DEVELOPMENT OF AN ADVANCED GENERATION BREEDING STRATEGY FOR *EUCALYPTUS NITENS* (DEANE & MAIDEN) MAIDEN

Tammy-Lyn Swain

Submitted in fulfillment of the academic
requirements for the degree of
Doctor of Philosophy in
Plant Breeding
in the
School of Agricultural, Earth and Environmental Sciences
College of Agriculture, Engineering and Science
University of KwaZulu-Natal
Pietermaritzburg

South Africa
October 2013

FACULTY OF SCIENCE AND AGRICULTURE

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PREFACE

The experimental work described in this thesis was carried out at the Institute for Commercial Forestry Research, situated in Pietermaritzburg, South Africa, under the supervision of Professor Mark D Laing (University of KwaZulu-Natal) and Dr Steve D Verryn (Creation Breeding Innovations and University of Pretoria).

These studies represent original work by the author and have not been submitted in any other form to another University. Where use has been made of the work of others, it has been duly acknowledged in the text.

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Tammy Swain

I hereby certify that this statement is correct.

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Professor Mark D Laing

Supervisor

.....

Dr Steve D Verryn

Co-supervisor

PUBLICATIONS AND PRESENTATIONS FROM THIS THESIS

Peer-reviewed publications

Swain T-L. 2011. Genetic gain in a breeding population of *Eucalyptus nitens* in South Africa. *ICFR Technical Note* No. 07/2011. Institute for Commercial Forestry Research, Pietermaritzburg.

Swain T-L, Verryn SD, Laing MD. 2013a. A comparison of the effect of genetic improvement and seed source and seedling seed orchard variables on progeny growth in *Eucalyptus nitens* in South Africa. *Tree Genetics and Genomes* 9(3): 767-778. DOI 100.1007/ s11295-013-0593-0.

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Field day presentations

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Swain T-L. 2011. Genetic gain in the ICFR's *Eucalyptus nitens* breeding programme. *Central/Mpumalanga Regional Interest Group Field Day*, 13 October 2011, Warburton, South Africa.

ACKNOWLEDGEMENTS

I would like to express my gratitude to the following people:

My supervisor, Professor Mark Laing, for your patience and guidance, which have been invaluable in achieving closure to this marathon.

Dr Steve Verryn, my co-supervisor, who has been involved since the beginning and has stuck for the long haul. Your mentorship, constructive criticism, patience and kindness are greatly appreciated. I have sincere respect for your knowledge in Tree Improvement and Quantitative Genetics and your mentorship abilities. Thank you!

Professor Colin Dyer for your belief, support, personal investment and guidance along the way, and for allowing the time to complete this study.

Past and current ICFR Tree Improvement Technical Staff who have been involved in the field trials over the years; Colin Chiappero, Chris Komakech, Shadron Naicker, David Hockly, Eric Wallace, Patrick Thabethe, Thulani Nsindane and Zama Ngidi.

Professor Annabel Fossey for your enthusiasm and support in starting this study.

Gert van den Berg for your willingness to assist with problematic SAS® procedures, and debate thereof.

Desiree Lamoral for your unflagging support and kindness in accessing (probably excessive!) publications for this study.

Robin Gardner for taking over Programme Management duties for substantial periods, for advice on flowering aspects and support throughout.

Dr Ryan Nadel and Dr Louis Titshall for your kindness and skills in graphing in Statistica®.

Dr Michael Bairu for sharing your “thesis” experience and for casting your eyes over the important beginning and end parts!

Sidhika Naidoo for your kindness and thoroughness in checking through, and editing, all my references.

Sally Upfold for your positive support throughout, editorial advice and for helping to pull it all together at the end.

The ICFR and funders of the Tree Improvement Programme for supporting and funding this study, without which it would not have been possible.

My parents for your belief and excitement throughout.

Finally, my husband, Paul, and daughters, Jessica and Bridget, for your patience and understanding. Paul, particularly for your support in the last twelve months, and the girls for doing so well without a proper Mom for ages.

THESIS SUMMARY

The objective of this study was to develop and implement an advanced generation breeding programme at the Institute for Commercial Forestry Research (ICFR) to manage and integrate the many and disjunct breeding and production populations of *Eucalyptus nitens* established by various entities over the past 30 years at multiple sites in South Africa. To develop such a breeding strategy, a good understanding of the population genetics, and the underlying assumptions made by tree breeders about the species, was needed.

Eucalyptus nitens is an important forestry species grown for pulp and paper production in the temperate, summer rainfall regions of South Africa. A tree improvement programme has been ongoing at the ICFR for three decades. The measurement and statistical analysis of data from eight F1 trials established during the 1980s and 1990s have enabled characterisation of the ICFR's breeding population. Provenance testing showed that the more northerly New South Wales (Australia) *Eucalyptus nitens* provenances of Barren Mountain and Barrington Tops are distinctly better suited to growth in South Africa than the southern New South Wales provenances and the Victorian provenances, Penny Saddle and Bendoc. Generally, the species was not badly affected by *Coniothyrium* canker. High Type B genetic correlations for all sites pairs, except one comparison, ranged from 0.75 to 0.99 for diameter at breast height at 76 to 113 months, indicating very little, or no, genotype by environment interaction for diameter at breast height for the genotypes tested in the F1 generation. Narrow sense heritability estimates ranged from 0.01 to 0.34, indicating that the species provides a breeding opportunity for improvement of diameter growth. High genetic correlations of greater than 0.90 between diameter measurements at 52 to 62 months after establishment and diameter measurements at 94 or 113 months were found, indicating that selections can be made reliably at five or six years. Diameter measurements at both 60 months and full rotation (94 to 113 months) were highly correlated with the final height measurements in these trial series (r_g > 0.71 and > 0.83, respectively). Predicted genetic gains for the F2 over the F1 generation were highest in the trials at Goedehoop and Arthur's Seat, with predicted increases in diameter at breast height of 3.07 cm (17.1%) and 3.17 cm (20.7%), respectively, at full rotation.

Genetic improvement in the species has been slower than anticipated due to delayed and infrequent flowering and seed production. Three genetic gain trials were established, firstly, to quantify the gains that have been made in the first generation of improvement in the breeding programme; and secondly, to establish whether a number of seed source and orchard variables influence the performance of the progeny. These variables were: the number of flowering trees in the seed orchard, year of seed collection, seed orchard origin and composition of seed orchard seed bulks. Diameter at breast height and tree height were measured in the trials at between 87 and 97 months after establishment, and timber volumes and survival were calculated. Improved seed orchard bulks performed significantly better ($p < 0.01$) than unimproved controls in the field trials, and genetic gains ranging from 23.2 to 164.8 m^3ha^{-1} were observed over the unimproved commercial seed. There were significant differences ($p < 0.01$) in progeny growth between the levels of flowering, with higher levels of flowering (≥ 40 %) producing substantially greater progeny growth than lower flowering levels (≤ 20 %). The seed orchard origin had no effect on progeny growth in this trial series. This suggests that seed collected from any of the four seed orchards tested will produce trees with significant improvement in growth.

Various scenarios investigating a range of assumptions were developed and used to predict genetic gain in the F2 populations. These were compared with realised gains achieved in the genetic gain trials. The family nested within provenance scenarios proved to be closer to realised gain than the family across provenance predictions. Two scenarios were used for family nested within provenance: Firstly, actual flowering for family nested within provenance; and secondly, estimated flowering after a 30% roguing of poor families. For both scenarios, a coefficient of relationship of 0.33 predicted gains closest to the realised gains. Indications were that the effects were additive, and that very little or no heterosis had occurred. The statistical information suggested that outcrossing in the seed orchards was > 80%. This study provides an objective and quantitative assessment of the underlying assumptions used for estimating genetic parameters, and predicting gain in this population of *Eucalyptus nitens.*

At the same time that genetic gain trials were established, F2 trials were planted, using seedlots collected from F1 seed orchards. Analysis of the two F2 trials showed that realised gains for diameter at breast height at 87 months were close to the predicted values and ranged from 1.02 cm to 1.90 cm. Two exceptions were the sites at Helvetia and Babanango, where gains were under- and over-predicted, respectively. Realised heritability estimates,

which are related directly to the realised gain and the actual selection intensities used in the seed orchards, reflected this trend. Estimation of breeding values allowed for selection of elite individuals in top families. Both grand-maternal provenance origin and F1 maternal effects were significant in the F2 trials. A Type B genetic correlation of 0.61 for diameter at 87 months indicated the possible presence of genotype by environment interactions for the two F2 sites. A low narrow sense heritability estimate of 0.06 for diameter at breast height at 87 months at one F2 site indicated that more emphasis should be placed on family information rather than individual information at this site. A heritability estimate of 0.17 for diameter at breast height at 87 months at the second site, however, indicated that further improvement is possible in this population of *Eucalyptus nitens*.

Modelling of predicted genetic gain using various breeding strategy scenarios can be a useful tool in assisting with the decision on which strategy or management plan will deliver the most genetic gains per unit time. Such modelling, using the parameters established in the first part of the study, played an important role in developing the advanced generation breeding strategy for *Eucalyptus nitens*. In addition, the modelling exercise highlighted various management options which could be used to increase gains in the existing production populations or orchards. Indications are that additional roguing of 1) existing Clonal Seed Orchards based on results of F2 trials (i.e., backward selection); and 2) F1 Breeding Seed Orchards based on stricter provenance selection, will markedly increase the quality of the seed produced from these orchards within one season. This study also highlighted the importance of shortening the breeding cycle in *Eucalyptus nitens*, particularly in view of the delays caused by reticent flowering and seed production in the species.

The information and understanding gathered from this study led to the development of a proposal for an advanced generation breeding strategy in *Eucalyptus nitens.* This proposal uses parental reconstruction of open-pollinated progeny to secure pedigree information of forward selections, thus combining the benefits of increased genetic gain with a shortened breeding cycle. Recommendations on the management and adaption of current production populations to increase gains have been made, because establishment and management of improved material in seed orchards to ensure a sustainable supply of improved seed to the South African forestry industry, is a key objective of the ICFR *Eucalyptus nitens* breeding programme.

Keywords

E. nitens, genetic parameters, heritability, genetic correlations, genotype-environment interaction, predicted gain, genetic gain, tree improvement, flowering levels, seed bulk composition, advanced generation breeding, coefficient of relationship, outcrossing

CONTENTS

THESIS INTRODUCTION

Background to the study

Eucalyptus nitens is an important cold tolerant eucalypt (CTE) species grown commercially in South Africa. It is recognised as the most snow hardy of the CTEs grown in South Africa (Gardner and Swain 1996) and currently, there are no suitable alternative commercial species for sites prone to moderate frost and heavy snowfalls. The species is primarily grown for production of bleached hardwood pulp and paper, although it is still a key species for mining timber. Site-species interaction and tree improvement trials have shown that significant variation exists between the provenances grown in South Africa for a range of growth, reproductive and wood property traits, making this species suited to improvement through breeding, with the objective of meeting the requirements of the diverse forestry sites available in South Africa. However, a key constraint to breeding for improved cultivars in the species, and for any achieved genetic gain being deployed commercially, is the poor and erratic flowering, and subsequent inadequate seed production, in this species (Gardner 2003).

Eucalyptus nitens is also a significant plantation species in other temperate regions of the world, with active breeding of the species in Australia, Chile and New Zealand. Open-pollinated breeding programmes are common for the species, with parameter estimates available for several traits, such as growth, wood property and tree architecture traits (Hamilton and Potts 2008). Parameter estimates from control-pollinated progeny trials are rare because flower size and the limited amount of flowering constrain control-pollinated breeding programmes in the species (Tibbits 1989).

The *E. nitens* breeding programme at the Institute for Commercial Forestry Research (ICFR) has been ongoing since the mid-1980s, and is currently funded by a consortium of commercial forestry companies in South Africa, with the primary objective of producing improved seed of the species for the South African forestry industry. These improvements include increased growth, improved stem form, and pest and pathogen tolerance. In addition to producing improved seed, germplasm in the form of pollen or scion is needed by the commercial forestry companies for use in their in-house pure-species or hybrid-breeding programmes.

The first generation of improvement in the ICFR *E. nitens* breeding programme has been completed and the remaining F1 material currently comprises five F1 trials that have been converted to seed orchards (BSOs), one F1 seedling seed orchard and three F1 clonal seed orchards (CSOs). Seed was collected from three of the F1 BSOs over several years and partial progeny trials (F2) were established at two sites in 1999 as part of the breeding strategy to embark on a recurrent selection breeding programme. These trials included only seed from those 80 individuals that had flowered and produced seed by that time. Since then, extensive seed collections were completed in six ICFR F1 *E. nitens* BSOs and one CSO. Subsequently, an F2 trial series was established on sites in Mpumalanga and KwaZulu-Natal in 2008. This series comprises 169 new seedlots collected from the F1 seed orchards and 13 controls/treatments in common with the F2 trials established in 1999. The two trial series were established nine years apart, due firstly to poor flowering in the orchards causing delays in sufficient seed production from all families, and secondly, due to financial constraints in the latter few years. Associated seed orchards were established with each trial series. The biological constraints have resulted in a disjunct series of trials representing the same nominal level of improvement, and a range of production populations/orchards with varying levels of improvement.

Significance of the study

The ICFR identified the need to develop and implement an advanced generation breeding programme to manage and integrate the many and disjunct breeding and production populations. To develop such a breeding strategy, a good understanding of the population genetics and underlying assumptions of the species are necessary (Tibbits and Hodge 1998). These assumptions, as well as biological constraints, affect the gain predicted from a breeding strategy, as well as the gain ultimately realised. The key biological factor that is possibly constraining genetic gain being realised in commercial plantations is that of poor and inconsistent flowering in *E. nitens*, with resultant poor outcrossing and seed production. However, this key assumption has not been proven in the South African breeding programmes of the species, and investigating the validity of this assumed constraint was an important aspect of the research.

The large differences found in the *E. nitens* provenances tested in South Africa (Swain et al. 1988, Gardner 2001) have been useful in matching Australian seed to South African sites until improved seed became available from local breeding programmes. The wide range of

provenances tested have also allowed for the development of a broad genetic base breeding population in South Africa. It was uncertain, however, whether the mixing of provenances in F1 seed orchards would result in a type of “hybrid-vigour” in the F2 progeny, or a loss of certain positive traits such as cold, frost or drought tolerance, which are more apparent in one provenance than another (Gardner 2001, Swain 2001). Information was thus needed to assist with the decision on whether to keep F1 orchards separate by provenance or to continue to allow provenance mixing.

Modelling of predicted genetic gain using various breeding strategy scenarios can be a useful tool in assisting with the decision on which strategy or management plan will deliver the most genetic gains per unit time (Verryn et al. 2000). Such modelling, using the parameters established in the first part of the study, plays an important role in developing the advanced generation breeding strategy for *E. nitens*.

Objective of the study

The primary objective of the study was to develop and implement an advanced generation breeding programme to manage the many and disjunct breeding and production populations of *E. nitens.*

To do this, the following secondary objectives were identified:

- To develop a good understanding of the population genetics and underlying assumptions of the species, this being key to developing a suitable breeding strategy.
- To study the role that poor and inconsistent flowering in seed orchards is playing in realised genetic gain in *E. nitens*.
- To determine the role that Australian provenance is playing in the breeding population, so that this can inform the decision on whether to keep F1 orchards separate by provenance or to allow provenance mixing.
- To investigate ways to optimise genetic gain without increasing breeding cycle length.

It was determined that this study should be done using conventional breeding methodology, and that the results of the study might lead to future studies in molecular genetics, to provide adjunct information.

Structure of the thesis

Chapter 1 of the thesis is a Literature Review that covers the relevant literature on the topics necessary to develop such an advanced generation breeding strategy. Chapter 2, the first of

the empirical chapters, uses the results from F1 trials in South Africa to characterise the *E. nitens* population from a genetic perspective and to identify provenance differences for growth. Estimates of variances and narrow sense heritabilities are calculated to determine the breeding potential of the population under consideration, to inform the breeding strategy and for use in selection of superior families and individuals. Estimates of genetic correlations for both juvenile-mature and trait-trait measurements are useful in determining the age at which early selections for growth can be reliably made, and which growth trait measurements are correlated, respectively. If such genetic correlations are high, the former allows for shortening of the breeding cycle, whilst the latter enables a decrease in research costs. The existence of genotype by environment interaction in the F1 informs whether separate populations of *E. nitens* should be developed for production of improved seed across the varying South African forestry landscape. Gains are predicted from the F1 orchards for F2 progeny.

Chapter 3 presents results from a series of genetic gain trials comprising seedlots from the F1 orchards, and quantifies the gain made in the first generation of improvement. As poor flowering may hamper gain in the species, the genetic gain trials include comparisons of flowering levels and various seed orchard factors that may impact on gain. Chapter 4 uses the results from F2 trials to estimate genetic parameters for that generation and predict breeding values for both families and individuals, which can then be used for the selection of elite material in the F2. Realised gains achieved in the F2 genetic gain trials are used to calculate realised heritabilities, which are useful in determining whether some of the assumptions used in the F1 were correct and how effective selection has been. The role of genotype by environment interactions are investigated in the F2, to determine whether this holds true to that found in the F1 trials. The significance of the role of Australian provenance, maternal effect and South African seed orchard is investigated, and the former may hint at the presence or absence of heterosis or hybrid vigour in the population. Chapter 5 uses the gains achieved in the genetic gain trials to investigate whether the assumptions used in the estimation of F1 genetic parameters were correct, or whether different assumptions should be applied in future estimations. This is done by comparing realised genetic gains with gains predicted from various scenarios using different coefficients of relationship, provenance and flowering scenarios.

Chapter 6 uses relevant information gathered in the earlier chapters to develop a strategy for advanced generation breeding for *E. nitens* at the ICFR. This includes modelling of various breeding and production population scenarios, using appropriate modeling software, to predict genetic gain per unit time. Recommendations on management and adaptation of current production populations to increase gains are provided in this chapter, as the establishment and management of improved material in seed orchards to ensure a sustainable supply of improved seed to the South African forestry industry, is a key objective of the ICFR *E. nitens* breeding programme.

The study is based on growth data obtained from field trials and statistical analysis thereof. A molecular genetics study to complement the findings of the field research and statistical analyses would have been very useful. However, it was beyond the scope of this thesis and its specific goals. The fieldwork and the population genetics uncovered by this thesis will provide the basis for a future study using tools of molecular genetics to generate the genotypic data to synchronise with the phenotypic data collected here.

The referencing system used in the chapters of this thesis is based on the Harvard system of referencing (De Montfort University), and follows the specific style used in “Southern Forests: a Journal of Forest Science”. The exception to this is Chapter 3, which has already been published in “Tree Genetics and Genomes”. In this case, Chapter 3 has followed the referencing and formatting style used by “Tree Genetics and Genomes”. The term “treatment/s” has been used throughout all chapters, except in Chapter 3, where the Journal required the use of the term entry/entries”.

The thesis is in the form of discrete research chapters, each following the format of a stand-alone research paper (whether or not the chapter has already been published). This is the dominant thesis format adopted by the University of KwaZulu-Natal because it facilitates the publishing of research out of theses far more than the older monograph form of thesis. As such, there is some unavoidable repetition of introductory information and references between chapters. In addition, formal numbering of headings and sub-headings has not been used, as this is not standard in research papers.

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CHAPTER 1

Literature Review

Introduction

Commercial forestry is a significant contributor to South Africa's GDP, contributing ZAR 7.0 billion per annum, whilst utilising only 1.1% of the total area of the country. Three main genera comprise the commercial forests in South Africa, namely *Eucalyptus, Pinus* and *Acacia*. Of the 1 273 357 ha planted to commercial forests, *Pinus* species make up 51.1% (650 888 ha) of the plantations, with *Eucalyptus* making up 40.6% (516 638 ha) and *Acacia* 7.9% (100 606 ha), respectively (DAFF 2011). These forestry tree species are grown mainly for the production of pulpwood (56%), sawlogs (36%), and mining timber (4%), with the bark from *Acacia* species being utilised for the tanning and adhesives industry (DAFF 2011).

The genus *Eucalyptus*

Since the 1950s, plantings of eucalypts globally has resulted in this genus becoming the most common hardwood in plantations worldwide (Turnball 1999). Eucalypt plantations established for wood production now total 14 million ha, or 8% of the world's productive planted forests (FAO 2007).

Origin of eucalypts

Eucalyptus is a diverse genus of trees, the natural distribution of which is largely confined to the Australasian region (Pryor 1971). There are more than 700 species of *Eucalyptus* native to Australia (Fiona *et al.* 2005), with only two species exotic to Australia, from Papua New Guinea and Timor (Pryor 1981, Turnbull 1981). The members of this genus dominate the tree flora of Australia, and are adapted to the range of Australia's climatic conditions (Schönau and Gardner 1991).

The distribution of the genus covers a wide latitudinal range, from 7 ^{0}N to 43 ^{0}S, and this may partly explain the wide adaptation of the genus to a great diversity of sites, types of management systems and range of uses, both in natural forests and in plantations worldwide

(Eldridge et al. 1993). The various species of *Eucalyptus* are now amongst the most widely planted silvicultural plants in the world, the prime reason being that, under many conditions, suitably selected species grow rapidly and produce wood of value for either industrial use or to meet simpler needs such as building poles and fuel (Pryor and Johnson 1971). In addition, many eucalypts have the ability to withstand and recover from harsh environmental conditions. Both the intrinsic capacity for fast growth and the adaptability to a range of environmental conditions stems from the rainforest origins of the genus, and evolution over millions of years under a range of alternating extreme conditions. These origins allow eucalypts grown today to take advantage of periods favourable for rapid growth in between unfavourable periods (Eldridge et al. 1993), which is a requirement for successful timber species in South Africa.

Eucalypts in South Africa

Eucalypts were first introduced into South Africa in 1823 when nine seedlings of *Eucalyptus globulus* Labill. were brought to the Cape Colony from Mauritius by the new Governor of the Cape, Sir Lowry Cole (Poynton 1979). Since then, there has been a continuous introduction of new eucalypt species to the sub-continent (Poynton 1979). The most important species for the South African forestry industry has historically been *Eucalyptus grandis* W. Hill ex Maiden (Schönau 1991), with 78% of the total area being planted to this species by 1981 for pulp and paper production (Directorate of Forestry 1981). The species is relatively fast growing with good rooting ability, however it is not cold tolerant and can be killed by frost. The first forestry species to be planted in Mpumalanga for wood production were the "Cold Tolerant Eucalypts" (CTEs) and, from the beginning of the 20th century, these were planted widely by gold-mining companies and private farmers for production of mining timber. *Eucalyptus elata* Dehnh., *Eucalyptus fastigata* Deane & Maiden, *Eucalyptus macarthurii* Deane & Maiden and *Eucalyptus nitens* (Deane & Maiden) Maiden were the preferred species for mining timber, due to their physical strength and their ability to grow on high-altitude, temperate sites that were prone to cold and frosts (Purnell 1988).

In the last three decades, the increasing demand for hardwoods, particularly for the pulp and paper industry, has led to the expansion of plantings of hardwoods onto colder sites where species were traditionally grown for mining timber, and where *E. grandis* does not survive (Little and Gardner 2003; Pallet and Sale 2004). These areas include the colder, frost-prone highland areas of western KwaZulu-Natal, the north-eastern Cape and south-eastern Mpumalanga Highveld. Site-species interaction trials conducted by the Institute for

Commercial Forestry Research (ICFR) confirmed the suitability of a range of CTEs for pulp and paper production in these areas, in particular the species *Eucalyptus badjensis* de Beuz et Welch*, Eucalyptus benthamii* Maiden & Cambage*, Eucalyptus dunnii* Maiden, *E. macarthurii, E. nitens* and *Eucalyptus smithii* R.T. Baker (Schönau and Gardner 1991, Darrow 1994, 1996, Gardner 2001, Swain and Gardner 2003).

Interspecific hybrids of *E. grandis* have been developed during the past two decades for growth in the subtropical areas of South Africa. These hybrids were developed with selection for the following traits: Faster growth than *E. grandis*; resistance/tolerance to several pests and pathogens attacking *E. grandis*; and increased density of the species (Morris 2007).

