an altitude of 369 meters.

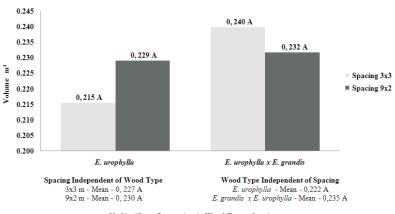
Data was collected in areas with similar soil characteristics, which are tropical sandy soils. In the experiment clones of the trees *Eucalyptus urophylla* and clones of the hybrid *Eucalyptus urophylla* x *Eucalyptus grandis* with an approximate age of 4,5 years were taken from stands of the two silvicultural managements mentioned above.

For the study a total of 20 trees (5 trees of 2 different species of 2 different managements) was collected. The trees were cut and total height, commercial height (in Brazil stem without crown), and diameter at breast height was taken. Then a disk of 3 cm thickness was cut from the base and at 25, 50, 75 and 100 % of the commercial height. This material was taken to the "Pulp and Paper"-laboratory of UNESP were dendrometric parameters according to Smalian were determined.

### **Results and Discussion**

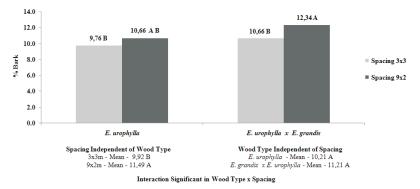
Figure 1 shows that there are no statistical differences at 5% level of significance for the volume of wood with bark. Still one can see that the hybrid of *E. urophylla x E. grandis* in the smaller spacing shows higher volumes with 0,240 m<sup>3</sup> where *E. urophylla* shows lower volumes with 0,215 m<sup>3</sup>. Another fact that stands out is an opposite trend in the behavior of species in relation to spacing. These values can be related to a lower age of the trees.

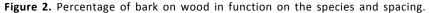
Comparing only the species, the hybrid *E. urophylla x E. grandis* shows a higher average with 0,235 m<sup>3</sup> than *E. urophylla* with 0,222 m<sup>3</sup>. These results seem to be



No Significant Interaction in Wood Type x Spacing

Figure 1. Volume of wood with bark in function for each species and spacing.





directly linked to the fact that hybrids of *E. urophylla x E. grandis* have better characteristics concerning adaptation to different forest sites and an increased productive capacity as already shown in MORAIS (2006), GOMINHO (2001), SILVA (2006).

Concerning the amount of bark, OLIVEIRA et al., (1999) mentioned that the percentage of bark is also important from a industrial point of view. Each of the used species show variations in amount, form and texture of the bark. Figure 2 shows that the hybrid *E. urophylla* x *E. grandis* in the 9 x 2 m spacing comes with the highest amount of 12,34% bark. The lowest value was found by the *E. urophylla* in the 3 x 3 m spacing with 9,76%.

### Conclusions

The hybrid *E.urophylla x E. grandis* stood out with greater volume of wood in both spacings.

The 9 x 2 m spacing produced a greater percentage of bark, especially for the hybrid *E. urophylla x E. grandis*.

There are significant interactions between type of wood and spacing. Most significant here is the difference in bark amount for the hybrid *E. urophylla x E. grandis* compared to *E. urophylla*.

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## POSTER SESSION 9. BIOTECHNOLOGY

## Culture Establishment and Multiplication of superior recombinants emanating of mature *Eucaltyptus* hybrid *E. citrodora* Hook. × *E. torelliana* F.v. Muell

Thakur, Ajay

### Introduction

*Eucalyptus* spp. is a desirable species for industrial wood in India and hence it is being planted in large area (estimated 2.5 million ha). This is most planted species supported by organized sector as well; ITC, Bhdrachalam is investing around \$ 100 million every year for plant improvement (http://www.indianow.org/Archives/ ViewArticles.aspx? artid=22303&catid=60). Hybrids in *Eucalyptus* opened a new dimension in tree improvement programme where the new interspecific combination showed better performance with desirable characters infused from both the parents. In case of hybrids, the individuals are unique and also they have to be maintained for future observation and original source material, micropropagation is the only way to multiply plant material *en masse*.So, this study was started to develop complete micropropagation protocol for a selected *Eucaltyptus* hybrid *E. citrodora* Hook.× *E. torelliana* F.v. Muell.