Eucalyptus plantations currently cover 516 638 ha of South African land *(*DAFF 2011), and *E. nitens* continues to be an important species grown in the temperate summer rainfall regions of South Africa

Eucalyptus nitens

Eucalyptus nitens in Australia

Eucalyptus nitens has a scattered natural distribution that extends from 30.5 ^{0}S in the Dorrigo area of New South Wales (NSW) in Australia to 38 ^{0}S in the Central Highlands of Victoria. The species occurs at elevations of between 600 and 1500 m, in disjunct populations in the Victorian Alps, eastern Victoria and southern NSW. Two small populations are also found at Barrington Tops and Ebor/Barren Mountain in northern NSW, at altitudes of up to 1600 m. The mean maximum temperature of the hottest month is 26 ^{0}C and the mean minimum temperature of the coolest month is -5 ^{0}C. Frosts are frequent and severe (50-150 frost events per annum), and snow is common. Rainfall is moderate to high (750-1750 mm per annum), varying in distribution between slight summer and winter maxima. The soils may be derived from a wide range of parent materials, but growth is best on those giving rise to friable clay subsoils. Landscapes vary from undulating tablelands to mountain slopes, where the species prefers the less exposed positions (Boland et al. 1992).

Pederick (1979) described two forms of *E. nitens*; “juvenile-persistent” and “early-adult”, the former because of its retention of juvenile foliage after the first year of growth, and the latter because it did not retain its juvenile foliage for long. The “early-adult” form came from Errinundra provenance and parts of Toorongo provenance in the Victorian central highlands,

and was found to have slower growth (Pederick 1979), poorer cold hardiness (Tibbits and Reid 1987, Raymond et al. 1992), different floral morphology (Tibbits 1989) and lower pulp yields (Williams et al. 1995) than the "juvenile-persistent" form. The "early-adult" form of *E. nitens* has subsequently been ascribed specific status and renamed *E. denticulata* I.O.Cook and P.Y.Ladiges by Cook and Ladiges in 1991 (Tibbits et al. 1997).

Cook and Ladiges defined three genetically distinct races of *E. nitens* in 1991: Central and Northern NSW; Southern NSW and Mt Kay; and Central Victoria. The populations in Victoria were further separated into three additional races by Dutkowski et al. in 2001 (Hamilton et al. 2008). These racial classifications are being utilised more commonly in genetic analyses than those described by Pederick (1979), because they represent populations that are distinct both geographically and genetically (Hamilton et al. 2008).

Eucalyptus nitens in South Africa

The species was first introduced into South Africa in 1929, but was not widely exploited because of the limited amount of seed available (Darrow 1984). *Eucalyptus nitens* was originally grown for mining timber, but more recently for pulp and paper production as demands by the consumers of forest products have changed. The species is classified as the most cold and snow tolerant of the eucalypts grown in South Africa (Herbert 2000) and currently, there is no suitable alternative commercial species to *E. nitens* in South Africa for sites prone to moderate frost and heavy snows. In addition, the species is an important hybrid partner of *E. grandis*. Commercial plantations of *E. nitens* currently cover approximately 45 000 ha or almost 9% of eucalypt plantation area in South Africa (Germishuizen pers comm[1]).

The species is most suited to cooler sites in the summer rainfall regions of South Africa, with a mean annual temperature (MAT) of 14 ^{0}C to 16 ^{0}C and minimum mean annual precipitation (MAP) above 825 to 950 mm for optimum growth (Herbert 2000, Swain and Gardner 2003). At the warmer end of the MAT range, MAP should be at least 950 mm, while 825 mm is sufficient at the cooler end of its range (Swain and Gardner 2003).The species is clearly unsuccessful in warmer areas and should only be planted on sites with a MAT less than 16 ^{0}C (Swain and Gardner 2003). *Eucalyptus nitens* is classified as frost tolerant, but is not

[1] Germishuizen I. 2012. Institute for Commercial Forestry Research, Pietermaritzburg, PO Box 100821, Scottsville, 3209, SOUTH AFRICA

as hardy as *E. macarthurii* (Darrow 1994, 1996) and should not be planted in low landscape positions, where extreme frosts are likely to occur. The species has clearly demonstrated good resistance to damage by all but the heaviest of snowfalls (Gardner and Swain 1996, Kunz and Gardner 2001). The summer rainfall provenances of *E. nitens* appear to tolerate strong winds and exposure to chill very well, making the species suitable for planting in exposed positions in the landscape, as long as still within the recommended MAT range.

When grown in the summer rainfall region of South Africa, the Victorian provenances of the species are susceptible to *Mycosphaerella* leaf blotch disease in their juvenile state (Purnell and Lundquist 1986). Generally the species has varying levels of susceptibility to *Endothia, Botryosphaeria* and *Phytophthora.* More recently, *Coryphodema tristis* (Drury) (cossid moth) has caused extensive damage to mature and over-age stands of *E. nitens* (van den Berg and Stanger 2007). The species is very sensitive to fire. *Eucalyptus nitens* does not coppice well, its ability to coppice decreasing with age, and being generally poor after eight years of age (Little and Gardner 2003, Swain and Gardner 2003). Micro- and macro-propagation of the species is difficult due to low-rooting success (Moncur 1988, de Little et al. 1992), although vegetative propagation of the species through grafting has had some success (Komakech 2002, Adejumo et al. 2012). The bark of *E. nitens* strips relatively easily.

There are significant differences with regard to provenance performances of *E. nitens* under different climatic conditions and for different traits, which will be detailed further in Section 1.3. As a species, *E. nitens* is recognised as having good kraft pulp yields (Clarke 2000), with dissolving pulp yields also being favourable, and ranging from 45.5 to 51.5% over a range of sites (Clarke 1995, Clarke et al. 1999).

Tree improvement

Tree improvement/breeding strategies

A breeding strategy is the overall concept of how to go about breeding. The essential objective is the improvement of the population by a combination of a particular type of selection with a particular type of mating, from the starting point of a well-adapted and broad genetic base (Eldridge et al. 1993). A breeding strategy is an ongoing and recurrent process, with selection of forest trees continuing past the first generation, and includes re-selection in generation after generation with interbreeding of selections to provide for recombination (McKinley and van Buijtenen1989). Recurrent selection is a way of making stepwise changes

in gene frequency within a population while maintaining sufficient genetic variability for continued selection (Otegbeye 1998). In most tree improvement programmes, first generation selection is generally through mass selection, whereas for second or advanced generation selection, both between and within-family (combined) selection occurs. The primary difference between first and advanced generation procedures/techniques is the amount of information available on which to base selection decisions (McKinley and van Buijtenen1989).

An effective breeding strategy involves the maintenance of three major types of populations, i.e., base, breeding and production populations. The breeding population comprises progeny trials and clonal archives in which the breeding cycle of selection and mating is repeated over many generations, and it is this population that is the breeder's main focus. It is the function of well-planned, long-term eucalypt breeding strategies to manage broadly-based and constantly improving breeding populations in which inbreeding is minimised (Eldridge et al. 1993). The production population for a given generation is composed of selected individuals from the breeding population, their function being to produce genetically-improved offspring for operational forestation. The increased yield realised from harvesting plantations established with these genetically superior trees is the primary benefit of most tree improvement programmes (White 1987). **Figure 1.1** shows an adaptation of the major components and activities of the breeding cycle as represented by White in 1987.

Effective selection in breeding populations is the basis for making genetic gains in a breeding programme. Cotterill and Dean (1990) stated that "maximising gains from advanced generation breeding is largely a matter of efficient selection". Both forward and backward selections are made in *E. nitens* breeding programmes globally (Hamilton et al. 2008). Forward selection involves the selection of the best individuals within the best families, based on information gathered from the individual and its family (siblings). Backward selection has been described as the selection of superior parents based on the performance of their progeny (Shelbourne et al. 1989). The optimum age at which selections can be made, as well as the best selection methods and traits to use, need to be considered in a breeding programme, and these topics have been well investigated in eucalypts (Greaves et al. 1997, Louw 2006, Harrand et al. 2009, Kien et al. 2009). In species where vegetative propagation is successful, such as *E. grandis*, superior selections can be cloned into clonal archives or clonal seed orchards for production of open-pollinated or control-pollinated elite seed.

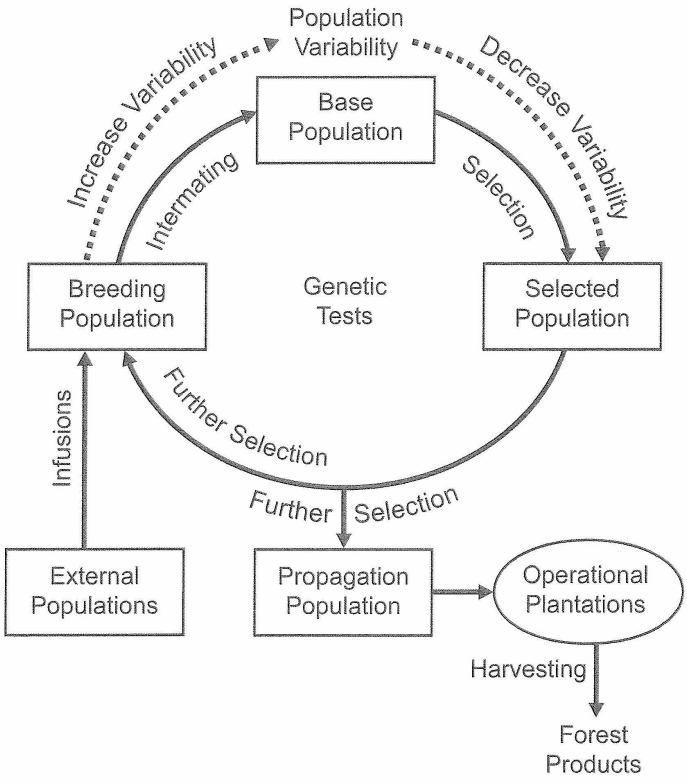

Figure 1.1 The breeding cycle of forest tree improvement programmes. Each of the core population types shown in the inner circle (base, selected and breeding populations) are formed once per cycle of improvement in the sequence shown, while the other population types may or may not be formed (White et al. 2007, reprinted from White 1987)

Mating allows for recombination of selections. Both open pollination (OP) and controlled pollination (CP) are options in viable breeding strategies. Controlled pollination has the advantage of controlled mating across trials, breeding lines or generations, because complete pedigree information is available. This reduces or avoids inbreeding, is good for identifying good specific combiners, provides good estimates of full-sib performance, and most selection is done within family (Otegbeye 1998, Hamilton et al. 2008). Pre-determined mating designs, through CP, are often considered essential for the creation of structured, pedigreed families for testing, thus facilitating accurate assessment of genetic parameters and the selection of superior genotypes for advanced breeding and establishment of seed orchards (Lambeth et al. 2001). However, in order to meet these objectives, a large number of crosses are necessary in rather complex schemes, which can create logistical difficulties,

increase costs and can take many years to complete. In many cases, the authenticity of the parents of the resultant offspring may contain errors (El-Kassaby and Lstibůrek 2009).

With OP, only the maternal pedigree is available and there is no control of inbreeding, which may result in decreased genetic variation due to increased relatedness. However, OP is suitable for both family and within-family selection, and can be used to evaluate General Combining Ability of parents/families (Otegbeye 1998).

***Tree improvement in* Eucalyptus nitens**

Eucalyptus nitens is a significant plantation species in temperate regions of the world, with active breeding programmes for the species in Australia, Chile, New Zealand (Hamilton et al. 2008) and South Africa (Swain et al. 2004, Swain 2008). It is possible to achieve establishment, selection and mating cycles of approximately 10 to12 years in *E. nitens* (de Little et al. 1992, Griffin 2001). However, biological constraints associated with flowering and seed production have resulted in delays in generation turnover in breeding strategies where breeding populations are maintained in discrete generations (Hamilton et al. 2008). This has lengthened breeding cycles beyond optimal time-frames in most breeding programmes.

Controlled pollination is difficult in *E. nitens*, because flower buds are small (Boland et al. 1992), making emasculation and CP tedious, time consuming and expensive (Venter and Sivlal 2007, Hamilton et al. 2008). Techniques developed for the large-scale production of elite CP seed from larger-flowered species such as *E. globulus* have not been successful with *E. nitens* (Williams et al. 1999, Venter and Sivlal 2007). In addition, the species is a reticent and inconsistent flowerer, particularly in South Africa (Gardner 2003, Pound et al. 2003), and a CP programme can take many years, causing delays in the breeding programme and variable mating of genotypes. Thus OP breeding programmes are common for the species, with parameter estimates available for several traits, such as growth, wood property and tree architecture traits (Hamilton and Potts 2008). Hodge et al. (1996) found few differences in genetic parameter estimates between *E. nitens* OP seed orchard stands and CP populations. These authors also found little inbreeding depression (1%) and less deleterious abnormalities at the seedling stage of *E. nitens* than was found in *E. globulus* by Potts and Jordan (1994).

Other difficulties associated with breeding *E. nitens* are that the species does not coppice reliably (Little and Gardner 2003) for vegetative propagation, and micro- and macro-propagation cutting techniques are limited due to low rooting success (de Little et al. 1992, Griffin 2001). However, grafting of selections can be done (Komakech 2002, Hamilton et al. 2008), allowing for the capture of superior genotypes in grafted clonal seed orchards.

Breeding of *Eucalyptus nitens* in South Africa

Tree breeding has a long history in South Africa (van Wyk and Roeder 1978). The former South African Forest Research Institute (SAFRI) pioneered eucalypt breeding, particularly with *E. grandis*, and also implemented provenance/progeny trials with several CTE species, including *E. nitens*, during the 1980s (SAFRI 1984). With the closure of SAFRI during the mid-1980s and the division of assets between various stakeholders, the CTE breeding trials and germplasm were taken over by the Institute for Commercial Forestry Research (ICFR). This included six F1 *E. nitens* trials on the Highveld of Mpumalanga. At the same time, tree breeding programmes for *E. nitens* were developed by some of the major forestry companies in South Africa (Darrow 1984). However, very little is known about these, because these are in-house research projects and research results are sporadic (van den Berg and Stanger 2007). Up until 1994, the ICFR breeding programme was the only *E. nitens* programme funded by both Government and commercial forestry companies, with information publically available. Since the former Department of Water Affairs and Forestry (DWAF) withdrew funding from the ICFR in 1994, the breeding programme has been funded by commercial forestry companies and growers in South Africa, with information and germplasm available to funders of the breeding programme.

Results from early provenance trials showed that the material from Victoria in Australia, which has a uniform to winter rainfall pattern, does not perform well in the summer rainfall regions of South Africa, due partly to the susceptibility of these provenances to *Mycosphaerella* leaf botch disease (Darrow 1984, Purnell and Lindquist, 1986). The fungus attacks juvenile leaves, particularly in areas with summer rainfall, and the “juvenile-persistent” forms of *E. nitens*, which predominate in central Victoria, are more susceptible than the “early-adult” (now *E. denticulata*) or more intermediate forms from NSW (Johnson 1996). Thus the majority of material included in South African breeding programmes is from NSW, i.e., from Barren Mountain, Ebor, Barrington Tops, Tallaganda, Badja and Glenbog

provenances. As a result, the improved *E. nitens* populations grown commercially in South Africa originate from these provenances in NSW.

Between these provenances, significant variation exists in South Africa for growth (Swain et al. 1998, Gardner et al. 2003), frost, cold (Gardner 2001, Swain 2001) and drought tolerance (Darrow 1996, Gardner 2001). The Ebor and Barren Mountain provenances appear to be the most cold tolerant of the *E. nitens* provenances tested in South Africa (Gardner 2001), while the Tallaganda provenance showed more drought tolerance than the Ebor provenance in high altitude site-species trials in KwaZulu-Natal (Darrow 1996, Gardner 2001). In addition, provenance differences exist for timing and abundance of flowering (Carlson et al. 2000, Jones 2002, Gardner and Bertling 2005) and seed production (Swain and Chiappero 1998, Jones 2002). The provenances also differ with regards to pulping properties. When four NSW provenances were tested for pulping properties, the Ebor provenance had the best pulp yield compared to the Brown Mountain, Barrington Tops and Tallaganda provenances, the latter having the lowest pulp yield (Clarke, 2000). These differences make this species ideally suited to genetic improvement.

These marked provenance differences formed the basis for the ICFR *E. nitens* breeding programme. In addition to the six F1 trials that the ICFR took over from SAFRI, further F1 trials were established, using seed collected from Barrington Tops in Australia. Over time, production orchards have been established either clonally through grafting, or with seedlings, and existing F1 trials have been converted to seed orchards. Seed was collected from F1 seed orchards and F2 trials established in two series in 1999 (Swain et al. 2004) and 2008 (Swain 2008). The breeding programme is now at the point of turning over the next generation, making selections and establishing new seed orchards. The information from two generations of trials are the subject of this thesis, and will be valuable in making informed choices for the development of an advanced generation breeding strategy.

Genetic characterisation of a population

A good understanding of a species, in terms of both its population genetics and biological constraints, is necessary to develop and implement a successful breeding programme. Both these areas affect the gain predicted from a breeding strategy, as well as those ultimately realised. The following section considers aspects of population genetics and how biological

constraints can impact on these and genetic gain. Thus the success of a breeding strategy can be compromised if these factors are not taken into account and estimated correctly.

Provenance effects

Comprehensive provenance representation has two main purposes in a breeding strategy. The first is to test material from different genetically-based geographical origins on target sites, such that specific characteristics have the potential to be expressed (Eldridge et al. 1993). The second is to ensure that maximum genetic diversity is captured in a base breeding population. In addition, the testing of material from different provenances in South African tree improvement trials has provided information on which seed should be purchased from Australia for establishment of commercial plantations, until improved and locally adapted seed became available from South African breeding programmes.

Heterosis (hybrid vigour) may result from mixing of provenances by outcrossing of families in seed orchards. However, although low levels of dominance effects have been found in selfed seedlots of *E. nitens* (Hardner and Tibbits 1998), Hodge et al. (1996) found negligible levels of non-additive effects in their *E. nitens* study and many authors assume negligible or zero non-additive effects in *E. nitens* (Hamilton et al. 2008).

Genotype by environment interaction

Genotype by environment interactions (GEI) occur when the relative performance of genotypes differs when grown in different environments (Zobel and Talbert 1984). This presents a dilemma for breeders, because GEI tends to hamper progress by necessitating larger replication in space, whilst conversely, a strong interaction offers the opportunity to increase gains by developing specific genotypes that will grow well in specific environments (Squillace 1969). However, it is generally difficult to define the environmental variables causing the interaction, and for specific genotypes to be matched to environments, those environments must be well defined and repeatable (Matheson and Cotterill 1990). This requires a large number of sites representing a range of defined environments, as well as a large number of common families. Thus it is difficult and expensive to manage GEI, with the result that most breeders and timber companies have ignored its potential to increase production (Barnes et al. 1984). Should strong GEI be identified, there are a few ways of managing the interaction. These are: Firstly, to eliminate unstable genotypes (at both the top and bottom ends of the ranking); secondly, stratification of environments, as discussed

above (Raymond and Namkoong 1990); and thirdly, to avoid environments that are associated with strong GEI (Verryn et al. 2000a). Generally, genetic and site effects are much stronger than the genotype by site interactions (Wright 1973) and thus, even if a significant GEI is present, significant progress can be made by identifying and selecting for good general performers (Kanzler 2002).

Genetic parameters

Information on population genetics and underlying assumptions of a species are important for designing breeding strategies (Tibbits and Hodge 1998). Estimates of variances and narrow sense heritabilities are calculated because this information is fundamental to determining breeding potential of the population under consideration, informs breeding strategies by prediction of breeding values (BV) and gains from various selection scenarios, and is used for purposes of selection of superior families and individuals (White 1987, Falconer and Mackay 1996, White 1996).

Although parameter estimates are available for several traits in *E. nitens* globally (Hamilton and Potts 2008), breeding of the species in South Africa is in its infancy, relative to agricultural crops and the comparative amount of information available to breeders. There is little published information on genetic parameters of *E. nitens* in South Africa, and it is important to establish whether the underlying assumptions on which current genetic parameter estimations are based, are correct.

Underlying assumptions

The degree to which the assumptions underlying the estimation of genetic parameters are not met will affect the utility of both genetic parameter estimates and BV predictions developed from OP progeny tests (Hodge et al. 1996). These assumptions are:

a) Families are true half-sib families;
   This assumption is likely to be unrealistic for insect-pollinated species (Hodge et al. 1996) and some inbreeding is likely to occur, resulting in the presence of some full-sibs (Squillace 1974). Various authors have investigated this in forestry species, and coefficients of relationship used in estimations in *E. nitens* OP populations range from 0.25 to 0.5 (Whiteman et al. 1992, Johnson 1996, Gea et al. 1997, Greaves et al. 1997, Tibbits and Hodge 1998, Kube et al. 2001, Sierra et al. 2001, Volker 2002, Hamilton and Potts 2008). In South Africa, van den Berg and Stanger (2007) used a coefficient of

relationship of 0.33 for their 2nd generation parameter estimations. In addition, complicating the use of OP progeny for genetic parameter estimates is the possibility that the rate of selfing/related matings and inbreeding depression due to this, can vary from family to family (Griffin and Cotterill 1988).

b) Random flowering and mating (panmixis);
Panmixis, or random mating, has been defined in Falconer and Mackay (1996) as the opportunity for all individuals to mate equally with any other individual in the population, and assumes equal contributions to the pollen pool from each tree (Moncur and Boland 2000, Hamilton et al. 2008). Asynchronous flowering has been found to exist between provenances of *E. nitens* (Tibbits 1989, Volker et al. 1990, Moncur and Boland 2000, Jones 2002), indicating that this assumption may not be correct. However, these flowering windows do overlap to some degree in South Africa (Jones 2002, Gardner 2003).

In addition to asynchronicity, flowering in *E. nitens* is subject to seasonal influences (Tibbits 1989, Moncur and Hasan 1994, Jones 2002, Gardner and Bertling 2005) and, as the stability, or greater influence, of female effects in reproductive success in some eucalypts has been noted (Tibbits 1989, Leal and Cotterill 1997, Suitor et al. 2009), it is possible that only a few effective males may contribute to pollination of particular trees in a seed orchard (Hodge et al. 1996, Suitor et al. 2009). Conversely, pollen from a few heavy-flowering individuals may have an exceptionally strong genetic influence on the progeny of adjacent female trees (Moncur and Boland 2000). Grosser et al. (2010) found that the parental contribution to progeny varied amongst clones in an *E. nitens* clonal seed orchard. Fecundity variation is common in seed orchards and can be caused by unequal parental representation during establishment of the orchard; mortality during the development of the orchard; and differences in parental reproductive output (Funda et al. 2009). Moncur and Boland (2000) suggested that if flowering intensity in the seed orchard canopy is too high, then the genetic influence of pollen sources on neighbouring females will negate the benefits of sophisticated orchard designs.

c) No genetic correlation between flowering and growth;
Varghese et al. (2009) found contrasting trends in their studies on *E. camaldulensis* and *E. tereticornis* in India. These authors found a negative genetic correlation between

flowering and outstanding growth performance in unimproved provenances in *E. camaldulensis*, but no such correlation was found in an improved seed orchard of *E. teretecornis*. Although there are currently no published results on such studies on *E. nitens* in South Africa, Gardner (pers comm[2]) did not find any correlations between flowering and growth in flowering studies in *E. nitens*, nor has Jones (pers comm[3]) found any strong correlations in *E. nitens* seed orchards.

The direct impact of this assumption not being met is that families with good flowering may inadvertently be selected against, because thinning and roguing of trials to seed orchards is usually done prior to flowering, and is based purely on growth traits. By contrast, flowering may be selected for in high-performance families unintentionally, because, in a shy-flowering species such as *E. nitens*, there is the risk that only those families that produce seed in time for the next generation of progeny trials will be included (Moncur and Boland 2000).

d) Absence of non-additive effects such as inbreeding or heterosis;
Additive variance effects have been found in growth studies on *E. nitens*, with negligible non-additive effects (Hodge et al. 1996, Hamilton et al. 2008). However, additive effects for wood property traits in *E. nitens* are lower than growth traits such as stem diameter at breast height (Hamilton and Potts 2008).