### **Material and Methods**

Explants were collected from a 24 years old tree of *Eucaltyptus*hybrid *E. citrodora* Hook. × *E. torelliana* F.v. Muell.Experiments for bud breaks using different media and sterilients were laid. Explants were treated with five sterilients; Bromine Water (1% v/v) Silver nitrate(1% w/v) Mercuric chloride (0.1 % w/v) Sterimax and sodium hypochlorite for three time periods; 3, 5, 10 and 15 minutes. Plants were grown on MS media supplemented with benzyladenine. For multiplication a combination of benzyladenine and 1-naphthaleneacetic acid. Rooting trials were started from15 mm or more long shoots of 2<sup>nd</sup> cycle onwards. Half strength MS media with indole butyric acid were used for rooting.

### **Results and conclusion**

Experiments for bud breaks using different media and sterilients shows that

- Sterimax and sodium hypochlorite were effective at 3min and 5 min.
- Bromine water changed the colour of explant to brown but no contamination

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was observed with HgCl, and Bromine water.

• Best result of successful bud break was obtained in Sodium hypochlorite (5%) for 5 minutes was most effective (45 %) on MS media supplemented with benzyladenine.

• No bud break was observed in MS basal medium.

• Best multiplication result was obtained with sodium hypochlorite (5%) for 5 minutes treatment on benzyladenine and 1-naphthaleneacetic acid.

• Best time of response was late March to April for bud break and multiplication.

• Culture was established successfully and in first cycle took 55 days to multiply one to many stage (9 shoots were more than 15 mm length). In later cycles it is takes 40 – 45 days.

• Cultures have completed 3 cycles and fourth cycle is going on.

No success in rooting was observed. Rooting trials will be repeated in fourth cycle as well.

### R2R3 MYB transcription factors controlling of wood formation in Eucalyptus

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With the current global focus on bioenergy, forest plantations are increasingly becoming important sources for second generation biofuel, where the whole plant lignocellulosic biomass is to be mobilized. The lignocellulosic biomass is mainly composed of secondary walls (SW) possessing unique characteristics (biochemical composition and tridimensional association of polymers), which govern the intrinsic properties of wood. They especially contain high amounts of lignins, hydrophobic phenolic polymers which constitute an obstacle to the optimal utilization of plant species in paper industry and for saccharification prior to bioethanol production. Among perennial species, *Eucalyptus* species grow very fast and produce high yields of lignocellulosic biomass. They represent the main industrial plantations in the worldand one of the most appealing lignocellulosic feedstock for bioenergy production. Dissection of the molecular switches controlling the coordinated lignin biosynthetic genes is therefore of utmost importance to understand the molecular mechanisms underlying tissue specific deposition of lignin and be able to improve secondary cell wall properties.

With the objective of improving Eucalyptus wood quality to better-fit industrial applications, we are focusing our efforts towards the identification and functional characterisation of regulatory genes controlling the biosynthesis of the cell wall polymers (mainly lignins).

We performed a precise mapping and functional characterization of the *cis*regulatory elements contained in the promoters of two genes encoding key and consecutive steps of the lignin biosynthetic pathway *i.e.* Cinnamoyl CoA reductase (CCR) and Cinnamyl Alcohol dehydrogenase (CAD) (Rahantamala *et al*, 2010). Our results supported a major role for the MYB transcription factors (TF) consensus sites in the control of the coordinated expression of these two genes. The functional analysis of two MYB factors (EgMYB1 and EgMYB2) preferentially expressed in *Eucalyptus* xylem revealed that they are able to bind specifically to these promoters

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and regulate transcription*in vivo*. EgMYB1 behaves as a repressor whereas EgMYB2 is an activator (Goicoechea *et al*, 2005, Legay et al, 2010) of the lignin biosynthetic genes but also of the secondary wall biosynthesis. Indeed, both MYBs were shown recently to be master genes regulating the entire secondary wall biosynthetic program including cellulose, xylan and lignin genes (Zhong *et al*, 2010; Legay *et al* 2010). The presence of both positive and negative regulators in *Eucalyptus* xylem offers the possibility of a combinatorial control of gene expression that could provide the necessary flexibility to ensure tight temporal and spatial regulation of lignin biosynthesis or secondary cell wall.