Tibbits and Hodge (1998) concluded that assumptions may change with each generation, particularly from the base population to the next generation, and that refinement in assumptions may be needed in subsequent generations of a breeding programme. Gea et al. (1997) found that the use of different coefficients of relationship for different generations of *E. nitens* proved an efficient tool for making heritability estimates comparable between the F1 and F2.

The value of genetic parameters, and subsequent BV predictions and selections, are dependent on whether these assumptions are met and if not, to what degree. Thus an understanding of the validity of these assumptions should be built up as part of the development of a tree improvement strategy.

[2] Gardner RAW. 2012. Institute for Commercial Forestry Research, Pietermaritzburg, PO Box 100281, Scottsville, 3209, SOUTH AFRICA
[3] Jones W. 2013. Shaw Research Centre, Tweedie, PO Box 473, Howick, 3290, SOUTH AFRICA

Heritability estimates

The narrow sense heritability estimates (h^2) for diameter at breast height (dbh) in published *E. nitens* OP trials range from 0.11 (pooled estimates, Gea et al. 1997, Volker 2002) to 0.78 (Hardner and Tibbits 1998) (**Table 1.1**). In 2008, Hamilton and Potts reviewed 24 publications on genetic parameters for growth, wood property, tree architecture and fitness traits in *E. nitens.* They estimated a mean h^2 of 0.26 for dbh of OP trials. These h^2 estimates are in the range of other eucalypt species (**Table 1.1**). There is thus a substantial body of evidence that there is sufficient genetic variability for improvement in the growth traits dbh, height and volume in *E. nitens*, as well as for a range of wood property and fitness traits. The few published heritability estimates in South African *E. nitens* breeding programmes tend to support this. Purnell (1986) reported h^2 of 0.11 and 0.19 for dbh and volume at 39 months, respectively, and van den Berg and Stanger (2007) estimated h^2 of 0.17 and 0.16 for volume at seven years at two sites, respectively.

Table 1.1 Range of narrow sense heritability estimates for diameter at breast height (dbh) in *Eucalyptus nitens* and other eucalypt species

Species	Heritability estimates (h^2)	Reference
E. nitens	0.11 (pooled estimates)	Gea et al. 1997, Volker 2002
	0.14	Johnson 1996
	0.18	Whiteman et al. 1992
	0.24 (converted from family h^2 of 0.80)	King and Wilcox 1988
	0.42	Greaves et al. 1997
	0.32 to 0.45	Kube et al. 2001
	0.37 and 0.57	Volker 2002
	0.78	Hardner and Tibbits 1998
E. camaldulensis	0.11	Mahmood et al. 2003
E. cladocalyx	0.41 to 0.47	Callister et al. 2008
E. grandis	0.11	Gapare et al. 2003
	0.16 to 0.34	Harrand et al. 2009
	0.60	Louw 2006
E. macarthurii	0.03 to 0.14	Ndlovu 2008
E. urophylla	0.10 to 0.31	Kien et al. 2009

Genetic correlations – juvenile-mature and trait-trait

Genetic correlations for juvenile-mature measurements (r_g) are a useful measure of which of the earlier measurements is the best predictor of top individuals and families at full rotation. This then enables early selection of elite individuals, thus decreasing the length of the breeding cycle. This is particularly relevant to *E. nitens*, where generation turnover and improvement in the species is constrained, worldwide, by delayed and poor flowering, with

small seed crops (Gardner 2003, Pound et al. 2003, Hamilton et al. 2008). Although there are many published reports of genetic correlations for inter-age measurements in *E. nitens*, there are only a few estimates between ages approximating selection age (5-6 years) and harvest age (8-12 years). These are r_g = 0.79, 0.98 and 1.00 (Kube et al. 2001), and Greaves et al. (1997) found r_g = 0.99 between 4 and 7 years. No published genetic correlations could be found for juvenile-mature measurements in South African *E. nitens* studies, these correlations potentially being very useful for decreasing breeding cycle length in South African breeding programmes.

Genetic correlations for trait-trait measurements indicate whether an increase in one measured trait will positively or negatively affect another measured trait, if at all (Cotterill and Dean 1990, Falconer and Mackay 1996). This information is useful in terms of both cost and time efficiency, as measurement for one trait can act as a surrogate for other positively correlated traits in the breeding programme and selection process. Tibbits and Hodge (1998) found genetic correlations among growth traits in three OP *E. nitens* trials to be strong, ranging from 0.97 to 1.0 for basal area and volume. However, genetic correlations between height and diameter in *E. nitens* are not as strong, ranging from 0.52 (Greaves et al. 1997) to 0.92 (Whiteman et al. 1992). There are a range of published estimates for genetic correlations among wood properties and between growth traits and solidwood/pulpwood traits. Hamilton and Potts (2008) have summarised these estimates, the details of which are not directly relevant to this study.

The areas described in this Section, “Genetic characterisation of a population”, have an impact on both the predicted and realised genetic gains in a breeding programme.

Genetic gain

The effectiveness of tree improvement programmes is called genetic gain, and it is necessary to quantify the gains made through selection and breeding at various stages of a breeding programme (White et al. 2007). Evaluation of actual gains is achieved by testing the yield and product quality of improved versus unimproved or previous generation plantations. These trials need to use large plots in order to achieve the kind of competitive conditions found in maturing stands. Due to the length of tree life-cycles and plantation rotations, information from genetic gain trials often measure progress from a point in the programme that has already been surpassed. Nevertheless, they serve a useful function in validating

gains on a per-unit-area basis in large plots treated in a truly operational manner (White 1987). Even at a relatively early age, such trials are useful as demonstrations to managers and funders of tree improvement programmes, of the potential for genetic gains in the breeding programmes.

Predicting genetic gain

The prediction or estimation of potential gains can assist the breeder in determining which breeding scenario to utilise (White 1996) and which strategy is best suited to a breeder's purpose in terms of genetic gain, length of breeding cycle, population size, etc (Verryn et al. 2000a). Predictions of gain are also useful to funders of tree improvement programmes, because these indicate whether investments in the breeding programme are justified.

Estimates of genetic gains from OP *E. nitens* seed orchards are generally derived from the predicted BVs of orchard genotypes, assuming that the underlying assumptions described in the section above on "Genetic parameters" are correct. Genetic gains can be predicted manually using the formulae described by Namkoong et al. (1966) and Falconer and Mackay (1996), which have been adapted by Shelbourne (1991) and Gea (1997) into more complex equations that allow gain predictions for a range of OP, full-sib and cloned breeding and production populations. The tables developed by Becker (1975 and later editions) are generally used to provide information on selection intensities in these estimations. However, a large number of calculations involving many variables are required, and a number of computer programmes have been developed to assist the breeder (Mullin and Park 1995, Rezende and Olivieira 1997, Verryn et al. 2000b, McRae et al. 2004).

Factors affecting predicted gains

Actual or realised genetic gains can differ from those predicted using quantitative genetic theory if the underlying assumptions are not met. These assumptions are described in the section "Genetic parameters" above, but there is further detail that is relevant to *E. nitens:*

a) The assumption of a coefficient of relationship of 0.33 to allow for the presence of full sibs within the open-pollinated families, and that at least some inbreeding may have occurred (Squillace 1974);

   Moncur et al. (1995) estimated an outcrossing rate of 75% in this species, and Pound et al. (2003) found that the levels of self-incompatibility in *E. nitens* ranged from 25.8 to 93.6%. The species demonstrates preferential outcrossing and appears to have a late-

acting self-incompatibility system operating to reduce the production of selfed seed (Tibbits 1989, Pound et al. 2003). However, self-pollination is possible in *E. nitens*, as demonstrated in controlled self-pollination experiments (Tibbits 1989). Inbreeding depression has been reported in nine-year old trees originating from the controlled self-pollinations of *E. nitens*. This inbreeding depression was not for survival, but for mature tree growth characteristics (Hardner and Tibbits 1998).

b) Realised selection intensity;

The delayed and poor flowering that is endemic to *E. nitens* has potentially important consequences on the realised selections intensities, as stratified selection based on flowering may occur in *E. nitens* seed orchards. Selection of poorer-performing individuals may be forced should there be insufficient flowering in higher-performing families and individuals. In addition, should less families or individuals per family be contributing, this will result in a smaller population from which to select parents. Consequently, the selection intensity in the orchard will have been higher than estimated in the predictions, resulting in lower realised genetic gains.

c) Length of breeding cycle;

Biological constraints associated with flowering and seed production have resulted in delays in generation turnover in *E. nitens* breeding strategies where breeding populations are maintained in discrete generations (Hamilton et al. 2008). This has lengthened breeding cycles beyond optimal time frames in most *E. nitens* breeding programmes worldwide, and can result in disjunct generations of trials. It has become apparent that shortening the breeding cycle plays a key role in increasing gain in *E. nitens*. Several ways this can be addressed are by; utilising juvenile-mature genetic correlations so that individual tree and family selections can be made early (White et al. 2007), improving the grafting success of superior individuals so that all selections can be captured in CSOs within a short time period, and successful implementation of new flowering-enhancement technologies to encourage early flowering and seed production in seed orchards (Gardner et al. 2011, Adejumo et al. 2012, Gardner 2012, Gardner et al. 2013, Germishuizen and Gardner 2013).

Realised genetic gain and heritability estimates

It is particularly important to measure the extent to which breeding has been successful in the South African *E. nitens* breeding populations, given the problems associated with flowering in the species and the lack of clarity regarding whether the assumptions underlying the estimation of genetic parameters, have been met. Once realised gains have been calculated, and if the selection differential from the previous generation of selection is known, the realised heritability can be estimated (White et al. 2007). Realised heritability is a good test of the effectiveness of selection (Hettasch et al. 2007), and can also be used to provide an independent validation of the heritability for a trait estimated in a previous generation (White et al. 2007).

Realised genetic gains in the region of 0.67 standard deviation points for *E. nitens* diameter were reported in New Zealand (Gea et al. 1997), and a 5.3% increase in *E. nitens* volume was reported in Chile (Velilla et al. 2007). In South Africa, van den Berg and Stanger (2007) reported average individual tree volumes of 0.168 m^3 in a 2^{nd} generation *E. nitens* breeding programme, although percentage increases were not published.

Flowering and seed production

The end product of any breeding programme is the improved material to be used in propagating plants for afforestation or reforestation, and thus seed orchards represent the link between tree breeding and operational forestry (Lstibůrek and El-Kassaby 2010). Although successes in vegetative propagation of some eucalypt species have decreased the demand for improved seed, seed still plays a major role in plantation establishment of those species that are difficult to propagate vegetatively (Eldridge et al. 2003). Eldridge (1978) stated that a sound knowledge of the breeding systems of a species is of fundamental importance to efficient seed orchard management and controlled-pollination. An understanding of the breeding system in *E. nitens* has been developed over time, but is not yet complete. Key studies are: Tibbits (1988, 1989) who performed CP studies in *E. nitens*; Barbour et al. (2002) who studied gene flow between introduced and native eucalypt species; Pound et al. (2003) who investigated pollen tube growth and early ovule development following both controlled crosses and self pollination; and Gea et al. (2007), and Grosser et al. (2010), whose groups performed parental analysis in a breeding population using microsatellites, and a paternity analysis in an *E. nitens* clonal seed orchard, respectively, which provided information on outcrossing and parental influence. Even utilising the

information that is available on the breeding system of the species, and working within the constraints of the species, flowering and seed production still continue to be challenges to breeders and seed orchard managers (Hamilton et al. 2008).

Description of flowering and associated problems in Eucalyptus nitens

Worldwide, the reticent or shy flowering of *E. nitens* (Gardner 2003) has hindered breeding programmes and the production of improved seed for plantation establishment (Hamilton et al. 2008). Globally, the species is known as a light and infrequent flowerer and produces small seed crops (Pound et al. 2003). In South Africa, the species usually becomes reproductively mature at only 10 to 15 years of age if grown in a plantation situation (Eldridge et al. 1993; Gardner 2003). If flowering is to occur earlier, then winter chilling is required, or hormonal treatments to replace the chilling (Gardner and Bertling 2005). The use of OP seed orchards to turn over generations in conventional breeding is therefore slow and difficult, and can result in inconsistent commercial seed production. Reticent flowering may also affect realised or actual gain, in that only certain families may contribute as pollen parents, potentially reducing gains relative to predicted gains. On the contrary, if different or additional families start flowering with each advancing year, gains may vary significantly on an annual basis and may result in the OP seed orchards failing to produce consistently high quality seed.

Factors affecting flowering and seed production

Location of seed orchards

The siting of orchards affects flowering and resultant seed production (Moncur and Boland 2000). Accumulated chilling is a requirement for flowering in *E. nitens* (Gardner and Bertling 2005) and this can be achieved by siting seed orchards specifically for cumulative cold prior to appearance of flower buds (Gardner and Germishuizen 2012, Germishuizen and Gardner 2013, Gardner et al. 2013).

Flowering enhancers

The growth regulator, paclobutrazol, can be used to reduce vegetative growth and enhance flower-bud production in *E. nitens.* Paclobutrazol is a broad spectrum, xylem-mobile plant growth retardant that inhibits the biosynthesis of gibberellins (GAs), and thus reduces the rate of cell division and expansion (Griffin et al. 1993). The implications for breeding programmes are that breeding cycle length will be decreased significantly due to flowering

occurring at an earlier age in trees, in addition to increased seed production in orchards (Moncur and Boland, 2000). A certain level of chilling is required for paclobutrazol to be effective, as reported by Moncur and Hasan (1994), who found that grafts maintained in a warm greenhouse over winter did not produce flower buds, despite paclobutrazol-induced reduction in GA concentration of the apical tissue. Gardner et al. (2013) found that, although paclobutrazol can increase flowering on sites with a moderate amount of chilling, this flowering enhancer is not effective on warm sites. Similarly, at sites with high levels of winter chilling, paclobutrazol has a negligible effect on the flowering of both seedling orchard trees and grafts (Gardner and Bertling 2005). Despite the successful use of paclobutrazol under certain conditions, use of the plant growth retardant or its generic equivalents is expensive (Chambers et al. 1997). In addition, species respond variably to different rates and methods of application of the chemical (Griffin et al. 1993), and the compound persists actively in the soil for several years, which is attracting pressure from environmentalists to discontinue its use (Reid et al. 1995, Jones pers comm[4]).

Pollination

Once flowering occurs, successful pollination may be affected by several factors (Moncur and Boland 2000), including the distance of pollen transfer, diversity in flowering times and intensity, and the number of pollen vectors present at flowering. Prolific flowering does not necessarily lead to high seed yields and these are often poor due to a lack of suitable pollinators (Moncur et al. 1995).

Eucalypts with small flowers, such as *E. nitens*, are predominantly insect pollinated (Ford et al. 1979), the most common of which are bees (Armstrong 1979). Flies and ants have been observed visiting *E. nitens* flowers in South Africa (Gardner pers comm[5]). Species such as *E. globulus* have been found to respond to an increase in the number of pollinating agents through the placement of honeybee hives in seed orchards, with a resultant increase in seed numbers per capsule, although the outcrossing rate was unchanged (Moncur et al. 1995). The same treatment in *E. nitens* resulted in no change in seed production, but an increased outcrossing rate. In South Africa, *E. nitens* is largely a winter-flowering species and it has been suggested by Jones (2002) that there are insufficient insect pollinators drawn to orchards at the time of flowering due to inadequate amounts, and poor quality, of nectar and pollen.

[4] Jones WR. 2010. Shaw Research Centre, Tweedie, PO Box 473, Howick, 3290, SOUTH AFRICA
[5] Gardner RAW. 2013. Institute for Commercial Forestry Research, Pietermaritzburg, PO Box 100281, Scottsville, 3209, SOUTH AFRICA

Outcrossing

Outcrossing in an orchard is a function both of the flowering levels and flowering patterns in the seed orchards, as well as the abundance and behaviour of insect pollinators (House 1997). The actual outcrossing rate for any particular seed crop from an individual tree will be determined by a variety of genetic and environmental factors including self-fertility (Eldridge and Griffin 1983), flowering phenology relative to neighbouring trees in the stand (Griffin 1980) and weather conditions during the flowering period that can influence pollinator activity (Griffin and Cotterill 1988, Hingston and Potts 1998, Jones 2002). Many authors have reported trends where increased flowering in seed orchards resulted in increased levels of outcrossing (Jones 2002, Butcher et al. 2004, Patterson et al. 2004, Grosser et al. 2010), but Varghese et al. (2009) suggest that increased flowering may decrease levels of outcrossing, because there is a higher possibility of crossing with a relative.

Self-incompatibility is believed to be a major factor determining outcrossing, and the factor with the most predictable effect on outcrossing rate (Patterson et al. 2004). In *E. globulus*, this is variable and under genetic control (McGowen et al. 2010), with higher levels of self-incompatibility (SI) being associated with higher outcrossing rates. It has been suggested that screening genotypes in grafted seed orchards for SI and then restricting seed collections to those individuals with high SI, could increase outcrossing rates, provided SI is repeatable across years and ramets. It is not known whether the SI level of the parents would affect outcross-progeny performance (Patterson et al. 2004).

Outcrossing may differ according to the position of flowers in the canopy. Patterson et al. (2001) found that outcrossing levels were higher and less variable higher up in the canopy in *E. globulus*, which is consistent with the behaviour of bird pollinators. There is evidence that stand density and tree size can affect the degree of outcrossing (Moran et al. 1989). Parents classified as being isolated individuals had significantly lower outcrossing rates than parents from open or closed forests in *E. regnans* (Hardner et al. 1996).

Several studies have been done to determine outcrossing rates in eucalypts, but to date there have been only three reported estimates of average outcrossing rates in *E. nitens* seed orchards; 0.75 from a seed orchard in Tasmania (Moncur et al. 1995), 0.87 from a CSO in New Zealand (Gea et al. 2007), and 0.85 from a CSO in Victoria, Australia (Grosser et al. 2010).

Seed orchards

Given that vegetative propagation is difficult in *E. nitens* (de Little et al. 1992, Moncur 1998), OP seed orchards have been widely established, globally, for the production of improved *E. nitens* seed. Once breeding programmes comprising broad collections from natural forests had commenced, it was recommended that large orchards containing many genotypes should be located at sites conducive to heavy flowering. This was in order to ensure the capture of most of the newly introduced alleles in OP breeding populations (Zobel et al. 1988). However, a compromise is normally required to achieve a balance between high genetic gain with maintenance of genetic diversity (El-Kassaby and Askew 2004), low levels of inbreeding, and shortened breeding cycles. Thus the relatedness of trees in an orchard and their relative contributions to flowering and seed production are important considerations when designing a seed orchard or thinning a trial into an orchard (Lindgren and Prescher 2005).

The establishment and management of seed orchards is extremely important, as this is where the gains achieved in tree improvement are ultimately captured (Moncur and Boland 2000). There are many designs available for OP seedling and clonal seed orchards (e.g., Eldridge et al. 1993, Hodge and White 1993, Barnes 1995, Tibbits and Hodge 1998, Lstibůrek and El-Kassaby 2010). In addition, many authors have investigated options for, and provided recommendations on, converting trials to seed orchards (e.g., Cannon and Shelbourne 1993, Eldridge et al. 1993, Johnson 1996). Open-pollinated seed orchards that have been developed by selectively thinning progeny trials to remove inferior trees and inferior families are still commonly employed as an important component of eucalypt breeding programmes (White et al. 2007).

Clonal seed orchards have an important role to play in production of improved seed, both for breeding purposes and for commercial deployment. Cloning captures superior individuals into elite seed orchards which, in addition to forward selection, can then be used for backward selections from progeny trials and improved performance in commercial plantations (Hamilton et al. 2008). In *E. nitens*, grafting is far more successful than rooted cuttings through micro- and macro-propagation, with the advantage that grafting overcomes delays due to juvenility and thus grafts flower and produce seed at an earlier age than seedling trees (Moncur and Boland 2000), which shortens the breeding cycle. Thus the longer cycle needed to graft elite material can be positively offset by combining greater gains

than those achieved in seedling seed orchards, with a shorter time to flowering and seed production in the grafted elites.

Although cloning, in the form of grafting, is widely used in *E. nitens* breeding programmes, and despite extensive research into alternative propagation and deployment strategies, improved *E. nitens* genotypes are still almost universally deployed as seedlings derived from OP seed orchards (Hamilton et al. 2008).

Inheritance of flowering

Flowering and fecundity have been found to be heritable in some eucalypts (Hodge et al. 1996, Varghese et al. 2009). In *E. globulus*, these reproductive traits were found to be highly heritable, were not affected by GEI, and appeared to be under stronger genetic control than other traits such as growth, survival and whole tree density (Chambers et al. 1997). Gore and Potts (1995) also found strong genetic control of flowering period in *E. globulus.* Varghese et al. (2009) found that fecundity greatly increased after one generation of domestication in both *E. camaldulensis* and *E. tereticornis*. Tibbits (1989) and Jones and van Staden (2001) found a good correlation in *E. nitens* flowering times from one year to the next, suggesting strong genetic control of flowering. In addition, provenance differences exist for timing and abundance of flowering in South Africa (Carlson et al. 2000, Jones 2002, Gardner and Bertling 2005). Therefore, in theory, breeders can select directly for flowering precocity in the first few generations of a breeding programme, with the aim of bringing the age of first selection and flowering into synchrony. This has been suggested by several authors, but could involve a loss of gain in other traits that may not be positively correlated with flowering, and/or loss of genetic diversity (Chambers et al. 1997).

Molecular genetics

In order to accurately predict the genetic gain or genetic quality of seed produced from OP seed orchards where the parents have known genetic worth or breeding values, it is necessary to have information on three main factors. These are: The relative paternal contribution of the trees in the orchard; the level of inbreeding; and the level of contamination in the orchard by pollen of lower genetic worth (Grosser et al. 2010). This is one of the roles of genetic markers or genotyping in tree improvement, in addition to the verification of identity and pedigrees of genotypes. Genotyping can assist with seed orchard management by estimating levels of pollen contamination, selfing rates and inbreeding, determining mating

patterns and gene flow within the orchard, as well as the effects of orchard management practices such as tree spacing and capsule location within the crown for seed collection (Moriguchi et al. 2004; Hansen and Kjær 2006, Gea et al. 2007). Although many techniques are available, microsatellites have been widely used in eucalypt population genetics (Byrne 2007), and have recently been used in studies of eucalypt seed orchards as they are highly polymorphic and co-dominant (Chaix et al. 2003, Patterson et al. 2004, Gea et al. 2007, Jones et al. 2008, Grosser et al. 2010).

Early screening of progeny using molecular markers to screen for desirable traits may enable earlier selection of elite material for use in orchards (Moncur and Boland 2000, Shimelis and Laing 2012). Marker-assisted selection is particularly attractive for timber breeding due to the long reproductive cycles and time to expression of mature traits, relative to annual or perennial field crops (Thavamanikumar et al. 2013). The sequencing of the eucalypt genome (Eucagen 2011) may make this applicable for a range of traits (Grattapaglia et al. 2011).

There are numerous techniques for the deployment of molecular markers based on DNA sequences (SNP, AFLP, RFLP, etc.). However, these markers are effective only when the traits are monogenic or oligogenic traits, which are usually qualitative traits. Where the trait is quantitative and governed by polygenic additive genes residing on many chromosomes, then DNA-based marker technologies may not always be deployed effectively. The challenge is that many key traits such as yield, drought tolerance and many forms of disease resistance are governed by additive genes. Proteomic markers may provide a solution to this problem, because the multiple additive genes may be expressed in a few proteins which can be tracked using modern quantitative proteomic tools to trace the expression of both constitutive and induced proteins (Thelen and Peck 2007, Que at al. 2011).

Optimising genetic gain through selection and breeding and production strategies

The challenge in any breeding programme is to formulate a breeding strategy that considers both genetic gain and relatedness, and then to apply a selection procedure that provides an optimal compromise between the two (Lindgren and Mullin 1997). In addition, one of the key factors affecting genetic gain is generation interval or breeding-cycle length, and it is important that this is decreased in order to increase genetic gain per unit time. The earliest age at which major traits can be reliably assessed, and the time to reproductive maturity, are

the two main influences on generation interval (Chambers et al. 1997). Although this may be as early as four years of age in species such as *E. globulus* (Borralho et al. 1992), only a small percentage of individuals will have reached reproductive maturity at this stage. In breeding programmes with generation intervals ranging from 5 to 15 years, such as *E. nitens*, a delay of even one year represents a 5 to 20% decrease in gain per unit time (Borralho and Dutkowsi 1998). Biological constraints associated with breeding in *E. nitens* have lengthened breeding cycles beyond optimal time-frames in most breeding programmes.

Optimising the balance of high genetic gain with the maintenance of genetic diversity (El-Kassaby and Askew 2004), and a low level of inbreeding in seed orchards, combined with shorter breeding cycles, is the core objective of tree breeders managing OP breeding programmes.

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CHAPTER 2[1]

Genetic characterisation of a *Eucalyptus nitens* base breeding population in South Africa

T-L Swain[2]*, SD Verryn[3], MD Laing[4]

[2] *Institute for Commercial Forestry Research, P.O. Box 100281, Scottsville, Pietermaritzburg 3209, South Africa.*

[3] *Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa and Creation Breeding Innovations, 75 Kafue St, Lynnwood Glen, 0081, South Africa.*

[4] *School of Agricultural Earth and Environmental Sciences (SAEES), University of KwaZulu-Natal, PO Box X01, Scottsville, 3209, South Africa.*

* Author for correspondence: T-L Swain

Telephone: +27 33 386 2314

Fax: +27 33 386 8905

Tammy.Swain@icfr.ukzn.ac.za

[1 **Published as:**
Swain T-L, Verryn SD, Laing MD. 2013. Genetic characterisation of a *Eucalyptus nitens* base breeding population in South Africa. *Southern Forests* 75(3): 155-167. DOI 10.2989/ 20702620.2013. 823717. Formatting and referencing style has been retained as per Journal style.]

Summary

The measurement and statistical analysis of data from eight *Eucalyptus nitens* trials established in the summer rainfall forestry region of South Africa during the 1980s and 1990s, have enabled the characterisation of the Institute for Commercial Forestry's breeding population. Provenance testing showed that the more northerly New South Wales (Australia) *Eucalyptus nitens* provenances of Barren Mountain and Barrington Tops are distinctly better suited to growth than the southern New South Wales provenances and the Victorian provenances, Penny Saddle and Bendoc. Generally, the species was not badly affected by *Coniothyrium* canker. High Type B genetic correlations for all sites pairs, except one comparison, ranged from 0.75 to 0.99 for diameter at breast height, indicating very little, or no, genotype-environment interaction for diameter at breast height for the genotypes tested in this study. Narrow sense heritability coefficients ranged from 0.01 to 0.34, indicating that the species generally exhibits sufficient breeding opportunity for improvement of diameter growth. High genetic correlations of greater than 0.90 between diameter measurements at 52 to 62 months after establishment and diameter measurements at 94 or 113 months were found, indicating that selections can be reliably made at five to six years.

Predicted genetic gains were highest in the trials at Goedehoop and Arthur's Seat, with predicted increases in diameter at breast height of 3.07 cm (17.1%) and 3.17 cm (20.7%), respectively, at full rotation.

Keywords

E. nitens, genetic parameters, heritability, genetic correlations, genotype-environment interaction, predicted gain

Introduction

Historically, *Eucalyptus grandis* has been the most important hardwood species for the South African forestry industry. However, increasing demand by the mining timber sector during the early 1900s and by the pulp and paper industry in the 1980s, led to the expansion of hardwood forestry into colder areas, often at altitudes exceeding 1 400 m and prone to frosts and snow, which are not suitable for growth of *E. grandis* (Darrow 1994). *Eucalyptus nitens* has become important on such high altitude, temperate sites in the summer rainfall forestry regions of South Africa, grown originally for mining timber, but more recently for pulp and paper production, as demands have changed. Plantation areas of *E. nitens* in South Africa currently cover approximately 46 600 ha (Germishuizen, pers comm[1]).

In its natural habitat in Australia, *E. nitens* occurs between 600 and 1200 m elevation in disjunct populations in the Victorian Alps, eastern Victoria and southern New South Wales (NSW). Two small populations are also found at Barrington Tops and Ebor/Barren Mountain in northern NSW, at altitudes of up to 1600 m. Rainfall is moderate to high, ranging in distribution between summer maxima in northern NSW to winter maxima in eastern Victoria. Thus there are a wide range of provenances available for testing for suitability to growth in the summer rainfall regions of South Africa. Landscapes vary from undulating tablelands to mountain slopes, where the species prefers the less exposed positions (Boland et al. 1992).

Generally in South Africa, *E. nitens* appears ideally suited to colder areas with a mean annual temperature (MAT) greater than 14 °C for optimal growth (Swain and Gardner 2003). The species is clearly unsuccessful in warmer areas and should only be planted on sites with a MAT less than 16 °C (Swain and Gardner 2003). At the warmer end of the MAT range, mean annual precipitation (MAP) should be at least 950 mm, while 825 mm is sufficient at the cooler end of its range (Swain and Gardner 2003). Although *E. nitens* is classified as frost tolerant, it is not as frost-hardy as *E. macarthurii* (Darrow 1994, 1996) and thus should not be planted in low landscape positions where extreme frosts are likely to occur. Provenance differences for growth under different climatic conditions have been found in Institute for Commercial Forestry Research (ICFR) site-species interaction trials. Ebor and Barren Mountain appear to be the most cold tolerant of the *E. nitens* provenances tested in South Africa (Gardner 2001) and seem to tolerate strong winds and exposure to chill very

[1] Germishuizen I. 2012. Institute for Commercial Forestry Research, Pietermaritzburg, PO Box 100821, Scottsville, 3209, SOUTH AFRICA

well, as long as the sites fall within the recommended MAT range. Tallaganda provenance showed the most drought and frost tolerance, although Ebor tolerates lower temperatures without frost better than Tallaganda (Darrow 1996, Gardner 2001). The diverse range of Australian provenances, the varying performance of these provenances in South African site-species trials and the disparate array of South African forestry sites, indicate that the presence of genotype by environment interaction (GEI) is a possibility in *E. nitens* populations in South Africa, and should be investigated further.

Eucalyptus nitens is recognised as the most snow tolerant, or snow hardy, of the Cold Tolerant Eucalypts (CTEs) grown in South Africa, and has clearly demonstrated good resistance to damage by all but the heaviest of snowfalls (Gardner and Swain 1996, Kunz and Gardner 2001). Currently, there is no suitable alternative commercial species to *E. nitens* on sites prone to moderate frost and heavy snows. The species is, however, very sensitive to fire. The species has good kraft pulping properties (Clarke 2000) and dissolving pulp yields ranging from 45.5 to 51.5%, over a range of sites (Clarke 1995, Clarke et al. 1999).

Provenance/progeny trials for *E. nitens* seedlots imported from Australia were established by the South African Department of Forestry during the 1980s. The ICFR took over these trials in 1989 and established additional trials at the end of the 1980s and early 1990s to determine which Australian provenances are best suited to South African summer rainfall growing conditions, and to identify seedlots which could be included in a tree improvement programme to selectively improve this species.

This paper uses the results from the range of trials to characterise the *E. nitens* population from a genetic perspective and to identify provenance differences for growth. Estimates of variances and narrow sense heritabilities were calculated as this information is fundamental to determining breeding potential of the population under consideration, to designing breeding strategies and is used for purposes of selection of superior families and individuals (White 1987, Falconer and Mackay 1996).The potential gains estimated from such calculations are important to funders of the *E. nitens* tree improvement programme, as these indicate whether investments in the breeding programme are justified. Genetic correlations for both juvenile-mature and trait-trait measurements were estimated, as the former is a very useful measure of which of the earlier measurements is the best predictor of top individuals

and seedlots at full rotation (van Buijtenen 1992), whilst the latter correlation indicates whether an increase in one measured trait will positively or negatively affect another measured trait, if at all (Cotterill and Dean 1990, Falconer and Mackay 1996).

The existence of GEI in this population was explored, as the presence of GEI will influence the breeding strategy and how the population should be managed, i.e., as discrete populations for specific environments in the presence of GEI (Squillace 1969) or by the exclusion of unstable genotypes (Kanzler 2002). The knowledge obtained from this study will inform the development of the breeding programme for the next generation of trials such that gains are maximised over a range of sites.

Materials and methods

Field trials

One hundred and sixty-five *E. nitens* seedlots were imported from Australia by the South African Department of Forestry during the 1980s, representing seedlots from eight provenances in NSW and Victoria i.e., Ebor, Barrington Tops, Barren Mountain, Badja, Tallaganda, Glenbog, Bendoc and Penny Saddle. These seedlots were established in four series of provenance/progeny trials on two sites for each trial series. **Table 2.1** provides details of the sites where the trials were established, as well as trial design information. **Table 2.2** provides information on the origins of the provenances represented in the trials. Details of seedlots allocated to each site can be found in Swain et al. (1998) and **Appendices 1a** and **1b**. The majority of seedlots are represented at both sites within each trial series, but the seedlots vary considerably between the different trial series. As early results from the E88/05 and E88/06 trial series indicated that there were significant differences between seedlots collected from two different areas within the Barrington Tops provenance area in Australia (Stanger 1991), a further comprehensive collection was done at Barrington Tops, and a fifth trial series (E88/07) established in South Africa in 1992, including 56 new imports from Australia. Details of these trials are also included in **Table 2.1**, as are the origins of the material in **Table 2.2** and **Appendix 1b**. All trials were planted at a spacing of 2 m x 3 m, with an initial planting density of 1667 stems per hectare. Trees were established in single row line plots of 5 to 10 trees. Controls in the first four trial series (E88/01 to E88/06) included 17 landrace seedlots collected from South African *E. nitens* plantations (**Table 2.2**), and an improved bulk from a private breeding programme was included in the E88/05 and E88/06 trial series (M1278). The E88/07 trial series included top

Table 2.1 Site and trial information of five *Eucalyptus nitens* provenance/progeny trial series in South Africa

Trial no.	Location	Latitude (S)	Longitude (E)	Altitude (m)	MAP (mm)	MAT (°C)	Soil depth (mm)	Planting date	No. of seedlots	Trial design	No. of reps	No. trees/ plot
E88/01	Jessievale, MPU	26° 15' 20.02''	30° 31' 29.48''	1706	873	14.5	850	08/12/1982	42	6x7 latt	10	10
	Amsterdam, MPU	26° 8' 36.02''	30° 43' 7.39''	1691	864	14.8	1200	14/12/1982	36	6x6 latt	10	10
E88/03	Daspoort, MPU	26° 13' 13.30''	30° 39' 55.75''	1618	874	14.8	1000	18/01/1985	49	7x7 latt	8	6
	Helvetia, MPU	25° 34' 8.44''	30° 18' 23.81''	1646	789	15.6	1000	19/03/1985	49	7x7 latt	8	6
E88/05	Woodstock, MPU	26° 23' 25.75''	30° 41' 14.15''	1578	867	14.4	1000	11/02/1988	141	2(8x9) latt	6	6
E88/06	Babanango, KZN	28° 18' 27.37''	31° 4' 57.43''	1338	780	16.6	1000	29/03/1988	92	2(7x8) latt	9	5
E88/07	Goedehoop, MPU	26° 10' 28.94''	30° 39' 30.69''	1737	884	14.4	1000	01/12/1992	100	10x10 latt	4	8
	Arthur's Seat, MPU	26° 18' 4.46''	30° 37' 33.15''	1645	871	15.2	1000	14/12/1992	100	10x10latt	4	8

MPU = Mpumalanga province, KZN = KwaZulu-Natal province, MAP = Mean Annual Precipitation, MAT = Mean Annual Temperature, latt = lattice

performing *E. nitens* families from the first four trial series, 2nd generation selections from the then New Zealand Forestry Research Institute (NZFRI), selections from a site-species trial in Lesotho and two *E. grandis x nitens* (*E. GxN*) hybrids.

Table 2.2 Origins of *Eucalyptus nitens* provenances and control seedlots established in five South African provenance/progeny trials

Trial no.	Location of trials	Provenances/Control	Latitude (S)	Longitude (E)	Altitude (m)
E88/01	Jessievale, MPU	Tallaganda, NSW	35°48'	149°31'	1280
		Barren Mountain, NSW	30°23'	152°28'	1460
	Amsterdam, MPU	Badja, NSW	36°00'	149°36'	1300
		Penny Saddle, VIC	37°47'	146°16'	900
		Woodbush, SA (landrace)	23°35'	29°59'	1780
E88/03	Daspoort, MPU	Barrington Tops (Mt Carson), NSW	30°57'	151°30'	Unknown
		Barren Mountain, NSW	30°23'	152°28'	1250-1560
	Helvetia, MPU	Badja, NSW	36°10'	149°31'	880-1300
		Penny Saddle, VIC	37°47'	146°16'	900
		Belfast, SA (landrace)	25°39'	30°02'	1880
		Perdestal, Jessievale, SA (landrace)	26°14'	30°31'	1750
		Nelshoogte, SA (landrace)	25°50'	30°50'	1400
E88/05	Woodstock, MPU	Ebor, NSW	30°29'	152°24'	1560
E88/06		Tallaganda, NSW	35°49'	149°30'	1180-1450
	Babanango, KZN	Badja, NSW	36°01'	149°34'	880-1240
		Glenbog, NSW	36°38'	149°24'	900-1200
		Barrington Tops, NSW	31°55'	151°30'	1450
		Bendoc, VIC	37°12'	145°52'	790-1040
		Nelshoogte, SA (landrace)	25°48'	30°47'	1450
		E. nitens improved bulk (M1278)	-	-	-
E88/07	Goedehoop, MPU	Barrington Tops (Kholwa Fire Trail), NSW	31°38'	151°30'	1200-1300
		Barrington Tops (Mt Carson), NSW	31°55'	151°30'	1450
	Arthur's Seat, MPU	Badja, NSW	35°59'	149°34'	1100-1200
		Glenbog control (Family 37209 & 37224)	36°38'	149°24'	900-1200
		Ebor controls (Families 37255, 37650 & 37651)	30°29'	152°24'	1560
		NZFRI (2nd generation selections ex New Zealand)	-	-	930
		Thaba Putsoa (selections ex abandoned species trial, Lesotho)	-	-	-
		Perdestal, Jessievale, SA (landrace)	26°14'	30°31'	1750
		E. GxN natural hybrid M, SA	-	-	-
		E. GxN hybrid H, SA	-	-	-

MPU = Mpumalanga province,
KZN = KwaZulu-Natal province,
NSW = New South Wales,
VIC = Victoria,
SA = South Africa,
NZFRI = New Zealand Forestry Research Institute,
E. GxN = *E. grandis x nitens* hybrid

Data collection and statistical analyses

Trials were routinely measured for height, using expandable height rods, at one or two years after establishment, and at approximately three, six and nine years after establishment for diameter at breast height (dbh), using diameter tapes. Final measurements of the trials were

done at 101 months in the E88/01 trial series, 110 and 94 months in the E88/03 Daspoort and Helvetia trials, respectively, 76 and 110 months in the E88/05 and E88/06 series, respectively, and at 113 months in the E88/07 series. Following the presence of *Coniothyrium* canker (formerly *Coniothyrium zuluense*, now *Teratosphaeria zuluense*) being noted in some of the trials, the E88/01 and E88/03 series were scored for the presence of lesions on the stem, indicating possible infection by *T. zuluense*, at 101 months and 86 months, respectively. Although commercial stands of *E. nitens* appear not to be particularly susceptible to this stem disease, as is *E. grandis*, symptoms can appear in areas of high rainfall and optimal growth (FABI 2000). A subjective scoring system of 0 to 2 was used, where 0 indicated no lesions or sign of disease; 1 indicated the presence of some lesions and 2 indicated marked signs of the disease. The disease scores were analysed both at a provenance and individual seedlot level.

Each site in a trial series was analysed separately in order to obtain single-site genetic parameter estimates. Statistical analyses were conducted using SAS® Institute Inc. Software 9.2 (2002-2008). To test for normality for dbh, residuals were plotted against fitted values. None showed any detectable trends or patterns and it can therefore be said that the condition $\varepsilon_{ijklm} \sim$ iid $(0,\sigma^2)$ were met for these data, and the standard ANOVA assumptions are valid. Provenance and family means were calculated for all sites individually using Proc GLM, as this procedure is recommended for unbalanced designs (Hettasch et al. 2007). Significant replication effects were corrected for and F-statistics were calculated to test for significant differences among families and provenances. Comparisons for differences between treatments were made using Fisher's test for Least Significance Differences (LSD) for $\alpha = 0.05$, as this test allowed for expression of more differences than tests such as Student-Newman-Keuls (SNK) multiple range test. Provenance means for both dbh and survival were calculated in order to provide information to South African growers on which seed to import from Australia until the South African breeding programmes could provide sufficient quantities of improved seed within the country.

Estimation of genetic parameters

Estimates of variances and narrow sense heritabilities were calculated for the individual sites. Controls were removed from the data before the variance components were estimated using Restricted Maximum Likelihood Method (REML) (Patterson and Thompson 1971) and

the VARCOMP procedure in SAS® Institute Inc. Software 9.2 (2002-2008), with the following model:

$$y_{ijklm} = \mu + R_i + B_{j(i)} + P_k + f_{l(k)} + e_{ijklm},$$

where y_{ijklm} = individual phenotypic observation for the trait of the l^{th} seedlot/family within provenance k in the j^{th} block within replication i, μ = overall mean, R_i = i^{th} replication effect (fixed), $B_{j(i)}$ = block (within rep) effect (fixed), P_k = provenance effect (fixed), $f_{l(k)}$ = seedlot/family effect within provenance (random) and e_{ijklm} = random error effects. Additional analyses were done ignoring the effect of provenance, as selections were actually made for best individuals and seedlots irrespective of, or across, provenances. The model was adjusted accordingly.

When the genetic parameters for half-sibs were calculated, a coefficient of relationship (*cr*) of 0.33 was used. This was based on the assumption that full-sibs do occur in matings from open-pollination, and at least some inbreeding is assumed to occur in natural stands (Squillace 1974, Verryn 1993). The ICFR have accepted the assumptions stated above and standardised on a coefficient of relationship of 0.33 when calculating genetic parameters for growth traits in *E. nitens*. Therefore, the additive genetic variance was estimated as:

$$\hat{\sigma}^2{}_A = \hat{\sigma}^2{}_f / 0.33\,,$$

where σ^2_f is the seedlot/family variance.

Single-site narrow sense individual heritability (h^2) and within-family (h^2_{wf}) heritability estimates were calculated, respectively, for all traits using the formulae (Falconer and Mackay 1996):

$$h^2 = \frac{1}{0.33}\,\hat{\sigma}^2_f / \hat{\sigma}^2_{phen}$$

and

$$h^2{}_{wf} = \frac{(1-0.33)h^2}{1-(0.33h^2)},$$

where σ^2_{phen} is the phenotypic variance. Provenance effects were included in the heritability estimations, and estimations were then repeated excluding provenance effects, as final selections were made in top performing families across provenances. Standard errors of

genetic variance components and of heritabilities were estimated as follows (Becker 1975):

$$\sigma = \sqrt{\text{var}\,\sigma^2}\ ,$$

where σ = standard error of variance components and σ^2 = additive, family or error variance, and

$$SE(h^2) = \frac{m\sqrt{\text{var}\,\sigma^2{}_f}}{\sigma^2{}_{phen}}$$

where $SE(h^2)$ = standard error of the heritability estimate and m = inverse of the coefficient of relationship.

Genotype by environment interaction (GEI)

Type B genetic correlations were estimated for dbh, both nested within and across provenance, to give an indication of any potential genotype by environment interaction (GEI). Where two traits are measured on different individuals within genetic groups, for example a genetic correlation between trees of the same family grown in different environments, the correlation can be designated a Type B genetic correlation. A Type B genetic correlation (r_{Bg}) of 0.67 is the level at which the GEI variance represents 50% of the total additive variance, and is the point where it is postulated that the GEI variance may be a cause for concern among tree breeders (Shelbourne 1972). Type B correlations at the family level (r_{Bg}) were estimated for all possible site pairs as follows (Burdon 1977):

$$r_{Bg} = \frac{\sigma_f}{\sigma_f^2 + \sigma_{site*f}^2}$$

The variance components were estimated using PROC MIXED in SAS® Institute Inc. Software 9.2 (Copyright © 2002-2008 SAS Institute Inc.), as it was not possible to directly estimate the site x family covariance for family nested within provenance using the VARCOMP/CORR procedures in SAS with this dataset. The only restrictions on the site comparisons were that the sites being paired should have at least 15 families in common (Kanzler and Hodge 2000). As there were 20 seedlots in common across all the E88/01, E88/03 and E88/05 trials, and 16 in common across the E88/03, E88/05 and E88/06 trials, correlations were carried out on all site pair combinations except the E88/07series.

Combined site analysis

A combined site analysis was done on 71 seedlots (numbers 1 to 34840) to determine overall performance of these common families across the range of sites and environments

represented over the trial series. Only these seedlots were included in the comparison, as they were present on at least four sites. The E88/07 trial series at Arthur's Seat and Goedehoop had very few families in common with the earlier trial series, and were therefore excluded from the combined site analysis. The following model was used to estimate family means for dbh:

$$y_{hiklm} = \mu + S_h + R_{i(h)} + P_k + f_{l(k)} + (S_h \times f_{l(k)}) + e_{hiklm},$$

where y_{hiklm} = mean for the trait of the m^{th} tree in the l^{th} family within provenance k in the i^{th} replication at the h^{th} site; μ = overall mean, S_h = h^{th} site effect (fixed), R_i = i^{th} replication within h^{th} site effect (fixed), P_k = provenance effect (fixed), $f_{l(k)}$ = family within provenance effect (random), $S_h \times f_{l(k)}$ = interaction between the h^{th} site and l^{th} family within provenance (random), and e_{hiklm} = random error effects. Significant site effects were corrected for.

Juvenile-mature and trait genetic correlations

Juvenile-mature genetic correlations (r_{g12}) were estimated at all sites using the following formula:

$$r_{g12} = \frac{\mathrm{cov}_{12}}{\sqrt{\mathrm{var}_1 \times \mathrm{var}_2}},$$

where cov_{12} = the family covariance of the trait at age 1 and age 2, var_1 = the family variance of the trait at age 1 (similarly for age 2). Standard errors were calculated according to Becker (1975).

Predicted genetic gain

The potential genetic gain to be obtained through selection varies according to the heritability of the trait under consideration, the present phenotypic variation for that trait in the population and the intensity of selection. Gains in dbh were predicted for the progeny of each trial as if selection was applied by roguing the poorest 30% of the families from each trial, then leaving the best tree per plot of the remaining 70% families standing, followed by bulking of seed from the top 15 to 36 families in each trial. This was done using the formula (Verryn et al. 2000):

$$\Delta G \quad = 0.5\left(\Delta G_f\right) + 0.5\left(\Delta G_m\right), \text{ where}$$

$$\Delta G_f \; and \; \Delta G_m = 0.5\left[SI_b \times cr \times \frac{\sigma^2{}_A}{\sigma_{fm}} \right] + \left[SI_{wf} \times \frac{(t-1)}{t} \times \left(1 - cr\right) \times \frac{\sigma^2{}_A}{\sigma_{wf}} \right],$$

and the predictions are the predicted genetic gains from female and male selection in a population, respectively (ΔG = predicted genetic gain or response to selection, ΔG_f = predicted genetic gain from female selection, ΔG_m = predicted genetic gain from male selection, SI_b = selection intensity between/among female or male families, respectively, cr = coefficient of relationship, σ^2_A = additive genetic variance, σ_{fm} = standard deviation among half-sib families, SI_{wf} = selection intensity within female or male families, respectively, within plots, t = number of trees per plot, and σ_{wf} = standard deviation within families). The selection intensity for male and females differed as, in addition to roguing and thinning, the top 15 to 36 families would be selected to make up bulk seed. Male and female selection intensities between/among families and within families within plots were determined using the standardised selection intensity tables of Becker (1975).