To address this question and get a deeper insight the complex regulation of the SW formation in *Eucalyptus*, we are now studying the regulation of these two MYBs including fine spatial and temporal expression, identification of their direct targets genes and of their protein partners. We have constructed a yeast-two-hybrid library from *Eucalyptus* xylem and we are currently screening for EgMYB1 and EgMYB2 interactants.

Thanks to the recent release of the *E. grandis* genome (*Eucalyptus grandis* Genome Project 2010, http:://www.phytozome.net/eucalyptus), we have performed a genomewide survey of the large R2R3-MYB superfamily. The phylogenetic comparison of this family with *Arabidopsis*, rice, poplar and grapevine showed a marked expansion of some clusters putatively involved in wood-related processes. Some R2R3 MYB genes seem to be specific of woody plants. Expression analysis and EST database surveys are currently underway to explore the transcript levels of each member in the different organs and tissues of *Eucalyptus* at key developmental stages as well as in response to hormonal treatments and to environmental stresses

Further functional genomics studies conducted on new R2R3 candidate transcription factors will help identifying major factors underpinning the physicochemical properties of cell walls, the recalcitrance of which remains a key scientific challenge for establishing highly efficient, sustainably produced, secondgeneration biofuels.

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# Optimal time for the adventitious rooting of *Eucalyptus* benthamii mini-cuttings

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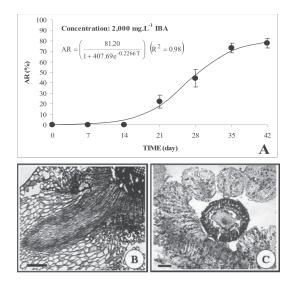
*Eucalyptus benthamii* is a forest species of economic interest, mainly by adaptability in cold regions and tolerance to frost [1]. However, there is difficulty to obtain seeds, and is considered difficult rooting when propagated by cuttings or mini-cuttings [2]. Currently, the protocols for adventitious rooting of cuttings or mini-cuttings have not been satisfactory to meet the demand of clones required by the market, affecting the breeding programs of species. We aimed to determine the percentage of adventitious rooting of *Eucalyptus benthamii* mini-cuttings in relation at IBA (indole-3-butyric acid) concentrations and the optimal time of stay of minicuttings rooted in greenhouse. We use the shoots of mini-stumps (clones: BP101, 118 and 120) grown in clonal mini-garden with semi-hydroponic system for preparation of mini-cuttings. The mini-cuttings presented a length of  $6 \pm 1$  cm, containing two pairs of leaves reduced to 50% leaf area, with the apex (tip shoot). The basal portion of mini-cutting was composed of two pairs of buds without leaves. The basal region of the mini-cuttings was treated with IBA (0 – free of plant growth regulator; 1,000; 2,000; 3,000 and 4,000 mg.L<sup>-1</sup>). Every seven days (0 – moment of planting, 7, 14, 21, 28, 35 and 42 days) proceeded destructive sampling of minicuttings to evaluate the percentage of rooting. The sampling for histological evaluation was performed until 28 days. Data were submitted to Hartley test (P<0.05) to verify the homogeneity of variance between treatments.

Then proceeded to analysis of variance (P<0.01 and P<0.05) and according to the significance, the data were submitted to logistical regression analysis (P<0.05). The rooting percentage did not differ in relation the concentrations of IBA, regardless of the clone evaluated at 45 days. The concentration of 2,000 mg.L<sup>-1</sup> IBA induced higher speed and percentage of rooting, being indicated the range of 35 to 42 days for the permanence of mini-cuttings in the greenhouse (Figure 1A). We observed induction of adventitious root showing connection with the vascular cambium in the treatments with 0; 1,000; 2,000 and 3,000 mg.L<sup>-1</sup> IBA, at 28 days (Figure 1B),