Results and discussion

Provenance and family performance

There were significant differences between Australian provenances for dbh at all sites ($p < 0.0001$). **Tables 2.3** and **2.4** present provenance comparisons for dbh and percentage survival for the five *E. nitens* trial series. The more northern New South Wales (NSW) provenance of Barren Mountain performed significantly better ($p < 0.05$) than the southern NSW provenances of Tallaganda and Glenbog, and the Victorian provenances of Penny Saddle and Bendoc, at all sites excepting Helvetia. Barren Mountain provenance was not included at Goedehoop and Arthur's Seat. This provenance also performed significantly better ($p < 0.05$) than the more southern NSW provenance of Badja at three of the six sites where it was included. Generally, the more northern NSW provenances of Barren Mountain and Barrington Tops performed better than the southern *E. nitens* provenances of Penny Saddle, Bendoc, Glenbog and Tallaganda. Stand density at final measurement varied between site and provenance, being generally lower at Helvetia and Woodstock. Final stand density of Badja provenance was poor at three of the six sites, yet the provenance did not perform significantly worse for dbh than Tallaganda, which ranked second for dbh at Jessievale and Amsterdam. Survival of Victorian provenance, Penny Saddle, was poor at

Table 2.3 Final mean diameter at breast height (dbh) (with percentage survival in brackets) for *Eucalyptus nitens* provenances and controls in four trial series over six sites in South Africa. Treatment means which do not differ significantly from each other bear the same letter of the alphabet

Trial number	**E88/01**		**E88/01**		**E88/03**			**E88/03**		**E88/05**			**E88/06**	
Site	**Jessievale**		**Amsterdam**		**Daspoort**			**Helvetia**		**Woodstock**			**Babanango**	
Assessment age	**101 months**		**101 months**		**110 months**			**94 months**		**76 months**			**110 months**	
Provenance	**No. of seedlots**	**dbh (cm) (% survival)**	**No. of seedlots**	**dbh (cm) (% survival)**	**Provenance**	**No. of seedlots**	**dbh (cm) (% survival)**	**No. of seedlots**	**dbh (cm) (% survival)**	**Provenance**	**No. of seedlots**	**dbh (cm) (% survival)**	**No. of seedlots**	**dbh (cm) (% survival)**
Barren Mountain	11	17.95 (75.8) a	11	18.49 (76.4) a	Barren Mountain	9	20.24 (81.3) a	8	17.87 (72.5) ab	Barren Mountain	9	15.13 (74.7) ab	3	19.49 (78.9) b
Tallaganda	13	16.48 (72.5) b	6	17.25 (72.7) ab	Barrington Tops	10	20.13 (89.2) a	10	18.39 (72.5) a	BTops[4] (Mt Carson)	9	14.66 (73.5) bc	6	20.46 (83.2) a
Badja	15	16.38 (68.3) b	16	16.66 (68.0) bc	Nelshoogte, SA[1] (l/race)[2]	8	17.57 (82.6) bc	8	16.36 (54.9) bcd	Badja	18	14.64 (69.1) bc	9	19.17 (75.6) bc
Woodbush, SA (l/race)	1	15.09 (42.0) c	1	18.90 (66.0) a	Badja	12	16.98 (83.3) cd	13	16.35 (55.1) bcd	Tallaganda	40	14.52 (69.3) bc	31	18.23 (76.2) cd
Penny Saddle	2	14.75 (44.5) c	2	15.15 (33.0) c	Penny Saddle	1	16.22 (85.4) cd	1	17.12 (54.2) abc	Glenbog	28	14.43 (67.9) bcd	20	17.95 (70.0) d
					Belfast, SA (l/race)	3	15.78 (74.3) d	4	15.47 (49.5) cd	Nelshoogte,SA (l/race)	4	14.41 (75.0) bcd	5	18.23 (75.9) cde
					Jessievale, SA (l/race)	4	11.36 (49.5) e	3	15.07 (21.5) d	Ebor	14	14.07 (73.2) cd	7	18.76 (75.6) bcd
										BTops[4] (Kholwa FT[5])	9	13.96 (69.4) cd	8	18.33 (64.7) cd
										Bendoc	5	13.71 (61.1) d	-	-
Mean		16.78		17.39			18.09		17.19			18.34		14.47
SD[3]		4.299		5.145			5.298		4.450			4.571		3.279

[1] South Africa, [2] Landrace, [3] Standard deviation, [4] Barrington Tops, [5] Kholwa Fire Trail

Jessievale, Amsterdam and Helvetia, but good at Daspoort. Although significant differences still existed between the two different collections of Barrington Tops at Babanango at final measurement ($p < 0.05$), the early measurements of this trial series having prompted the further collection of this provenance and the establishment of the E88/07 trial series, there were no significant differences ($p > 0.05$) between the two different Barrington Tops collections from Mount Carson and Kholwa Fire Trail in either of the subsequent E88/07 trials at Goedehoop and Arthur's Seat.

Table 2.4 Final mean diameter at breast height (dbh) (with percentage survival in brackets) for *Eucalyptus nitens* provenances and controls in the E88/07 trial series at two sites in South Africa. Treatment means which do not differ significantly from each other bear the same letter of the alphabet

Trial number	**E88/07**			**E88/07**	
Site	**Goedehoop**			**Arthur's Seat**	
Assessment age	**113 months**			**113 months**	
Provenance	**No. of seedlots**	**dbh (cm) (% survival)**	**Provenance**	**No. of seedlots**	**dbh (cm) (% survival)**
GxN H	1	20.84 (87.5) a	*GxN* M	1	18.86 (100.0) a
GxN M	1	20.58 (96.9) a	Thaba Putsoa, Lesotho	3	16.30 (85.4) b
Glenbog control 37209	1	19.66 (87.5) ab	Glenbog controls (37209 & 37224)	2	16.08 (81.3) bc
Barrington Tops (Mt Carson)	15	18.24 (76.3) ab	Badja	26	15.98 (88.0) bc
Badja	26	18.24 (81.0) ab	NZFRI[1], New Zealand	11	15.78 (83.5) bc
Thaba Putzoa, Lesotho	3	17.86 (77.1) b	Barrington Tops (Kholwa Fire Trail)	27	14.84 (81.3) bcd
Barrington Tops (Kholwa Fire Trail)	27	17.84 (80.2) b	Ebor controls (37255, 37650, 37651)	3	14.65 (70.8) bcd
Ebor controls (37255 & 37650)	2	17.73 (60.9) b	Barrington Tops (Mt Carson)	15	14.51 (80.0) cd
NZFRI[1], New Zealand	13	17.48 (74.8) b	Jessievale, SA[2] (l/race)	9	11.15 (53.8) e
Jessievale, SA[2] (l/race)[3]	9	14.59 (55.9) c			
Mean		17.79			15.12
SD[4]		4.790			4.346

[1] New Zealand Forestry Research Institute,
[2] South Africa,
[3] Landrace,
[4] Standard deviation

The Victorian provenance, Penny Saddle, did not perform as well as several of the other Australian provenances for dbh, except at Helvetia, where this provenance was one of the top performing provenances with Barrington Tops and Barren Mountain. The juvenile leaves of the Victorian provenance displayed susceptibility to *Mycosphaerella* leaf blotch disease, and it is likely that the trees never recovered after this early setback. Other authors have found this to be the case in *E. nitens* (Lundquist and Purnell 1987, Hunter et al. 2004) and in *E. globulus* subsp. *bicostata* (Komakech et al. 2009). In Australia, the Victorian provenances are tending towards a winter rainfall maximum with a drier summer and it is interesting that, of the sites in this South African trial series, the disease did not appear to be as prevalent at the Helvetia site, which has slightly lower rainfall than the other sites. This was also found in a South African trial series of *E. globulus* subsp. *bicostata*, where susceptible provenances

were less infected at 30 months at two sites with lower rainfall (≤ 900 mm) than sites with higher rainfall (> 950 mm) (Komakech et al. 2009).

ICFR site-species interaction trials have shown that Ebor and Barren Mountain appear to be the most cold tolerant of the *E. nitens* provenances tested, and Tallaganda the most frost and drought tolerant (Darrow 1996, Gardner 2001, Swain 2001). The results of these trials support this to some degree, as Barren Mountain was the top performing provenance at three of the five cold sites at which it was present, i.e., Jessievale, Amsterdam and Daspoort, and performed well at the other cold sites, i.e., Helvetia and Woodstock.

Disease assessments for *T. zuluense* were done in the E88/01 and E88/03 trials and significant provenance differences ($p < 0.05$) were found (**Table 2.5**). Although the ranking of the provenances changed over site with respect to presence of the disease, it appears that Badja and Tallaganda were the provenances least affected by the disease. Generally however, with the exception of a few seedlots which had scores of 1.5, i.e., 32097 at Jessievale (ex Barren Mountain), 32079 at Daspoort (ex Badja) and 34832 (ex Barrington Tops), these populations of *E. nitens* do not seem to be badly affected by the canker. Disease seems to have been worse at Amsterdam than at the other three sites (mean score 1.02). Trees of those seedlots which were badly affected at more than one site were rogued from the ICFR trials when the trials were converted to seed orchards (seedlots marked in **Table 2.8**).

Table 2.5 Assessment for *Teratosphaeria zuluense* in four *Eucalyptus nitens* provenance/progeny trials in South Africa. Infection scores: 0 = no lesions, 1 = presence of some lesions, 2 = marked signs of the disease (Swain et al. 1998). Scores which do not differ significantly from each other bear the same letter of the alphabet

Trial number	**E88/01**			**E88/03**			
Site	**Amsterdam**	**Jessievale**		**Daspoort**		**Helvetia**	
Assessment age	**101 months**	**101 months**		**86 months**		**86 months**	
Provenance	**Disease score**	**Provenance**	**Disease score**	**Provenance**	**Disease score**	**Provenance**	**Disease score**
Penny Saddle	0.8 a	Tallaganda	0.9 a	Jessievale, SA	0.8 a	Nelshoogte, SA	0.7 a
Badja	1.0 ab	Badja	1.0 ab	Badja	0.9 a	Barren Mountain	0.8 a
Tallaganda	1.0 b	Woodbush, SA	1.0 ab	Nelshoogte, SA	1.0 a	Badja	0.9 a
Woodbush, SA[1]	1.0 b	Barren Mountain	1.1 ab	Barren Mountain	1.0 a	Jessievale ,SA	0.9 a
Barren Mountain	1.2 b	Penny Saddle	1.2 b	Belfast, SA	1.0 a	Penny Saddle	0.9 ab
				Barrington Tops	1.0 a	Belfast, SA	0.9 ab
				Tallaganda	1.1 a	Barrington Tops	1.2 b
				Penny Saddle	1.1 a		
Mean	1.02		0.97		0.96		0.91

[1] South Africa

Significant differences existed ($p < 0.05$) between the top seedlots at Amsterdam, Daspoort, Babanango, and between top and bottom performing seedlots at all sites. Although *E. nitens* is not recommended for areas where the altitude is lower than 1400 m and where MAT is greater than 16 °C (Herbert 1993), the average growth at Babanango (altitude 1325 m and MAT 16 °C) was better than that of the other seven sites, all of which can be described as high altitude sites (**Tables 2.3** and **2.4**).

Genetic parameters

Variance components and individual heritability estimates for dbh are presented in **Table 2.6**. Narrow-sense heritabilities ranging from 0.10 to 0.30 are considered intermediate to high, and generally indicate that moderate to good genetic gains can be expected from individual tree selection (Namkoong 1979, Cotterill and Dean 1990). This is the range most commonly found in eucalypts (Cotterill and Dean 1990). On the contrary, heritabilities less than 0.10 are considered low in forestry, resulting in poor genetic gains from selection (Falconer and Mackay 1996). The individual heritability coefficients (h^2) obtained for dbh in *E. nitens* in this study ranged from 0.01 to 0.34 when families were nested within provenance, and from 0.11 to 0.63 for families across provenance, depending on age of trees and site (**Table 2.6**). With regards to the heritability estimates of family nested within provenance, estimates were highest at Arthur's Seat at all ages (h^2~0.27 – 0.34 for dbh); for families irrespective of provenance, heritability estimates were highest at Daspoort at all ages (h^2~0.58 – 0.63). The site with the lowest heritabilities generally for families nested within provenance was Helvetia (h^2~0.01 – 0.08); whilst Jessievale had the lowest estimates for families across provenance (h^2~0.11 - 0.19).

The dbh heritability estimates fell within the intermediate range for eucalypts, with the exception of Jessievale and Helvetia, and compared well with those estimated in previous *E. nitens* studies for dbh: 0.24 (converted from family h^2 of 0.80, King and Wilcox 1988), 0.18 (Whiteman et al. 1992), 0.14 (Johnson 1996), 0.11 (pooled estimates, Gea et al. 1997) and 0.39 (Kube and Raymond 2001). However, all sites had heritabilities lower than those found in seven year old *E. nitens* trees in Victoria, Australia; 0.42 (Greaves et al. 1997). The heritability estimates from this South African study were also within the range described by Hamilton and Potts (2008) and demonstrate that this breeding population of the species exhibits sufficient potential for selections to result in progeny improved for diameter growth.

Table 2.6 Variance components and genetic parameters with standard errors for *Eucalyptus nitens* at eight sites in South Africa, for diameter at breast height (Dbh) at different ages

Site (age in months)	σ^2_A	σ^2_f	σ^2_e	σ_p	σ_{wf}	h^2		$h^2_{wf(prov)}$
						$h^2_{fam(prov)}$	h^2_{fam}	
Dbh:								
Jessievale (39)	0.35 ± 0.144	0.12 ± 0.048	6.51 ± 0.163	2.57	2.15	0.05 ± 0.022	0.19 ± 0.052	0.04
Jessievale (101)	1.06 ± 0.464	0.35 ± 0.153	20.99± 0.551	4.62	3.84	0.05 ± 0.022	0.11 ± 0.034	0.03
Amsterdam (39)	0.91 ± 0.354	0.30 ± 0.117	6.50 ± 0.243	2.61	2.23	0.13 ± 0.052	0.19 ± 0.062	0.09
Amsterdam (101)	4.06 ± 1.618	1.34 ± 0.534	27.97 ± 1.136	5.41	4.63	0.14 ± 0.055	0.20 ± 0.066	0.10
Daspoort (30)	0.57 ± 0.186	0.19 ± 0.061	3.99 ± 0.123	2.05	1.75	0.14 ± 0.044	0.63 ± 0.141	0.10
Daspoort (60)	1.83 ± 1.912	0.60 ± 0.631	12.52 ± 0.410	3.62	3.10	0.14 ± 0.146	0.63 ± 0.146	0.10
Dasport (110)	3.90 ± 1.348	1.29 ± 0.445	28.48 ± 0.955	5.45	4.66	0.13 ± 0.045	0.58 ± 0.137	0.10
Helvetia (24)	0.16 ± 0.217	0.05 ± 0.023	2.10 ± 0.066	1.47	1.23	0.08 ± 0.032	0.34 ± 0.083	0.05
Helvetia (62)	0.17 ± 0.314	0.05 ± 0.104	10.80 ± 0.418	3.30	2.71	0.02 ± 0.029	0.23 ± 0.073	0.01
Helvetia (73)	0.16 ± 0.390	0.05 ± 0.129	13.80 ± 0.534	3.72	3.06	0.01 ± 0.028	0.21 ± 0.068	0.01
Helvetia (94)	0.20 ± 0.589	0.07 ± 0.194	22.78 ± 0.887	4.78	3.93	0.01 ± 0.026	0.14 ± 0.051	0.01
Woodstock (36)	0.93 ± 0.171	0.31 ± 0.056	3.97 ± 0.093	2.07	1.81	0.22 ± 0.040	0.26 ± 0.044	0.16
Woodstock (76)	1.61 ± 0.368	0.53 ± 0.122	10.81 ± 0.261	3.37	2.89	0.14 ± 0.032	0.15 ± 0.033	0.10
Babanango (35)	1.22 ± 0.236	0.40 ± 0.078	3.65 ± 0.088	2.01	1.81	0.30 ± 0.058	0.32 ± 0.060	0.22
Babanango (62)	3.18 ± 0.662	1.05 ± 0.219	13.65 ± 0.329	3.83	3.36	0.22 ± 0.045	0.28 ± 0.054	0.16
Babanango (110)	4.04 ± 0.886	1.33 ± 0.292	20.98 ± 0.512	4.72	4.10	0.18 ± 0.040	0.25 ± 0.049	0.13
Goedehoop (36)	1.00 ± 0.215	0.33 ± 0.005	3.46 ± 0.010	1.95	1.73	0.26 ± 0.057	0.29 ± 0.060	0.19
Goedehoop (52)	2.35 ± 0.518	0.78 ± 0.171	8.86 ± 0.253	3.10	2.74	0.24 ± 0.054	0.26 ± 0.055	0.18
Goedehoop (77)	3.14 ± 0.733	1.03 ± 0.242	14.11 ± 0.407	3.89	3.40	0.21 ± 0.048	0.21 ± 0.047	0.15
Goedehoop (113)	5.78 ± 1.444	1.91 ± 0.477	24.21 ± 0.760	5.11	4.48	0.22 ± 0.055	0.21 ± 0.053	0.16
Arthur's Seat (36)	1.09 ± 0.224	0.36 ± 0.074	3.02 ± 0.087	1.84	1.66	0.32 ± 0.066	0.39 ± 0.075	0.24
Arthur's Seat (52)	2.68 ± 0.552	0.88 ± 0.182	7.06 ± 0.208	2.82	2.56	0.34 ± 0.069	0.41 ± 0.079	0.25
Arthur's Seat (77)	4.03 ± 0.853	1.33 ± 0.282	11.43 ± 0.344	3.57	3.22	0.32 ± 0.067	0.38 ± 0.074	0.24
Arthur's Seat (113)	5.67 ± 1.275	1.87 ± 0.421	19.50 ± 0.596	4.62	4.12	0.27 ± 0.060	0.30 ± 0.064	0.20
Height:								
Daspoort (17)	0.13 ± 0.038	0.04 ± 0.013	0.59 ± 0.018	0.79	0.70	0.21 ± 0.060	0.69 ± 0.154	0.15
Helvetia (12)	0.03 ± 0.010	0.01 ± 0.003	0.25 ± 0.008	0.51	0.43	0.11 ± 0.031	0.27 ± 0.067	0.08
Babanango (12)	0.03 ± 1.124	0.01 ± 0.002	0.14 ± 0.003	0.39	0.34	0.20 ± 0.041	0.25 ± 0.047	0.14
Goedehoop (113)	4.60 ± 1.057	1.52 ± 0.349	14.37 ± 0.453	3.99	3.57	0.29 ± 0.067	0.30 ± 0.067	0.21
Arthur's Seat (113)	4.65 ± 0.940	1.54 ± 0.310	9.98 ± 0.309	3.39	3.13	0.40 ± 0.082	0.44 ± 0.086	0.31

σ^2_A = additive variance,
σ^2_f = family variance nested within provenance,
σ^2_e = error variance,
σ_p = phenotypic standard deviation,
σ_{wf} = standard deviation within families,
h^2 = heritability estimates of individual values (narrow-sense) for a) family nested within provenance *(fam(prov))* and b) family excluding provenance *(fam)* effects,
$h^2_{wf(prov)}$ = within family heritability estimates for family nested within provenance

There was a trend for the individual heritability estimates in this study to decrease with age across sites, with the exception of the Jessievale, Amsterdam and Daspoort sites, where there was no apparent change in estimates with age. With regards to the other sites,

differences amongst ages were generally within the standard error of the estimates. Although a similar, if negligible trend was found in *Eucalyptus* hybrid populations in the Congo (Bouvet et al. 2009), this trend is in contrast to previous eucalypt studies in *E. nitens* (Greaves et al. 1997), *E. grandis* (Gapare et al. 2003) and *E. urophylla* (Wei and Borralho 1998, Kien et al. 2009), although older heritabilities in these *E. grandis* and second *E. urophylla* studies were found to have been inflated due to thinning of the trials. As none of the trials in this South African series were thinned during the rotation, being grown as pulpwood stands, the decline in heritability estimates is likely to have occurred at the time when the site was captured and competition between trees set in, resulting in a decrease in variation between trees and a related decrease in heritability. A marked increase in heritability estimates between early and older ages would decrease relative reliability of early selections (Harrand et al. 2009), thus this opposite trend, although unusual, is encouraging for early selection in these *E. nitens* populations. The within-family heritability (h^2_{wf}), representing the regression of an individual's true breeding value on the deviation of its phenotypic value from the family mean, is invariably lower than individual heritability for dbh (Cotterill and Dean, 1990), as was found in this study (**Table 2.6**). This statistic is relevant for estimating gains when selecting top individuals from within each family (Falconer and Mackay 1996).

Genotype by environment interaction (GEI)

Type B genetic correlations (r_{Bg}) between pairs of sites were high for dbh when provenance effect was ignored, generally ranging from 0.75 to 0.99 (**Table 2.7**). The exception to this was the correlation between Babanango and Woodstock (r_{Bg} of 0.33). A similar trend was found for correlations estimated using parameters for family nested within provenance, although there were not always sufficient degrees of freedom to successfully perform this calculation for all site pairs (**Table 2.7**).

Table 2.7 Type B correlation estimates (r_{Bg}) of *Eucalyptus nitens* provenance/progeny trials at eight sites in South Africa, for all possible site pairs for diameter at breast height (dbh) (and height in brackets) at final measurement age. Results for families nested within provenance are presented above the diagonal and families excluding provenance effects below the diagonal

Site	Jessievale	Amster-dam	Daspoort	Helvetia	Baba-nango	Wood-stock	Goede-hoop	Arthur's Seat
Jessievale	-	0.98	*	*	*	*	**	**
Amsterdam	0.95	-	*	*	*	0.68	**	**
Daspoort	0.99	*	-	0.88	0.84	*	**	**
Helvetia	0.99	0.75	0.87	-	0.94	*	**	**
Babanango	*	*	0.84	0.94	-	0.59	**	**
Woodstock	*	0.68	*	*	0.33	-	**	**
Goedehoop	**	**	**	**	**	**	-	0.89 (0.60)
Arthur's Seat	**	**	**	**	**	**	0.80 (0.70)	-

* no variance components for the site combination
** not enough families in common for GEI

An r_{Bg} of 1.00 would indicate a perfect correlation between the behaviours of genotypes on both sites and would suggest the complete absence of GEI. The high Type B correlations estimated for most site pairs indicated very little, or no, GEI for dbh for the *E. nitens* genotypes tested over these sites. The low correlations at Babanago and Woodstock are below the level at which GEI may start to be of concern for the breeder (Shelbourne 1972). These two sites are very different from each other in terms of altitude, MAT and MAP, and the presence of GEI between these two sites would not be unexpected. However, as it is unlikely that *E. nitens* will continue to be grown commercially on site types similar to Babanango, it can be assumed that the performance of this breeding population will not be affected by GEI effects on sites in southern Mpumalanga and the Highveld in South Africa. At the two sites where heights were measured, Goedehoop and Arthur's Seat, the r_{Bg} was 0.70 for families across provenance and 0.60 for family nested within provenance.