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**Figure 1.** Adventitious rooting of *Eucalyptus benthamii* mini-cuttings in relation at evaluation time and IBA concentration. (A) application of 2,000 mg.L<sup>-1</sup> IBA, AR = percentage of adventitious rooting; (B) transversal section of adventitious root induced with 2,000 mg.L<sup>-1</sup> IBA, 28 days, Barr: 100 $\mu$ m; (C) transversal section of adventitious root induced with 4,000 mg.L<sup>-1</sup> IBA, 28 days, Barr: 100 $\mu$ m.

similar to that observed for *Eucalyptus globulus* [3]. However, external callus induction occurred in the treatment with 4,000 mg L<sup>-1</sup> IBA with subsequent adventitious rooting (Figure 1C). The information obtained from this work will serve to gain knowledge of the adventitious rooting of *Eucalyptus benthamii* minicuttings for increase the commercial production of clones.

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POSTER SESSION 10. SOCIAL AND ENVIRON-MENTAL RESPONSABILITY

# Sustainable Intensification of *Eucalyptus camaldulensis* in Southern India

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### Background

Sustainability and optimal productivity of eucalypt plantations in short rotations on marginal lands in India has been a point of controversy given the poor management strategies and genetic stock. A negative balance of 9.7 M t of major nutrients in Indian agricultural land (Tondon 2007) is a major concern. There is a general tendency to disregard the nutrient content of soils or the uptake by plantations (nutrient depletion) and define site quality based on wood yield produced from an area. The impact of management practices on degradation of site quality to support tree growth was never addressed seriously. Long-term studies elsewhere have shown that improvements in productivity can be achieved through use of genetically superior planting stock and adoption of site management practices such as slash management, nutrient addition and weed control (Nambiar 1996, Tiarks et al. 2000, Hehir, and Nambiar 2010). Sustainability is no longer an option but a necessity to ensure better management of environment and economy.

A study was taken up to evaluate productivity and impact of management strategies on indigenous nutrient supply system of the soil, nutrient uptake and accumulation in different plant parts of E. camaldulensis. The study also aims to improve and sustain productivity of eucalypt plantations in southern India through efficient nutrient application.

### Material and Methods

The study sites were located at Hyderabad (Andhra Pradesh 17°30' 4.20" N and 78°16' 43.83" E) and Mettupalayam (Tamil Nadu 11°26' 23.35" N and 78°29' 20.79" E) in southern India.

The site characteristics (Table 1) and soil profile study were carried out and initial soil nutrient status (Table 2) was estimated at the time of initiation of the trials. A factorial combination of nitrogen, phosphorus and potassium in four replications was tested with control in both locations. Each plot comprised of 30 trees planted in a spacing of 3 X 1.5 m while growth and soil parameters were carried out on 12 central trees to maintain local control. Trees were felled from

Location (Clone No.)	ICRISAT (413)	Kovai (411)
Latitude	17°30' 4.20'' N	11°26' 23.35'' N
Longitude	78°16' 43.83″ E	78°29' 20.79" E
Altitude	545 m	410 m
Rainfall (mm yr⁻¹)	750	700
Soil texture	Clay loam	Clay loam
Soil Type	Red	Red

Table 2. Soil characteristics of the selected site.

Particulars	Site and pla	inted clone
	ICRISAT	Kovai
Su	rface soil properties (0-30 cm)	
pH (1:5 H <sub>2</sub> O)	6.4	8.4
Organic C (%)	0.25	0.30
Available N (kg/ha)	313	176
Available P (kg/ha)	22	17.8
Available K (kg/ha)	601.2	152.09
Ca (ppm)	1425	921.11
Mg (ppm)	129.7	68.9
Zn (ppm)	17.8	10.2
Fe (ppm)	99.9	86.3
Cu (ppm)	6.1	4.2
Mn (ppm)	78.7	84.33

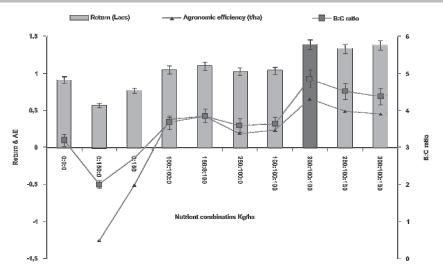


Figure 1. Wood yield, use efficiency and benefic cost ratio under nutrient management after 2 years of treatment.

each plot, portioned in leaf, wood and bark for biomass analysis and estimation of nutrient uptake. Soil was collected from a distance upto one meter periphery from the felled tree at a depth of 60 cm and analysed for available nutrients, nutrient

use efficiency and soil nutrient budgeting at the time of harvesting. Economics (Benefit: Cost) of nutrient application was also studied for deciding capital outlay for sustainable eucalyptus production.