Combined site analysis

The lack of GEI, except for the Woodstock-Babanango comparison, allowed for all sites in the E88/01 to E88/06 series to be analysed as one data set. The combined site analysis to determine overall performance of 71 common seedlots (numbers between 1 and 34840) across the range of five sites is presented in **Table 2.8**. There were significant differences ($p < 0.05$) between seedlots, the top seedlots generally being from Barrington Tops and Barren Mountain, supporting the findings of the provenance analyses (**Table 2.3**). Based on the performance of the seedlots in this analysis, top families and individuals within families were selected to form the next generation of progeny trials in *E. nitens.*

Table 2.8 Performance of *Eucalyptus nitens* seedlots (numbers 1 to 34840) across five sites in South Africa (Amsterdam, Jessievale, Helvetia, Daspoort and Babanango), as determined by final diameter at breast height (dbh) measurements. Treatment means which do not differ significantly from each other bear the same letter of the alphabet

Rank	Seedlot	Provenance	Dbh (cm)	Rank	Seedlot	Provenance	Dbh (cm)
1	34838	Barrington Tops	20.51 a	37	32089	Badja	16.91 def
2	32096	Barren Mountain	20.45 a	38	32083	Badja	16.77 def
3	34833	Barrington Tops	19.50 ab	39	31328	Tallaganda	16.73 def
4	34840[1]	Barrington Tops	19.45 ab	40	31334	Tallaganda	16.69 def
5	34831	Barrington Tops	19.19 abc	42	32078	Badja	16.69 def
6	34832[1]	Barrington Tops	19.17 abc	42	31329	Tallaganda	16.68 def
7	34835	Barrington Tops	18.94 abc	43	32084	Badja	16.66 def
8	34839	Barrington Tops	18.90 abc	44	26	Nelshoogte, SA	16.58 ef
9	32101	Barren Mountain	18.67 bc	45	32079[1]	Badja	16.55 ef
10	32099	Barren Mountain	18.63 bc	46	31327	Tallaganda	16.51 ef
11	32091	Badja	18.55 bcd	47	22	Nelshoogte, SA	16.36 ef
12	31339	Tallaganda	18.47 bcd	48	31335	Tallaganda	16.35 ef
13	32087	Badja	18.42 bcd	49	32086	Badja	16.33 ef
14	32100	Barren Mountain	18.41 bcd	50	32085	Badja	16.31 ef
15	32097[1]	Barren Mountain	18.38 bcd	51	25	Nelshoogte, SA	16.23 ef
16	34836	Barrington Tops	18.25 bcd	52	32076	Badja	16.21 ef
17	34815	Barrington Tops	18.08 bcd	53	31333	Tallaganda	16.20 ef
18	30	Nelshoogte, SA[2]	17.91 bcd	54	27	Nelshoogte, SA	16.18 ef
19	32093[1]	Barren Mountain	17.88 bcde	55	32090	Badja	16.02 ef
20	27832	Tallaganda	17.84 bcde	56	31189	Penny Saddle	15.99 ef
21	32095	Barren Mountain	17.82 bcde	57	40	Belfast, SA	15.93 f
22	32092	Barren Mountain	17.74 bcde	58	28	Nelshoogte, SA	15.90 f
23	32094[1]	Barren Mountain	17.67 bcde	59	29	Nelshoogte, SA	15.88 f
24	31338	Tallaganda	17.45 cde	60	32081	Badja	15.79 f
25	34837	Barrington Tops	17.41 cde	61	32088	Badja	15.77 f
26	31332	Tallaganda	17.35 cde	62	42	Belfast, SA	15.76 f
27	31337	Tallaganda	17.33 cde	63	24	Nelshoogte, SA	15.69 f
28	34834	Barrington Tops	17.25 de	64	41	Belfast, SA	15.63 f
29	32082	Badja	17.24 de	65	32080	Badja	15.31 fg
30	32102	Barren Mountain	17.19 def	66	31188	Penny Saddle	13.88 gh
31	32119	Woodbush, SA	17.16 def	67	43	Belfast, SA	13.54 h
32	31331	Tallaganda	17.15 def	68	14	Jessievale, SA	12.63 hi
33	31330	Tallaganda	17.02 def	69	1	Jessievale, SA	11.60 ij
34	32098	Barren Mountain	16.96 def	70	2	Jessievale, SA	10.76 j
35	32077	Badja	16.95 def	71	13	Jessievale, SA	10.38 j
36	31336	Tallaganda	16.91 def				
Mean							17.17
SD[3]							4.802

[1] Some trees of these seedlots showed infection by *Teratosphaeria zuluense*, refer to **Table 2.5**,
[2] South Africa,
[3] Standard deviation

Juvenile-mature and trait genetic correlations

Table 2.9 presents genetic correlations (r_g) for a range of dbh and height measurements at different ages. Mid-rotation (five to six year) dbh measurements were not done at Jessievale and Amsterdam. The trial at Woodstock was converted to a seed orchard seven years after establishment, thus age-age correlations at more than seven years are not possible for the

latter site. As expected, the genetic correlations became stronger with decreasing differences between age of measurement. The mid-rotation dbh measurements (≈ six year) were highly correlated with the final dbh measurements at nine years (r_g > 0.90), and the earlier dbh measurements at ≈ three years were also positively correlated with the final dbh measurements (r_g > 0.72). This indicates that early selections for seed/vegetative production could be made as early as three years of age in *E. nitens*, although these authors would recommend making selections after four and a half years. These results concur with what has been found in previous *E. nitens* studies (r_g ≈ 0.99) (Greaves et al. 1997) and in other eucalypt species such as *E. grandis* (r_g ≈ 0.98) (Harrand et al. 2009) and *E. urophylla* (r_g ≈ 0.83) (Kien et al. 2009).

Table 2.9 Genetic correlations (below the diagonal) and Pearson's phenotypic correlations (above the diagonal) of early measurements of *Eucalyptus nitens* with a range of later measurements at eight trial sites in South Africa. All phenotypic correlations were significant for $p < 0.0001$

Age	Ht1	Dbh3	Dbh6	Dbh9	Ht9
Ht1	-	-	Das 0.63 Bab 0.34	Das 0.59 Hel 0.47	-
Dbh3	Das 0.94 ± 0.016 Bab 0.73 ± 0.012	-	Das 0.84 Hel 0.65 Woo 0.88 Bab 0.76 Goe 0.85 Art 0.88	Jes 0.80 Ams 0.75 Das 0.80 Hel 0.59 Bab 0.72 Goe 0.80 Art 0.80	Goe 0.66 Art 0.62
Dbh6	Das 0.79 ± 0.021 Hel 0.44 ± 0.025 Bab 0.54 ± 0.018	Woo 0.83 ± 0.004	-	Das 0.96 Bab 0.97 Goe 0.93 Art 0.90	-
Dbh9	Das 0.80 ± 0.019 Hel 0.26 ± 0.022 Bab 0.43 ± 0.020	Jes 0.79 ± 0.006 Ams 0.94 ± 0.002 Das 0.93 ± 0.006 Hel 0.75 ± 0.007 Bab 0.73 ± 0.009 Goe 0.83 ± 0.006 Art 0.83 ± 0.007	Das 1.00 ± 0.000 Hel 0.95 ± 0.002 Bab 0.98 ± 0.001 Goe 0.93 ± 0.002 Art 0.91 ± 0.003	-	Goe 0.83 Art 0.82
Ht9	-	Goe 0.62 ± 0.018 Art 0.76 ± 0.014	Goe 0.72 ± 0.013 Art 0.81 ± 0.012	Goe 0.84 ± 0.009 Art 0.96 ± 0.003	-

Ht1 = height at 12 to 17 months,
Dbh3 = dbh at 24 to 39 months,
Dbh6 = dbh at 52 to 76 months,
Dbh9 = dbh at 94 to 113 months,
Ht9 = height at 113 months,
Das = Daspoort,
Bab = Babanango,
Hel = Helvetia,
Woo = Woodstock,
Jes = Jessievale,
Ams = Amsterdam,
Goe = Goedehoop,
Art = Arthur's Seat

With regards to trait genetic correlations, the early height measurements at 12 months were strongly correlated with the first dbh measurements at three years (r_g > 0.72), but the 12 month height measurements were less strongly correlated with the six year dbh measurements (r_g 0.44 - 0.79). As the six year and final dbh measurements were highly correlated with the final height measurements in the E88/07 trial series (r_g > 0.71 and > 0.83, respectively), it could be regarded as sufficient to make selections based solely on dbh for growth at six years, as this would represent both dbh and height at full rotation.

Predicted genetic gains

The predicted genetic gains from the trials, following roguing, thinning and bulking of the top 15 to 36 families from each trial, are described both as an increase in dbh (cm) and percentage improvement over the trial mean (**Table 2.10**). The predicted gains ranged from 0.29 cm (1.7%) increase in dbh at Helvetia at 94 months to 3.17 cm (20.7%) increase at Arthur's Seat at 113 months. It should be noted that, as the predicted gains are calculated using single-site genetic parameters, these might slightly overestimate genetic gain on future sites, as they do not account for GEI. However, the negligible GEI effects in this study suggest this may not be worth pursuing further. Due to the low selection intensity in the trials with the larger number of families, highest genetic gains will be achieved by using seed obtained from the E88/07 trials at Goedehoop and Arthur's Seat and the E88/05 trial at Babanango. It should be noted however, that the predicted gains for Helvetia and Woodstock are for 94 and 76 months, respectively, and would be expected to increase with age.

Table 2.10 Predicted gains for diameter at breast height (dbh) (cm) in the next generation of *Eucalyptus nitens* from eight provenance/progeny trials in South Africa. Selection intensity between families (SI_b) comprises two parts which are used to predict gains incrementally i.e., i) accounts for selection due to 30% roguing and ii) accounts for female selection when bulking the top 15 to 36 families of the remaining families

Trial sites: age at measurement[1]	$h^2_{fam(prov)}$ ± SE	Selection Intensity between families (SI_b)		Selection Intensity within families (SI_w)	Predicted gain in dbh (cm) (% gain)
		i) For 30% roguing	ii) For bulk composition (nos. in bulk)		
E88/01 Jessievale: 101 months	0.05 ± 0.022	0.466	0.777 (top 15)	1.539	0.99 (6.2%)
E88/01 Amsterdam: 101 months	0.14 ± 0.055	0.493	0.216 (top 36[2])	1.539	1.25 (7.4%)
E88/03 Daspoort: 113 months	0.13 ± 0.045	0.497	0.826 (top 16)	1.267	2.05 (11.4%)
E88/03 Helvetia: 94 months	0.01 ± 0.026	0.497	0.826 (top 16)	1.267	0.29 (1.7%)
E88/05 Babanango: 112 months	0.18 ± 0.040	0.474	1.536 (top 15)	1.163	2.52 (14.0%)
E88/06 Woodstock: 76 months	0.14 ± 0.032	0.474	1.536 (top 15)	1.267	1.55 (10.7%)
E88/07 Goedehoop: 113 months	0.22 ± 0.055	0.492	1.128 (top 20)	1.424	3.07 (17.1%)
E88/07 Arthur's Seat: 113 months	0.27 ± 0.060	0.492	1.128 (top 20)	1.424	3.17 (20.7%)

$h^2_{fam(prov)}$ = heritability estimates of individual values (narrow-sense) for family nested within provenance,
SE = Standard error of h^2,
[1] Different ages at time of measurement should be noted
[2] Poor families were not rogued from this seed orchard before seed was collected

With regards to trial series, the progeny of the E88/07 series should produce the best growth of the five series. The gains predicted from these trials are low to intermediate, but additional gains should be recognised in terms of improved survival/stocking, as is common with improved material. The predicted gains were calculated using the conservative family within provenance heritabilities, as provenance could otherwise inflate estimates if not accounted for. Family selection intensities were used, disregarding nesting within provenances.

Once actual gains have been measured in progeny trials established from seed collected from these trials, realised heritabilities can be calculated, and it will then be possible to determine whether the estimation of genetic parameters for families nested within provenance was appropriate, or whether the provenance effect should have been ignored.

Conclusions

This study has been invaluable in providing 1st generation benchmarking information on growth, genetic stability across sites, genetic parameters and potential gains in this population of *E. nitens*. Provenance testing at the eight sites has indicated that the more northerly NSW *E. nitens* provenances of Barren Mountain and Barrington Tops are distinctly

better suited to growth in the South African summer rainfall region than the southern NSW provenances of Tallaganda and Glenbog, and the Victorian provenances, Penny Saddle and Bendoc. Survival appears to have played a role in provenance performance, as those provenances with better survival at final measurement generally performed better than those with lower survival. Material from Penny Saddle provenance is not recommended for establishment in the summer rainfall areas of South Africa due to its poor performance in most trials, probably due to early susceptibility of the juvenile leaves to *Mycosphaerella* leaf blotch disease.

Provenance differences also exist for tolerance to *T. zuluense* (formerly *Coniothyrium* canker), with Badja and Tallaganda appearing to be the provenances least affected by the disease overall. Generally however, the species was not badly affected by the disease at the four sites assessed and the few seedlots which were affected should be removed from existing seed orchards and excluded from selections.

Analyses of the final measurements of the *E. nitens* provenance/progeny trials have identified the top performing seedlots at the eight different sites. The high Type B genetic correlations estimated for all sites pairs indicated very little, or no, GEI for dbh for the *E. nitens* genotypes tested in this study, which implies high genetic stability across sites. This lack of GEI allowed for a combined site analysis of 71 seedlots to be done, thus identifying the top performing seedlots across five sites. These top seedlots were generally from Barrington Tops and Barren Mountain, supporting the findings of the provenance analyses. Collection of seed from these seedlots will form a good base for progeny testing, although care should be taken to include families from other provenances in order to maintain a broad enough genetic base for advanced generation breeding.

The generally intermediate heritability estimates obtained for *E. nitens* in this study demonstrate that the species exhibits sufficient levels of additive variance for selections to result in progeny improved for diameter growth using conventional breeding strategies. High genetic juvenile-mature correlations of dbh measurements, at 52 to 62 months after establishment, with dbh measurements at 94 or 113 months, have shown that individual tree and family selections can be made as early as five or six years. This suggests that the breeding cycle could be decreased by at least three years, with selections based on growth traits being made for seed or vegetative production after the mid-rotation measurement. This would aid greatly in decreasing the time required to turn over generations in the breeding

programme, as well as in the production of improved commercial seed, as flowering in *E. nitens* is slow and erratic (Gardner 2003). Any time gained by early thinning of seed orchards to promote early flowering and subsequent seed production, or by early grafting of elite selections for establishment of Clonal Seed Orchards and seed production will greatly benefit the breeding programme and the South African Forestry Industry. Earlier juvenile-mature correlations, of three year dbh measurements with full-rotation measurements, are encouraging, and should be investigated further.

Genetic gains predicted from the progeny of these trials ranged according to site, with the highest gains predicted by using seed or vegetative material from the E88/07 trials at Goedehoop and Arthur's Seat and the E88/05 trial at Babanango. Measurement of actual gain achieved in the progeny of these trials will indicate whether the estimation of genetic parameters for families nested within provenance was appropriate in this base breeding population, or whether the provenance effect could be ignored. The realised heritabilities and genetic parameters of future generations will also help inform the presence/absence of non-additive effects between provenances, whether the co-efficient of relationship used was correct, and generally indicate whether there are strong digressions from the assumptions made in this benchmarking study.

In summary, this breeding population of *E. nitens* exhibits sufficient variation for dbh between and within seedlots, as well as sufficient levels of additive variance, for significant improvements to be possible using selection of top individuals in top seedlots/families, both within and across provenances. Earlier selections at mid-rotation will shorten the breeding cycle, partially overcoming one of the shortfalls of breeding in this species in South Africa, namely the reticent flowering and resultant delays in seed production of *E. nitens*. Due to this potentially shortened breeding cycle and the genetic stability of the species across sites, these selections and ensuing improved genetic material will result in gains made through the breeding programme being deployed more rapidly commercially.

Acknowledgements

The foresters and research staff at Komatiland Forests (Pty) Ltd, Mondi Ltd, Sappi Forests and York Timbers Ltd are gratefully acknowledged for their assistance with the establishment and long term maintenance of these trials, many of which have since become seed orchards. The anonymous reviewers of this manuscript are also acknowledged for their useful comments.

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CHAPTER 3[1]

A comparison of the effect of genetic improvement and seed source and seedling seed orchard variables on progeny growth in *Eucalyptus nitens* in South Africa

Tammy-L Swain[2]*, Steve D Verryn[3] and Mark D Laing[4]

[2] *Institute for Commercial Forestry Research, P.O. Box 100281, Scottsville, Pietermaritzburg 3209, South Africa.*

[3] *Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa and Creation Breeding Innovations, 75 Kafue St, Lynnwood Glen, 0081, South Africa.*

[4] *School of Agricultural Earth and Environmental Sciences (SAEES), University of KwaZulu-Natal, PO Box 375, Pietermaritzburg, 3201, South Africa.*

* Author for correspondence: T-L Swain

Telephone: +27 33 386 2314

Fax: +27 33 386 8905

Tammy.Swain@icfr.ukzn.ac.za

[1 **Published as:**
Swain T-L, Verryn SD, Laing MD. 2013. A comparison of the effect of genetic improvement and seed source and seedling seed orchard variables on progeny growth in *Eucalyptus nitens* in South Africa. *Tree Genetics and Genomes* 9(3): 767-778. DOI 100.1007/s11295-013-0593-0.
Formatting and referencing style has been retained as per Journal style.]

ABSTRACT

Eucalyptus nitens is an important forestry species grown for pulp and paper production in the temperate, summer rainfall regions of South Africa. A tree improvement programme has been ongoing at the Institute for Commercial Forestry Research for two decades, but genetic improvement in the species has been slow due to delayed and infrequent flowering and seed production. Three trials were established to firstly, quantify the gains that have been made in the first generation of improvement in the breeding programme; and secondly, establish whether a number of seed source and orchard variables influence the performance of the progeny. These variables are: the amount of flowering trees in the seed orchard, year of seed collection, seed orchard origin and composition of seed orchard seed bulks. Diameter at breast height and tree height were measured in the trials at between 87 and 97 months after establishment and timber volumes and survival were calculated. Improved seed orchard bulks performed significantly better ($p < 0.01$) than unimproved controls in the field trials. Genetic gains ranging from 23.2 to 164.8 m^3ha^{-1} were observed over the unimproved commercial seed. There were significant differences ($p < 0.01$) in progeny growth between the levels of seed orchard flowering, with higher levels of flowering (≥ 40 %) producing substantially greater progeny growth than lower flowering levels (≤ 20 %). The seed orchard had no effect on progeny growth in this trial series. This suggests that seed collected from any of the four seed orchards tested will produce trees with significant improvement in growth.

Keywords

E. nitens, genetic gain, tree improvement, breeding, flowering levels, seed bulk composition

INTRODUCTION

Eucalyptus nitens remains one of the most important commercial cold tolerant eucalypt (CTE) species currently grown for pulp and paper production in the summer rainfall regions of South Africa. Significant variation exists among the provenances grown in South Africa for growth (Swain et al. 1998; Gardner et al. 2003) and drought (Darrow 1996; Gardner 2001), frost and cold tolerance (Gardner 2001; Swain 2001); timing and abundance of flowering (Carlson et al. 2000; Jones 2002; Gardner and Bertling 2005); seed production (Swain and Chiappero 1998; Jones 2002) and pulping properties (Clarke 2000). This makes the species ideally suited to genetic improvement.

In South Africa, *E. nitens* grows optimally where the mean annual temperature (MAT) is greater than 14^{o}C and less than 16^{o}C (Swain and Gardner 2003). The species is classified as frost tolerant, but is not as hardy as *Eucalyptus macarthurii* (Darrow 1994; 1996), and is recognised as one of the most snow hardy of the CTEs grown in South Africa (Gardner and Swain 1996; Kunz and Gardner 2001). Currently, there is no alternative commercial species to *E. nitens* for sites prone to moderate frost and heavy snows.

The *E. nitens* populations grown commercially in South Africa originate from several provenances in New South Wales (NSW) in Australia, as provenance trials have shown that the material from Victoria in Australia does not perform well in the summer rainfall regions of South Africa (Swain et al. 1998). A breeding programme for *E. nitens* has been ongoing since the early 1980s, when the Institute for Commercial Forestry Research (ICFR) took over a series of provenance/progeny trials from the South African Department of Forestry. These trials tested a range of seedlots and provenances imported from Australia, with additional trials being established by the ICFR to assess new Australian seed imports at the end of the 1980s (Swain et al. 1998).

As vegetative propagation is difficult in *E. nitens* (de Little et al. 1992; Moncur 1998), open-pollinated seed orchards have been established for the production of improved seed. The reticent or shy flowering of *E. nitens* (Gardner 2003) has hindered the breeding programme and the production of improved seed, for plantation establishment. Globally, the species is known as a light and infrequent flowerer and produces small seed crops (Pound et al. 2003). In South Africa, the species often only becomes reproductively mature at 10 to 15 years of age if grown in a plantation situation (Eldridge et al. 1993; Gardner 2003) and requires winter chilling, or hormonal treatments to replace the chilling, if flowering is to occur earlier (Gardner and Bertling 2005). The use of open-pollinated seed orchards to turn over generations in conventional breeding is therefore slow and difficult, and can result in inconsistent commercial seed production. Shy flowering may also affect realised or actual gain, in that only certain families may be contributing as pollen parents, potentially causing differences from predicted gain. On the contrary, if different or additional families start flowering with each advancing year, gain may vary significantly on an annual basis. The mixed mating system of eucalypts, where outcrossing is preferential but selfing is not uncommon (Griffin et al. 1987; Sedgley et al. 1989), in conjunction with the erratic flowering of the species, may result in the open-pollinated seed orchards failing to produce consistently high quality seed.

A series of genetic gain trials was established in 2001, firstly, to quantify the gain that has been made in the first generation of improvement in *E. nitens* and, secondly, to establish whether there is any relationship between level of flowering in an orchard, family composition of the seed orchard bulk, the seedling seed orchard and the genetic gain in progeny derived from the ICFR's *E. nitens* advanced generation seedling seed orchards.

MATERIAL AND METHODS

Three genetic gain trials were established on temperate sites in KwaZulu-Natal (KZN) and Mpumalanga (MPU) in South Africa early in 2001, i.e. Balgowan, Amsterdam and Lothair. Details of the trial sites and trial designs are included in **Table 3.1**. All trials were planted at 1667 stems per hectare stocking (2 x 3 m), with four replicates of treatments or entries in square plots of 5 x 5 trees, and only the inner 9 trees (3 x 3) being measured in order to exclude inter-treatment/entry competition effects. Twenty-five to 28 entries, details of which are in **Table 3.2**, were included in the trials. The improved material originated from four ICFR seedling seed orchards, i.e. Amsterdam, Helvetia, Jaglust and Jessievale. These were former provenance/progeny trials that were thinned to seed orchards using a 30 % roguing of poor families and a thinning to the best tree per plot of remaining families. After roguing, there were only three common families across all four of these seed orchards which could potentially act as pollen parents, and an additional six that were common to three of the orchards.

Table 3.1 Site and trial design details of three *E. nitens* genetic gain trials in South Africa

Plantation, Province	Date Planted	Latitude (°S)	Longitude (°E)	Altitude (m a.s.l.)	MAP[a] (mm)	MAT[b] (°C)	Soil depth (mm)	No. of entries	Design
Balgowan, KZN[c]	05/02/01	-29.4044	30.02417	1498	1002	15.3	1000-1200	28	5x6 unbal.[e] latt[f]
Amsterdam, MPU[d]	20/02/01	-26.5728	30.72778	1478	881	14.8	700	26	5x5 unbal. latt
Lothair, MPU	22/02/01	-26.4833	30.63333	1600	869	14.6	800	25	5x5 triple latt

[a] Mean Annual Precipitation [b] Mean Annual Temperature [c] KwaZulu-Natal [d] Mpumalanga
[e] unbalanced [f] lattice

In addition to comparing improved with unimproved material, entries included seed orchard bulks comprising a mix of the same mother families originating from different seedling seed orchards, i.e. approximate half sibs, to determine if seed orchard plays a role in progeny performance. Common seed/mother trees ranged from eight to 15 families, depending on bulk composition, the low number of common pollen parents allowing for potential variation between bulks to be expressed. All bulks from a specific seedling seed orchard were also combined in another comparison, irrespective of flowering level, to further examine the relationship between seed orchard and genetic gain. In order to establish whether there was a relationship between the number of trees flowering simultaneously in a seed orchard and progeny performance (i.e. assuming increased outcrossing with increased flowering, above a certain level of flowering), entries were included that comprised bulks of the same families, but which were collected in different years to represent different levels of flowering in the orchards. Flowering assessments were made in these orchards over three years to acquire the necessary flowering figures, which were obtained by totaling the number of flowering trees in a seed orchard and calculating these as a percentage of all

trees in the orchard. Lastly, bulks comprising different family combinations were included to determine whether this played a significant role in achieved gain being commercially deployed. Flowering over the period fell between 15 and 20 % or 40 and 47 %, and was thus categorised into these two levels (≤20 % and ≥40 %, respectively) for the purposes of this study. Details of the treatment/bulk compositions, selection intensity and grouped comparisons are included in **Tables 3.2** and **3.3**.