### Results

### Productivity

Nutrient management had significant impact on productivity at the time of harvest. Mean annual wood production was about 27% lower (82.3t ha"1) when no external nutrient application (control) was made compared to yields achieved with a balanced dose of nutrient combination 300 : 200 : 150 kg N:P:K ha<sup>"1</sup> (i.e. 112.9 t  $ha^{(1)}$  which was on par with application of 250: 100 : 150 and 200 : 100 : 100 kg N:P:K ha<sup>71</sup> (i.e. 107.8 t ha<sup>71</sup> and 108.9 t ha<sup>71</sup>, respectively (figure 1). In general yield reduced when one or more major nutrient was omitted from the combinations in both locations. Overall growth rate and productivity across the sites was 22-27% higher in plots receiving balanced dose nutrients over the control. The highest wood volume nutrient ratio (WVNR) of 66.4 was obtained in plots receiving 200:100:100 kg N:P:K ha<sup>''</sup> which was followed by the treatments 250: 100 : 150 and 300 : 200 : 150 kg N:P:K ha-1 with an agronomic efficiency of 49.3 and 45.5, while lowest WVNR (-125.4 and -50.5) was noted in plots with omission of nutrients i.e. 0 : 150 : 0 and 0 : 0 : 150 (imbalance in nutrient application) (figure 1). The benefit cost ratio was also higher in the systems which received balanced dose of nutrients 200:100:100 (4.9), 250: 100: 150 (4.5) & 300: 200: 150 (4.4) kg N: P: K ha<sup>''1</sup> (figure 1).

### Impact of nutrient management on soil

One of the main aims of the study was to establish an experimental base to examine the relationship between conservation and depletion of site resources (nutrients) in different nutrient management regimes. Though there is negative balance in the plots with balanced nutrient application for all major nutrients potential they are likely to reach the initial levels in due course of time and reach a balance between nutrient demand by the tree and supply by the soil. The quantity of nutrients exported from plantations under different nutrient regimes shows high ratios between nutrients exported by harvesting and those available in soil, indicating limitation for major nutrients (N, P & K) over the long term. Current harvesting practices (removal of stem wood, leaves and bark from site) result in high rates of export of these nutrients (figure 2). Built up of soil nitrogen was

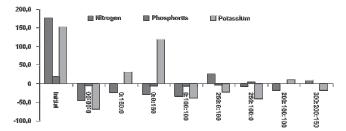


Figure 2. Soil nutrient balance sheet at the time of harvesting.

observed in treatment 300 : 200 : 150 kg N:P:K ha<sup>"1</sup> (7.7 kg/ha) while phosphorus and potassium levels were high in treatments 0 : 150 : 0 kg N:P:K ha<sup>"1</sup> (where only Single super phosphate was applied - 0.5 kg/ha) and 0 : 0 : 150 kg N:P:K ha<sup>"1</sup> (where only muriate of potash was applied -118.8 kg/ha) respectively. This was mainly due to sole application of the nutrients - phosphorus & potassium that resulted in buildup of these nutrients in soil' but did not realise into yield improvement.

### Conclusion

It is often assumed that the present intensified eucalyptus cropping in India is sustainable. The magnitude of nutrient transport from soil during harvest in the form of leaf, wood and bark will affect sustainability of eucalyptus plantations under current management system. Adequate fertilizer inputs are necessary in degraded soils worked on short rotations for sustained productivity. A management package involving balanced nutrient application will increase wood yield by 27% over present practice. Slash management in situ will have good impact on soil nutrient reserves for sustained productivity in subsequent rotations.

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# The Cultural objective and its Realization of *Eucalyptus* Plantation Management

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### Abstract

China has a 120-year history of eucalyptus cultivation. To meet the industrial requirements of pulpwood, the average area of new eucalyptus plantations has increased to 200,000 ha per year in southern China. But as a blinding flash of mass cognition and some false standpoints, the local damage of environment has been attributed to the fast development of eucalyptus plantation. Many people consider eucalyptus as a water-depleting plant and also the major culprit of the severe drought that affected Southwestern China. Because of these reasons, the local government has been cautious about planting eucalyptus and has reduced the funding for research on eucalyptus.