Table 3.2 Individual entry comparisons in *E. nitens* genetic gain trials at three sites

Entry no.	Origin and year seed collected (flowering percentage in previous year)	Entry/bulk composition	Level of female selection[a]
1	E88/01 Jessievale SO[b] A 1998 (15%)	27832 31332 31328 31337	8 top families from 42
2	E88/01 Jessievale SO A 1999 (40%)	31329 32101 31331 32098	8 top families from 42
3	E88/03 Helvetia SO B 2000 (44%)	32079 32093	8 top families from 49
4	E88/01 Jessievale SO B 1998 (15%)	32087 32095	8 top families from 42
9	E88/01 Jessievale SO B 2000 (45%)	32089 32097	8 top families from 42
10	E88/05 Jaglust SO B 2000 bulk (47%)	32090 32100	8 top families from 144
5	E88/01 Amsterdam SO C 1998 (20%)	Top 70% families	25 families from 34
6	E88/05 Jaglust SO D 1998 bulk (47%)	27832 31331 31338 32084 32087 32091 32092 32094 32095 32096 32097 32099 32100 32101 32102	15 top families from 144
7	E88/01 Jessievale SO D 1998 bulk (15%)		15 top families from 42
8	E88/03 Helvetia SO E 2000 (44%)	32087 32093 32095 32096 32100 34831 34832 34833 34834 34835 34836 34837 34838 34839 34840	15 top families from 49
11	E88/01 Jessievale SO 1998, top family	32097	
12	E88/01 Jessievale SO 2000, top family	32097	
13	E88/03 Helvetia SO 1999, top family	32097	
14	E88/03 Helvetia SO 2000, top family	32097	
16	E88/05 Jaglust SO 1998, top family	34832	
17	E88/03 Helvetia SO 2000, top family	34832	
18	E88/05 Jaglust SO 1998, top family	37232	
19	E88/05 Jaglust SO 1998, top family	37224	
20	Land race commercial bulk, ex Dorstbult SO, SA[c]	-	
21	Improved commercial bulk, ex Helvetia SO, SA	-	
22	Unimproved general bulk ex Australia	32083 32099 32091 32101 32092 34832 32093 34838 32096 37628	10 families
23	Unimproved average family ex Nelshoogte, SA	28	
24	Unimproved top family ex Badja, Australia	37232	
26	Unimproved top family ex Barren Mountain, Australia	32097	
27	Unimproved top family ex Barrington Tops, Australia	34832	
28	Unimproved local bulk *E. nitens* ex Perdestal, SA, 1989	-	
29	*E. grandis x nitens (GXN)* clone ex SA	-	
30	Controlled pollination seed ex SA	-	

[a] See text for level of male selection [b] Seedling seed orchard [c] South Africa

Table 3.3 Combination of entries for group comparisons in *E. nitens* genetic gain trials at three sites

Group comparison	Entries included	Group comparison	Entries included
i) Level of improvement		iv) Seed Orchard	
Unimproved	20, 21, 22, 28	Amsterdam	5
Improved	1 - 14	Helvetia	3, 8, 13, 14
ii) Flowering level		Jaglust	6, 10
≤ 20 %	1, 4, 5, 7	Jessievale	1, 2, 4, 7, 9, 11, 12
≥ 40 %	2, 3, 6, 8, 9, 10		
iii) Year of seed collection		v) Composition of Seed Orchard Bulk	
1998	1, 4, 5, 6, 7, 11	A	1, 2
1999	2, 13	B	3, 4, 9, 10
2000	3, 8, 9, 10, 12, 14	C	5
		D	6, 7

Measurements

Diameter at breast height (dbh) and tree height measurements were carried out at Lothair and Amsterdam at 87 months after establishment and at Balgowan at 97 months, which is just prior to full rotation for eucalypts grown on a pulp rotation in the temperate areas of South Africa. Formal stem form and disease assessments were not carried out because these traits were bred to the desired level in the first-generation trials (Swain et al. 1998). Individual-tree volume was calculated from these measurements using the equation developed by Schnöau (1982):

$\text{Log } V = b_0 + b_1 \log (D + \text{vald}) + b_2 \log H$

where V = total volume to 5 cm tip diameter in cubic decimetre, D = dbh in centimetre, H = total height in metre, b_0 = -2.17055, b_1 = 2.07516, vald = constant tree form value = 0, and b_2 = 1.42792. The assumption of constant tree form value throughout is satisfactory (Bredenkamp 2000). Total treatment/entry volumes per plot were calculated and then estimated per hectare, taking survival into account.

Statistical analysis

Statistical analysis was conducted using SAS® Institute, Inc., Software 9.2 (SAS 2002-2008). Dead or missing trees were removed from the dataset before analysis. To test for normality for dbh, height and volume, residuals were plotted against fitted values. None showed any detectable trends or patterns and it can therefore be said that the condition $\varepsilon_{ijkl} \sim \text{iid } (0,\sigma^2)$ were met for these data, and the standard ANOVA assumptions are valid. Analyses of variance for dbh, height, survival, individual-tree and total volumes were carried out for each site, as well as across sites, and F statistics were calculated to test for significant differences among entries. Proc GLM was used to calculate least squares means for dbh, height, survival, individual-tree and total volumes of each entry, as this procedure is recommended for unbalanced designs (Hettasch et al. 2007). Comparisons were made for differences between individual entries using Fisher's test for Least Significance Differences (LSD) at the 1 % significance level. Simple and partial phenotypic correlation statistics were estimated between traits using the combined site entry means.

In addition, individual entries were grouped, and statistical comparisons were made for levels of improvement, flowering levels, year of seed collection, seedling seed orchard origin and composition of seedling seed orchard bulk for all traits, across sites. Comparisons within the entry groups were made using pairwise t tests. The following model was used for the individual site, nine tree square plot analysis:

$y_{ijkl} = \mu + \text{rep}_i + \text{block}_j(\text{rep}_i) + \text{tmt}_k + \text{rep}_i*\text{tmt}_k + (\text{rep}_i*\text{tmt}_k) + \varepsilon_{ijkl}$

where y_{ijkl} = mean for the trait of the l^{th} tree in the i^{th} rep and k^{th} entry, μ = overall mean, rep = i^{th} rep effect (fixed), i = 1, …, 4; block = j^{th} block within i^{th} rep effect (fixed), j = 1,…, 5; tmt = k^{th} entry effect (random), k = 1,…, 14, 16,…, 25, 26 or 29; rep*tmt = interaction between the i^{th} rep and k^{th} entry (random plot effect); ε_{ijkl} = random error associated with i^{th} rep, j^{th} block within i^{th} rep, k^{th} entry and l^{th} tree where $\varepsilon_{ijkl} \sim$ iid $(0,\sigma^2)$.

The following model was used for the combined site analysis:

$y_{ijkl} = \mu + \text{site}_i + \text{rep}_j(\text{site}_i) + \text{tmt}_k + (\text{site}_i*\text{tmt}_k) + \varepsilon_{ijkl}$

where y_{ijkl} = mean for the trait of the l^{th} tree in the j^{th} rep and k^{th} entry at the i^{th} site; μ = overall mean; site = site effect (fixed), i = 1,…, 3; $\text{rep}_j(\text{site}_j)$ = j^{th} rep effect (fixed) within i^{th} site, j = 1,…, 4; tmt = k^{th} entry effect (random), k = 1,…, 14, 16,…, 25, 26 or 29; site*tmt = interaction between the i^{th} site and k^{th} entry (random); ε_{ijkl} = random error associated with i^{th} site, j^{th} rep within i^{th} site, k^{th} entry and l^{th} tree where $\varepsilon_{ijkl} \sim$ iid $(0,\sigma^2)$. Interactions between the grouped entries (i.e. level of improvement, flowering level, year of seed collection, seed orchard and bulk composition) were tested, and a regression analysis was performed on flowering levels with growth traits.

RESULTS AND DISCUSSION

Comparison of individual entries

Growth

Table 3.4 presents the final survival, individual-tree and total volume entry means for the three genetic gain trials. There were significant differences ($p < 0.01$) between entries for all traits at all three sites, and within-site replicate effects were significant for all traits except total volume. Block-within-replicate effects were not significant ($p > 0.01$) and replicate x entry interaction effects were only significant ($p < 0.01$) at Amsterdam for individual volume (details not shown). With regard to the combined site analysis, site effects were significant ($p < 0.001$) for all traits, but site x entry effects were only significant for total volume ($p < 0.001$) and survival ($p < 0.05$) (**Table 3.5**). **Table 3.4** also presents the across or combined site survival, individual and total volume entry means. Across all sites, top performing entries 11 (top improved family 32097 from Jessievale seed orchard) and 17 (top improved family 34832 from Helvetia seed orchard) significantly outperformed ($p < 0.05$) the majority of unimproved entries, the land-race commercial bulk (entry 20), the *E. grandis x nitens* (*GXN)* hybrid clone (entry 29) and the two first-generation top families from Jaglust (entries 18 and 19), for total volume. Entry 27 (unimproved top family 34832 ex Barrington Tops, Australia) was only present at two sites, but performed well overall, being the top entry in the combined site analysis for total volume. At first glance, this would seem to indicate that the more northernmost Australian provenance of Barrington Tops should be widely used in future breeding. Although this is supported by results of first-generation trials in South Africa (Swain et al. 2013), the northern provenance of Barren Mountain performed as well as Barrington Tops in the first-generation trials.

Table 3.4 Final percentage survival, total volume and individual-tree volume entry means, ranked for decreasing total volume, in three *E. nitens* genetic gain trials, and a combined site analysis

Balgowan (97 months)				Amsterdam (87 months)				Lothair (87 months)				Combined site analysis (87 months)			
Entry	Survival (%)	Total volume (m^3ha^{-1}) (p <0.05)	Indiv. tree volume[a] (m^3)	**Entry**	**Survival (%)**	**Total volume (m^3ha^{-1}) (p <0.05)**	**Indiv. tree volume[a] (m^3)**	**Entry**	**Survival (%)**	**Total volume (m^3ha^{-1})**	**Indiv. tree volume[a] (m^3)**	**Entry**	**Survival (%)**	**Total volume (m^3ha^{-1}) (p <0.001)**	**Indiv. tree volume[a] (m^3)**
17	93 a	404.8 a	0.262 a	17	63 bc	189.3 a	0.183 b	10	85 ab	240.2 a	0.164 ab	27	89 a	257.6 a	0.174 ab
11	83 ab	366.4 ab	0.264 a	13	75 a	189.2 a	0.151 b	5	81 ab	236.5 a	0.082 b	11	79 ab	252.5 a	0.195 ab
2	86 ab	331.2 ab	0.231 ab	8	72 ab	182.5 a	0.152 b	27	83 a	230.9 a	0.080 b	8	79 ab	249.7 a	0.195 ab
6	81 ab	322.7 ab	0.240 ab	1	78 a	179.1 ab	0.136 b	2	86 a	226.2 a	0.157 abc	17	70 abcde	241.7 ab	0.206 a
8	78 ab	321.9 ab	0.241 ab	11	67 b	173.0 abc	0.156 b	11	83 a	218.1 a	0.157 abc	2	76 abc	241.0 ab	0.186 ab
14	72 ab	308.7 ab	0.256 a	14	69 ab	169.8 abc	0.142 b	8	78 bc	209.9 ab	0.158 abc	5	73 abcd	226.6 ab	0.191 ab
9	89 ab	307.1 ab	0.207 ab	2	61 bc	165.6 abc	0.160 b	1	86 a	206.0 ab	0.144 bc	3	67 abcde	222.2 ab	0.178 ab
5	72 ab	296.0 ab	0.246 ab	3	75 a	160.2 abcd	0.127 b	12	81 ab	199.8 ab	0.149 bc	14	69 abcde	219.7 ab	0.185 ab
4	75 ab	290.6 ab	0.233 ab	9	58 bc	155.5 abcd	0.159 b	9	72 bc	191.0 ab	0.156 abc	6	74 abcd	218.8 ab	0.175 ab
1	82 ab	285.5 ab	0.211 ab	6	64 bc	153.7 abcd	0.144 b	17	64 bc	184.8 ab	0.172 ab	1	81 a	217.9 ab	0.160 abc
27	95 a	284.3 ab	0.181 ab	29	31 bc	148.1 abcde	0.290 a	14	72 bc	180.5 ab	0.149 bc	9	73 abcd	217.9 ab	0.179 ab
3	75 ab	284.1 ab	0.227 ab	5	61 bc	147.2 abcde	0.144 b	6	81 ab	180.0 ab	0.133 bc	13	68 abcde	216.4 ab	0.183 ab
16	64 ab	281.5 ab	0.264 a	10	61 bc	131.7 abcde	0.128 b	21	69 bc	178.2 ab	0.154 bc	10	72 abcde	213.2 ab	0.181 ab
12	78 ab	280.3 ab	0.216 ab	26	47 bc	122.8 abcde	0.154 b	7	75 bc	177.6 ab	0.145 bc	30	78 abc	200.9 abc	0.155 abc
10	70 ab	274.4 ab	0.237 ab	12	44 bc	112.3 abcde	0.159 b	20	78 bc	172.8 ab	0.142 bc	12	70 abcde	197.5 abc	0.175 ab
30	78 ab	262.8 ab	0.203 ab	21	50 bc	109.6 abcde	0.133 b	30	78 bc	169.9 ab	0.130 bc	7	73 abcde	181.9 abc	0.144 abc
7	76 ab	248.7 ab	0.182 ab	24	44 bc	105.3 abcde	0.150 b	29	42 c	155.9 ab	0.222 a	4	61 abcde	179.8 abc	0.166 abc
13	67 ab	243.5 ab	0.219 ab	4	67 b	103.2 abcde	0.092 b	28	75 bc	155.2 ab	0.121 bc	21	65 abcde	174.8 abc	0.163 abc
21	70 ab	215.9 ab	0.184 ab	7	67 b	102.6 abcde	0.094 b	22	75 bc	149.1 ab	0.118 bc	16	58 bcde	164.5 abc	0.178 ab
20	58 ab	210.2 ab	0.246 ab	20	56 bc	95.7 abcde	0.106 b	4	53 bc	145.6 ab	0.171 ab	20	57 cde	158.7 abc	0.154 abc
18	75 ab	209.3 ab	0.168 ab	22	36 cd	93.9 abcde	0.155 b	16	58 bc	129.5 ab	0.132 bc	22	62 abcde	144.3 abc	0.141 abc
23	67 ab	189.8 ab	0.171 ab	18	51 bc	93.6 abcde	0.106 b	24	50 bc	129.2 ab	0.162 abc	24	49 e	132.6 abc	0.151 abc
19	70 ab	188.8 ab	0.163 ab	16	44 bc	82.4 bcde	0.107 b	26	72 bc	118.7 ab	0.107 bc	29	51 e	130.8 abc	0.157 abc
22	67 ab	177.3 ab	0.160 ab	19	33 d	71.6 cde	0.129 b	23	50 bc	106.5 ab	0.120 bc	19	58 bcde	130.2 abc	0.152 abc
24	64 ab	163.4 ab	0.153 ab	23	39 cd	61.8 de	0.103 b	18	39 c	61.7 b	0.094 c	18	54 de	121.5 bc	0.133 bc
26	47 c	108.4 ab	0.138 ab	28	36 cd	49.5 e	0.083 b					26	54 de	116.2 bc	0.134 bc
29	72 ab	73.8 ab	0.061 b									23	50 e	114.9 bc	0.137 abc
28	30 c	38.4 b	0.078 ab									28	47 e	84.9 c	0.105 c
Trial mean	72.1	248.1	0.205		55.7	128.6	0.139		69.8	173.5	0.149		65.7	185.2	0.168
SD[b]	17.43	188.74	0.153		18.88	54.19	0.090		21.49	53.25	0.091		19.64	74.07	0.121

Values followed by the same letter of the alphabet within a column are not significantly different from each other (p > 0.01, unless indicated otherwise)

[a] Individual-tree volume [b] Standard deviation of entry means

Table 3.5 Analysis of variance for combined site percentage survival and growth, as well as growth within entry groups for the three genetic gain trials

	Trait	Source of variation	*df*	Mean Square	*F* value	*p* value	
Percentage survival	**Entry**	Site	2	8196.743	21.27	<0.0001	***
		Rep (site)	9	611.339	1.59	0.12	
		Entry	27	1165.615	3.02	<0.0001	***
		Site x entry	49	390.207	1.01	0.46	
		Error	213	385.403			
	Level of improvement (imp)	Site	2	4751.905	16.07	<0.0001	***
		Rep (site)	9	283.383	0.96	0.48	
		Improvement	1	7330.620	24.78	<0.0001	***
		Site x imp	2	1445.573	4.89	0.01	**
		Error	166	295.771			
Total volume	**Entry**	Site	2	354315.929	69.96	<0.0001	***
		Rep (site)	9	5559.452	1.10	0.37	
		Entry	27	25933.600	5.12	<0.0001	***
		Site x entry	49	7318.113	1.44	0.04	*
		Error	213	5064.653			
	Level of improvement (imp)	Site	2	62611.3621	12.44	<.0001	***
		Rep (site)	9	5708.6565	1.13	0.34	
		Improvement	1	207367.1799	41.19	<.0001	***
		Site x imp	2	29193.6384	5.80	0.004	**
		Error	166	5034.029			
	Flowering level (grouped ≤ 20 and ≥ 40 %)	Site	2	225973.664	56.22	<0.0001	***
		Rep (site)	9	6466.717	1.61	0.13	
		Flower	1	16351.056	4.07	0.05	*
		Error	102	4019.636			
	Year of seed collection	Site	2	305192.646	62.28	<0.0001	***
		Rep (site)	9	4718.353	0.96	0.48	
		Year	2	1929.537	0.39	0.68	
		Error	145	4900.134			
	Seed orchard	Site	2	304054.905	61.64	<0.0001	***
		Rep (site)	9	4735.810	0.96	0.48	
		Seed orchard	3	1350.865	0.27	0.84	
		Error	144	4932.819			
	Composition of bulk	Site	2	227622.635	57.42	<0.0001	***
		Rep (site)	9	6999.527	1.77	0.08	
		Bulk	4	8479.672	2.14	0.08	
		Error	99	3963.992			
Individual tree volume	**Entry**	Site	2	0.114	8.05	0.0006	***
		Rep (site)	9	0.010	0.74	0.67	
		Entry	27	0.015	1.10	0.36	
		Site x entry	45	0.016	1.10	0.35	
		Error	102	0.014			
	Level of improvement	Site	2	0.823	56.85	<0.0001	***
		Rep (site)	9	0.051	3.57	0.0002	***
		Improvement	1	0.240	16.58	<.0001	***
		Error	1145	0.014			
	Flowering level (grouped ≤ 20 and ≥ 40 %)	Site	2	0.596	42.90	<0.0001	***
		Rep (site)	9	0.033	2.38	0.01	*
		Flower	1	0.030	2.14	0.14	
		Error	751	0.014			
	Year of seed collection	Site	2	0.844	57.55	<0.0001	***
		Rep (site)	9	0.043	2.96	0.0017	**
		Year	2	0.008	0.51	0.60	
		Error	1035	0.015			
	Seed orchard (SO)	Site	2	0.091	7.31	0.0012	**
		Rep (site)	9	0.018	1.48	0.17	
		Seed orchard	3	0.012	0.97	0.41	
		Site x SO	6	0.033	2.68	0.02	*
		Error	88	0.012			
	Composition of bulk	Site	2	0.604	43.60	<0.0001	***
		Rep (site)	9	0.035	2.49	0.0082	*
		Bulk	4	0.024	1.73	0.14	
		Error	748	0.014			

df degrees of freedom; *, **, *** = significant at 5%, 1% and 0.1% levels of probability, respectively

With regards to poor performance, unimproved control entry 28 (unimproved South African *E. nitens* ex Perdestal) performed significantly worse ($p < 0.01$) than the majority of improved entries for most traits. The control-pollinated seed (entry 30) performed at or below the trial mean at the two sites where it was established. This performance may have been relatively poor, either because the seed was produced from early control-pollinated crosses, where the technique was still being established and the levels of contamination may have been high, or due to poor specific combining ability of the crosses.

Survival

It is notable that the ranking of many of the entries changed markedly once survival was taken into account, i.e. total volume per hectare was calculated with dead trees having a volume of zero. Survival differences were significant at varying levels at the individual sites, i.e. Balgowan ($p < 0.05$), Amsterdam ($p < 0.005$) and Lothair ($p < 0.1$), with no significant entry x replicate effects ($p > 0.1$) for the three sites (details not shown). In the combined site analysis, site effect was significant for survival ($p < 0.001$); yet the site x entry effect was non-significant ($p > 0.05$) **(Table 3.5)**. With the exception of entry 27 (unimproved top family 34832 ex Barrington Tops, Australia), survival or stocking of the improved entries was generally better than that of the unimproved and land-race material (**Table 3.4**). The *GXN* hybrid clone performed well below the trial average at Balgowan yet had good survival (72 %), and at Amsterdam, although survival was poor (31 %), individual-tree growth of surviving trees was significantly better ($p < 0.01$) than all other entries, as trees captured the open space around them. In contrast, entry 27 performed well at the two sites where it was planted, this performance being due in part to final survival of 95 and 83 %, respectively. All other unimproved entries (22, 23, 24, 26 and 28) had lower survival than most improved entries and low total volume, as if survival itself was behaving as a genetic trait. The positive impact of survival was expected after selection, as the previous generation of improvement focused on selection of trees that (1) had improved pest and/or disease tolerance, (2) were able to capture the site better, as measured by superior growth, and (3) had high survival. Selection for these traits has apparently resulted in increased stocking contributing significantly to the gain achieved through tree improvement.

Simple correlations (r) between survival, dbh and height, as well as partial correlations between survival and total volume, are presented in **Table 3.6**. These indicate that the correlation between survival and height ($r = 0.77$) is greater than that of survival with dbh ($r = 0.65$), and that there is a positive correlation between dbh and height for this trial series ($r = 0.74$). This latter correlation is lower than that obtained in first-generation trials of *E. nitens* ($r \geq 0.82$), the material being related to that included in this genetic gain trial series (Swain et al. 2013). With regard to the partial correlations of total volume with dbh and height (total volume being dependent on both dbh and height), the higher r of 0.90 for total volume with height for constant dbh supported the stronger correlation between survival and height. Swain et al. (2013) present further trait and juvenile-mature correlations for the related first-generation material over a range of sites and trial series, as well as genetic parameters for the *E. nitens* population.