Thus, in this study, we have described eucalyptus plantation management system as a complex social-natural-economic system and designed a model system (Figure 1). Here, we report the objective system for economic eucalyptus plantation, with due consideration to the effects of eucalyptus plantation on ecology, society, safety, and culture. The main objective of this study was to analyze the cultural objective of industrial plantation of eucalyptus and explain the concept, function, structure, and implementation of cultural objectives. On the basis of graph analysis, we determined 6 social correlating factors, namely, enterprises, government, research and education institutes, technique expansion units, farmers, and community. Appropriate management of these factors would facilitate the realization of the cultural objective of industrial eucalyptus plantation (Figure 2).

Further, we determined the relationship between management of these factors and the cultural objective. Leizhou Forestry Bureau, which has more than 50 years of experience in eucalyptus plantation, serves as an example for achieving the cultural objective of eucalyptus plantation. Leizhou Forestry Bureau has a longterm policy and gives importance to the cultural impact of industrial eucalyptus plantation, which results in higher yields of eucalyptus, higher staff income, and better cultural quality than at any point in history.

On the basis of the above analysis, we have drawn the following conclusions:

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1. Management of eucalyptus plantation is required to account for its effects on economy, society, safety, culture, and ecology. The primary objective of cultural development is the accumulation of knowledge and the accomplishment of cultural systems during the development of eucalyptus plantation. In addition, we also aimed to develop and use technical knowledge by developing basic research, enhancing scientific and technological progress, creating educational institutes,

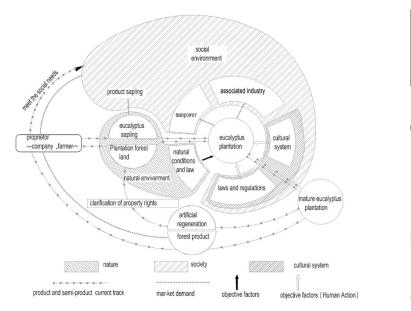


Figure 1. Structure Model for Ciclye management of Eucalytpus plantatins.

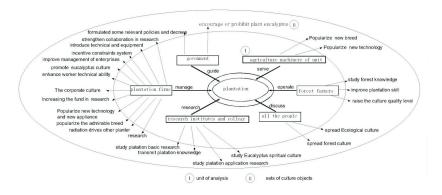


Figure 2. Decomposition of Cultural objective of Ecualyptus plantations management.

and inculcating of corporate culture and forest culture in the domain of cultural knowledge.

2. Plantation management is a complex social-natural-economic system for meeting the manifold needs of human beings and society. In particular, in developing countries, economic and social developments should not be based on exploitation of natural resources, and plantations must be performed to protect the nature.

We performed a systematic analysis of the process of plantation management. We found that plantation is an art in that people completely utilize natural conditions and harmonize the conflicts among society, economy, and nature by using novel technologies and policies and regulation systems. A recycle loop of afforestation, silvicultural cutting, and reforestation is performed to achieve sustainable management of plantations. To identify the optimal solutions for the existing problems in plantations, we must consider 3 aspects, namely, society, economy, and nature.

3. Achieving cultural objectives is an important part of plantation operation. Cultural objectives are realized through the commonly participating operators (enterprises, farmers), government, research and education institutes, technique expansion units, and the community. The important steps and factors in achieving cultural objectives include scientific collaboration, technology spillovers, institutional creation, management-level activities, culture education, development of employee skills, enterprise culture, research support, technology expansion, propagation of new varieties, effect of radiation, and indigenous R&D.

4. Case analysis indicated that the realization of cultural objectives in eucalyptus plantation management promotes simultaneous growth of forest resources and economic benefits and has an active effect on improving the operators' cultural qualities, personal income, and the economy of surrounding area. It also promotes favorable development of plantation and related industries.

**Key words:** *Eucalyptus* plantationÿ managementÿcultural objectiveÿRealization complex social-natural-economic system