Table 3.6 Selected simple phenotypic correlations (below the diagonal) and partial correlations (above the diagonal) between traits for final measurements of the three genetic gain trials

Trait	Pearson's phenotypic trait correlations ($p \leq 0.0002$) dbh	Height	Survival	Total volume
dbh	-	-		
Height	0.74	-		
Survival	0.65	0.77	-	0.78 (For constant height) 0.90 (For constant dbh)
Total volume	-	-	0.91	-

Gains

These results indicate that improvement has been made through the first generation of selection in the ICFR breeding programme, with the average increase in total volume of improved over unimproved material being 62.3 % (**Tables 3.4** and **Table 3.7**). Gains that can be made by using seed orchard bulks originating from any of the four ICFR seedling seed orchards included in these trials range from 9.3 to 94.4 % in total volume depending on site and bulk used, and expressed as a percentage of the unimproved and land-race bulk means, respectively (Shelbourne 1970). There were no significant differences ($p > 0.01$) between the improved bulks, although the bulk D from Jessievale (entry 7 (15 % flowering)) performed below the mean for all traits for the combined site analysis, and similarly, bulk B from Jessievale (entry 4 (15 % flowering)) performed just below the mean for total volume and individual-tree volume (**Table 3.4**). Both commercial bulks i.e. the improved commercial bulk from Helvetia (entry 21) and the land-race commercial bulk from Dorstbult (entry 20), performed at the trial mean for dbh but were below the mean for volumes and height in the combined site analysis (**Table 3.4**). As there were no significant differences between the different *E. nitens* seed orchard bulks in this study, nor the individual top-performing families, the homogeneity of the various entries was investigated. This showed that the range of dbh was similar for all entries across all sites, in the range of 15 to 20 cm, with only two exception: *GXN* (entry 29) had a narrower range of variation of 9 cm, as would be expected from a clone, and the unimproved South African bulk from Perdestal (entry 28) had a narrow range in the lower dbh range. Although the literature provides many comparisons between unimproved and improved eucalypt seedlots, most of which show significant improvements of the bred material over the unimproved material, very few comparisons have been found that displayed significant differences between improved eucalypt open-pollinated seed orchard bulks of the same species and nominal level of improvement. This is supported by findings in previous *E. macarthurii* (Swain et al. 1999) and *E. nitens* genetic gain trials (Jones, pers. comm[1]) in South Africa and in *E. camaldulensis* genetic gain trials in India (Varghese et al. 2009), where bulks of the same nominal level of improvement did not differ significantly from each other.

Although there were no significant differences ($p > 0.01$) between the improved seed orchard bulks, the yield improvement of these bulks over the unimproved controls varied markedly according to which bulk

[1] Jones W (2010) Shaw Research Centre, Tweedie, PO Box 473, Howick, 3290, SOUTH AFRICA.

was used in the comparison. The improved commercial bulk E from Helvetia (entry 8, 44 % flowering) produced an average of 91.0 and 164.8 m^3ha^{-1} more than the land-race commercial bulk from Dorstbult (entry 20) and the unimproved South African bulk from Perdestal (entry 28), respectively. By contrast, the bulk D from Jessievale (entry 7, 15 % flowering) produced only 23.2 and 97.0 m^3ha^{-1} more than the two controls, respectively.

Comparison of grouped entries

Tables 3.5 and **3.7** present comparisons of grouped entries for different levels of improvement, flowering, year of seed collection, seedling seed orchard and seed orchard bulk composition.

Table 3.7 Comparison of growth within entry groups in *E. nitens* genetic gain trials across all sites

	Grouping	Total volume (m^3ha^{-1})	dbh (cm)	Height (m)	Individual-tree volume (m^3tree^{-1})
Level of improvement	Improved	217.95 a	15.26 a	20.59 a	0.178 a
	Unimproved	114.61 b	13.60 b	18.33 b	0.125 b
Flowering percentage	≥ 40 %	227.62 a	15.50 a	20.77 a	0.183 a
	≤ 20 %	200.79 b	14.81 b	20.20 b	0.115 b
Year of seed collection[a]	1999	231.14 a	15.51 a	20.83 a	0.185 a
	2000	219.98 a	15.40 a	20.56 a	0.183 a
	1998	212.41 a	15.06 a	20.55 a	0.172 a
Seed orchard	Helvetia	228.51 a	15.60 a	20.59 a	0.187 a
	Amsterdam	226.57 a	15.77 a	20.60 a	0.190 a
	Jaglust	216.11 a	15.25 a	20.74 a	0.178 a
	Jessievale	212.20 a	15.03 a	20.55 a	0.172 a
Composition of seed orchard bulk[b]	E	249.69 a	15.78 a	20.48 a	0.195 a
	C	226.57 ab	15.78 a	20.61 a	0.191 ab
	A	229.95 ab	15.19 a	20.63 a	0.173 ab
	B	206.84 bc	15.23 a	20.60 a	0.176 ab
	D	199.60 bc	14.66 a	20.33 a	0.159 b

Values within an entry grouping within a column followed by the same letter of the alphabet are not significantly different from each other ($p > 0.05$)
[a] Refer to Table 4.2 for details of flowering in these years
[b] Refer to Table 4.2 for details of bulk composition
dbh = diameter at breast height

Levels of improvement

There were significant differences ($p < 0.01$) between the level of improvement for all traits (supporting the findings in **Tables 3.4** and **3.5**, and as detailed earlier).

Flowering level and outcrossing rate

Significant differences ($p < 0.01$) were found between flowering levels for all traits, with seed collected from seed orchards that had ≥ 40 % flowering producing progeny with significantly greater volume than seed that was collected from seed orchards with ≤ 20 % flowering (**Table 3.7**). It is unlikely that survival in the parent seed orchards would have affected flowering percentage, as the top 70 % of families were well represented in the seed orchards, despite subsequent poor flowering in a few of the orchards in some years. Mining of the survival data of the progeny for the different flowering levels did not show any

consistent resultant high or low survival for the ≥ 40 % or ≤ 20 % flowering entries, respectively, as these seemed to differ across site and with flowering level (**Tables 3.4** and **3.5**).

There is little research on the breeding system of *E. nitens*, but Moncur et al. (1995) estimated a 75% outcrossing rate in this species, and Pound et al. (2003) found that levels of self-incompatibility in *E. nitens* ranged from 25.8 to 93.6%. Self-pollination is definitely possible in *E. nitens* (Tibbits 1988), particularly in areas where the presence of natural pollinators is low, and pollen load is poor. This is despite selfing being controlled by a late–acting self-incompatibility system where ovule abortions occur after self-pollination (Pound et al. 2003), and resultant inbreeding depression has been reported in nine-year-old trees originating from controlled self-pollinations of *E. nitens* (Hardner and Tibbits 1998).

A regression analysis performed on the complete range of flowering levels for the four different growth traits indicated a slight significant positive trend ($p < 0.1$) between increasing levels of flowering and progeny tree growth for all traits except total volume. However, the R^2 values were very low for all traits, indicating a poor fit of the model, and no conclusions can be drawn from this analysis. A comparison of percentage improvement, as determined by flowering level, showed the following improvements in total volume over the 15 % flowering level: 40 % flowering (25.4 %), 45 % flowering (20.1 %), 20 % flowering (17.9 %) and 47 % flowering (12.0 %). The flowering levels happened to be specific to the design of each of the seed orchards that seed was collected from both in terms of family and final spacial distribution of parent trees; i.e. these were trials thinned to seed orchards based on family and individual performance and were not originally planted as seed orchards. This may partly explain the inconsistency of gain related to flowering level. Although a decrease in outcrossing rates has been linked to a decrease in progeny growth in forestry species (*E. nitens,* Hardner and Tibbits 1998; *Eucalyptus globulus,* Hardner and Potts 1995; Patterson et al. 2004; *Acacia mangium*, Butcher et al. 2004; Harwood et al. 2004), the flowering levels in this study do not necessarily represent the rate of outcrossing in the seed orchards, although the trends appear to be similar. Consequently, it could be assumed that an increase in flowering above a certain low level may result in increased gains in a population due to an increase in outcrossing rate, a decrease in selfing and subsequent decrease in inbreeding depression, but that additional flowering above this level may confer very little, if any benefit. Molecular studies would be necessary to determine the outcrossing rates in these seed orchards, and would be useful in understanding the link to gain.

Year of seed collection

The year of seed collection did not differ significantly ($p > 0.1$) (**Tables 3.5** and **3.7**).

Bulk composition

The composition of the seed orchard bulks differed in that bulk E performed significantly better ($p < 0.1$) than bulks D and B for total volume, and better than bulk D for individual-tree volume (**Tables 3.5** and **3.7**). Bulk E comprised a mix of 15 top-performing families where seed was collected in a year following ≥ 40 % flowering. By contrast, the poorer performing bulk D comprised two entries of 15 families representing the top 40 % of families during years of ≤ 20 % and ≥ 40 % flowering in two different seed

orchards, respectively. Bulk B comprised seven top and one average family in years following ≤ 20 % and ≥ 40 % flowering in three different seed orchards. Although this might imply that flowering level was influencing the bulk performance, bulk E did not perform significantly better than other bulks with low flowering levels, i.e. bulk A (15 and 40 %), bulk D (20 %).

Seed orchard

There were no significant differences ($p > 0.1$) in progeny growth based on seedling seed orchard. As not all seed orchards were represented by both high and low levels of flowering, which may have been biasing the data, the ≤ 20 % flowering levels were removed from a subsequent analysis so that only the higher flowering levels were represented in all seed orchards. This had no effect on significance, with seed orchard still showing no impact on progeny growth.

This could imply that, irrespective of flowering levels in these seed orchards, seed can be utilised from any of these four seed orchards to achieve the same appreciable level of gain and production in commercial plantations. This is similar to what was found in *Pinus taeda* (Sluder 1988). Unfortunately there were insufficient degrees of freedom for the seed orchard x flowering level interaction to be tested in the current study, which may have further informed this finding. Although the seed orchard x bulk interaction was not significant ($p > 0.05$) for dbh or volume, certain combinations of flowering level, bulk composition and seedling seed orchard resulted in marked differences in progeny growth, as discussed earlier (**Table 3.4**). Caution should thus be exercised when compiling bulks from seedling seed orchards with low flowering levels in any given year. It may be necessary to ensure that certain maternal families that produce high-yielding progeny are included in these, or all, seedling seed orchard bulks.

To this end, a study on the mating system of this population of *E. nitens* should be carried out to determine how many individuals or families are involved in pollination in these *E. nitens* seed orchards, the levels of outcrossing and how much self-incompatibility varies with genotype. This will add to an understanding of the degree of selfing and outcrossing which is occurring in the seed orchards and the effect on the genetic quality of the seed.

CONCLUSIONS

Significant improvements have been made over the first generation of selection in the ICFR *E. nitens* breeding population. It is therefore recommended that seed from any of the ICFR improved bulks be accessed for commercial deployment when available, rather than using unimproved or land-race material from Australia and South Africa, respectively.

Improvement in survival of the advanced-generation material plays an important role in the gains in total volume per hectare achieved. In addition, indications are that levels of flowering have an impact on progeny growth. These results suggest that seed orchards with 15 % flowering result in poorer progeny growth than those with ≥ 40 % flowering, although this is not consistent and it is thus difficult to draw any definite conclusions in this regard. Indications are that flowering above a certain low level may result

in increased gains in a population due to a decrease in selfing or related crosses, but that additional flowering above this level may confer very little, if any benefit. Further investigation of flowering levels should be carried out with larger numbers of observations per flowering level. Until then, it is recommended that seed should be collected, where possible, from seed orchards where 40 % or more flowering was observed in the previous year. This is supported by substantial percentage improvement in total volume of the progeny, generally being more than 20 % (and $p < 0.05$) at these higher levels of flowering.

The orchard from which the seed is collected appears to have no effect on progeny growth in this trial series, irrespective of flowering levels. This suggests that seed collected from any of the four ICFR seedling seed orchards tested in the trial series will produce trees with significant improvement in growth over the unimproved and commercial material. It should however be noted that certain combinations of seedling seed orchard and bulk composition, particularly at the lower levels of flowering, produced much better progeny growth than others, even if this difference is not statistically significant. It is thus recommended that such higher yielding bulk and seedling seed orchard combinations be used for commercial deployment. This will impact on management of ICFR seed orchards and future seed bulk composition.

Molecular studies in the *E. nitens* seed orchards will provide a better understanding of selfing and outcrossing in this breeding population. This, in turn, will allow for manipulation of current and future seed orchards to ensure that maximum gains are captured in the seed for commercial deployment.

Acknowledgements

The foresters and research staff at Mondi Group, NCT Forestry Co-Operative Ltd. and Sappi Forests are gratefully acknowledged for their assistance with the establishment and maintenance of these trials. Thank you to the Technical Staff in the ICFR Tree Improvement Programme for the establishment and measurement of these trials, and to the reviewers of this document for their constructive comments. We are also grateful to the two reviewers and associate editor who made important contributions to the improvement of this manuscript.

Conflict of interest

The authors declare that the experiments described in this research paper comply with the current laws of South Africa and that there is no conflict of interest between authors.

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CHAPTER 4

Realised genetic gains for growth in *Eucalyptus nitens* in South Africa

T-L Swain[1]*, SD Verryn[2], MD Laing[3]

[1] *Institute for Commercial Forestry Research, P.O. Box 100281, Scottsville, Pietermaritzburg 3209, South Africa.*

[2] *Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa and Creation Breeding Innovations, 75 Kafue St, Lynnwood Glen, 0081, South Africa.*

[3] *School of Agricultural Earth and Environmental Sciences (SAEES), University of KwaZulu-Natal, PO Box X01, Scottsville, 3209, South Africa.*

* Author for correspondence: T-L Swain

Telephone: +27 33 386 2314

Fax: +27 33 386 8905

Tammy.Swain@icfr.ukzn.ac.za

Summary

Analysis of two progeny and three genetic gain trials has provided a better understanding of the factors affecting genetic gain in the breeding of a *Eucalyptus nitens* population in South Africa. Significant increases in volume per hectare have been achieved over the first generation of improvement, ranging from 43.9 to 63.5% per seed orchard, with seed derived from a range of seed orchards. Genetic gains can be increased further by collecting seed from only the top 20 to 30% of families in a seed orchard, rather than the top 70%. Realised gains for diameter at breast height at 87 months were close to the predicted values and ranged from 1.02 cm to 1.90 cm. Two exceptions were the sites at Helvetia and Babanango, where gains were under- and over-predicted, respectively. Realised heritabilities, which are related directly to the realised gain and the actual selection intensities used in the seed orchards, reflected this trend, with the Helvetia site having a predicted heritability estimate of 0.01 and a realised heritability estimate of 0.15, and Babanango having a predicted heritability of 0.18 and a realised heritability of 0.12.

Both the grand-maternal provenance origin and F1 maternal effects were significant in the F2 trials. A Type B genetic correlation of 0.61 indicated the likely presence of a genotype by environment interaction between the two F2 sites. A low narrow sense heritability estimate of 0.06 for diameter at 87 months at the F2 site at In de Diepte indicated that emphasis should be placed on family information rather than individual selections at this site. A heritability estimate of 0.17 at the second site, however, indicated that further improvement is possible in this population of *E. nitens*.

Keywords

E. nitens, genetic parameters, advanced generation breeding, progeny trials, tree improvement, genotype by environment interaction

Introduction

Eucalyptus nitens is an important cold tolerant eucalypt (CTE) species grown commercially in the summer rainfall regions of South Africa. The species was originally grown for the production of mining timber in temperate, high altitude areas of the summer rainfall region due to its frost and snow tolerance, rapid growth and suitable wood properties. Suitability of *E. nitens* for bleached hardwood pulp and paper manufacturing further increased plantings in the early 1980s. *Eucalyptus nitens* is recognised as the most snow tolerant of the CTEs grown in South Africa, and has clearly demonstrated good resistance to damage by all but the heaviest of snowfalls (Gardner and Swain 1996, Kunz and Gardner 2001). Currently, there is no suitable alternative commercial eucalypt species to *E. nitens* in South Africa for sites prone to heavy snowfalls.

The improved *E. nitens* populations grown commercially in South Africa originate from several provenances in New South Wales (NSW) in Australia. Several provenance trials have shown that the material from Victoria in Australia does not perform well in the summer rainfall regions of South Africa. Thus the majority of material included in breeding programmes is from NSW, i.e., Barren Mountain, Ebor, Barrington Tops, Tallaganda, Badja and Glenbog provenances. Significant variation exists among these provenances tested in South Africa for growth (Swain et al. 1998, Gardner et al. 2003), frost and cold (Gardner 2001, Swain 2001), and drought tolerance (Darrow 1996, Gardner 2001), as well as for flowering (Carlson et al. 2000, Jones 2002, Gardner and Bertling 2005) and seed production (Swain and Chiappero 1998, Jones 2002). This genetic diversity provides potential for breeding for improved performance of the species in South Africa. Provenance differences also exist for pulp properties (Clarke 2000) and, as a species, *E. nitens* is recognised as having good kraft (Clarke 2000) and dissolving (Clarke 1995, Clarke et al. 1999) pulp yields in South Africa.

The current Institute for Commercial Forestry Research (ICFR) *E. nitens* breeding population originates from eight F1 provenance-progeny trials and one Breeding Seed Orchard (BSO) comprising seedlots imported from Australia that were established during the 1980s. Six of the trials were subsequently converted to BSOs, following final measurements in the trials (Swain et al. 2013b). The narrow sense heritability coefficients obtained for the F1 *E. nitens* population indicated that there is sufficient potential for breeding for increased diameter growth, and genetic gains were predicted for the F2 (Swain et al. 2013b). Although the F1

genetic parameters were estimated for families nested within provenance, to remove provenance effects (Tibbits and Hodge 1998), these differed markedly compared to those obtained when provenance effects were not included in the model (Swain et al. 2013b).

Continued recurrent selection in the *E. nitens* breeding programme included a series of F2 progeny trials and genetic gain trials, established with seed collected from the F1 BSOs. These trials allowed a comparison of the estimated and realised gains which can be used to evaluate the importance of provenance effects in estimates of F1 heritability.

This paper reports on the predicted gains and heritabilities versus their realised values, from three *E. nitens* BSOs, the role of provenance and genotype by environment interaction in the F2 population, as well as genetic parameters for the F2.

Material and methods

Field design

Seed collections were made in three ICFR F1 *E. nitens* open-pollinated BSOs during 1998, i.e., Jessievale, Jaglust and Helvetia. **Table 4.1** provides details of the three BSOs, which comprised the best tree per plot of the top 70% of families, based on the family means of the paired site analyses of the F1 trials (Swain et al. 2013b). However, due to delays in onset of flowering and limited seed production from many of the *E. nitens* families at these sites, seed was collected only from those trees and families that had flowered and produced seed by 1998. In some cases, seed was collected from the same F1 family in more than one BSO, resulting in more F2 families than F1 maternal families. The seed was used to establish a first progeny trial series (F2) at two sites, namely In de Diepte near Sabie (Mpumalanga (MPU)), and Mt Gilboa near Howick (KwaZulu-Natal (KZN)), early in 1999. Both trials were planted at 1667 stems per hectare (2 m x 3 m), in single row plots of six trees, with four replicates laid out in a rectangular lattice design (**Table 4.1**). Eighty F2 families and 10 controls were included in both trials and, of the F2 families, nine originated from the same grandmother, but from different maternal trees in two separate BSOs. **Table 4.2** provides a summary of the families included in the trials, grouped according to grand-maternal (provenance) origin, their BSO and F1 family origin, as well as descriptions of the controls. The controls comprised two improved ICFR bulk seedlots, two unimproved seedlots from Australia, two improved seed orchard bulks from New Zealand and three F1 commercial bulks supplying the South African Forestry Industry with seed at the time of trial

establishment. An *E. grandis x E. nitens* hybrid clone (*GxN*) was included at In de Diepte, but not at Mt Gilboa, due to insufficient numbers of cuttings at time of establishment.

Table 4.1 Site and trial design details of a) three F1 *Eucalyptus nitens* Breeding Seed Orchards (BSOs) in South Africa from which seed was collected for establishment of b) two F2 trials in South Africa

Locality	Planting date	Latitude (S)	Longitude (E)	Altitude (m a.s.l.)	MAP[1] (mm)	MAT[2] (°C)	Soil depth (mm)	Original no. of seedlots (nos. remaining after roguing)	Design
a) F1 BSOs[3]:									
Jessievale (MPU[4])	08/12/1982	-26.255562	30.524858	1706	873	14.5	850	42 (32)	6x7 lattice, 10 reps[5], now 1 tree/plot
Helvetia (MPU)	19/03/1985	-25.569012	30.306615	1646	789	15.6	1000	49 (37)	7x7 lattice, 8 reps, now 1 tree/plot
Jaglust (MPU)	21/01/1988	-26.163714	30.428449	1737	820	14.9	850	144 (107)	2(7x8) lattice, 9 reps, now 1 tree/plot
b) F2 trials:									
In de Diepte, Sabie (MPU)	02/02/1999	-25.039436	30.693807	1859	1010	14.6	500-600	90	9x10 lattice, 4 reps, 6 trees/plot
Mt. Gilboa, Howick (KZN[6])	27/01/1999	-29.248482	30.296161	1550	940	15.2	>900	90	9x10 lattice, 4 reps, 6 trees/plot

[1] Mean Annual Precipitation,
[2] Mean Annual Temperature,
[3] Breeding Seed Orchard,
[4] Mpumalanga,
[5] replicates,
[6] KwaZulu-Natal

Table 4.2 Summary of F2 families grouped by a) grand-maternal (provenance) origin, b) F1 Breeding Seed Orchard (BSO) origin and, c) F1 family origin; and description of controls included in *Eucalyptus nitens* progeny trials at two sites in South Africa

F2 families grouped by:

a) Grand-maternal (provenance) origin

Grand-maternal provenance	Number of F2 families	
	In de Diepte	Mt Gilboa
Badja	14	12
Barren Mountain	19	19
Barrington Tops	5	5
Bendoc	1	1
Ebor	8	8
Glenbog	1	1
Nelshoogte SA[2]	18	21
Tallaganda	1	1
Woodbush SA		

b) Breeding Seed Orchard origin

F1 BSO[1] origin	Number of F2 families	% flowering individuals in orchard (% families flowering)
Helvetia	5	26 (21)
Jaglust	46	47 (63)
Jessievale	29	15 (60)

c) F1 family origin

F2 treatment no.[3]	F1 maternal family no.	Grand-maternal (provenance) origin	BSO[1] of origin	F2 treatment no.	F1 maternal family no.	Grand-maternal (provenance) origin	BSO of origin
1	30	Nelshoogte, SA[2]	Helvetia, plot 34	47	34840	Barrington Tops	Helvetia, plot 20
2	27832	Tallaganda	Jessievale, plot 6	48	37198	Glenbog	Jaglust, plot 312
3	31327	Tallaganda	Jessievale, plot 24	49	37199	Glenbog	Jaglust, plot 411
4	31328	Tallaganda	Jessievale, plot 21	50	37203	Glenbog	Jaglust, plot 50
5	31329	Tallaganda	Jessievale, plot 377	51	37204	Glenbog	Jaglust, plot 199
6	31330	Tallaganda	Jessievale, plot 230	52	37206	Glenbog	Jaglust, plot 20
7	31331	Tallaganda	Jessievale, plot 20	53	37209	Glenbog	Jaglust, plot 109
8	31332	Tallaganda	Jessievale, plot 66	54	37212	Glenbog	Jaglust, plot 194
9	31334	Tallaganda	Jessievale, plot 46	55	37216	Glenbog	Jaglust, plot 35
10	31338	Tallaganda	Jessievale, plot 101	56	37218	Glenbog	Jaglust, plot 359
11	31336	Tallaganda	Jessievale, plot 283	57	37223	Glenbog	Jaglust, plot 363
13	32077	Badja	Jessievale, plot 181	58	37224	Glenbog	Jaglust, plot 156
14	32078	Badja	Jessievale, plot 402	60	37229	Badja	Jaglust, plot 24
15	32079	Badja	Jessievale, plot 41	61	37232	Badja	Jaglust, plot 290
16	32082	Badja	Jessievale, plot 81	67	37255	Tallaganda	Jaglust, plot 193
17	32083	Badja	Jessievale, plot 65	68	37257	Tallaganda	Jaglust, plot 98
18	32087	Badja	Jessievale, plot 353	69	37258	Tallaganda	Jaglust, plot 50
19	32087	Badja	Jaglust, plot 210	71	37266	Tallaganda	Jaglust, plot 18
21	32091	Badja	Jessievale, plot 61	72	37271	Tallaganda	Jaglust, plot 174
22	32092	Barren Mountain	Jessievale, plot 15	74	37278	Tallaganda	Jaglust, plot 384
23	32092	Barren Mountain	Jaglust, plot 137	76	37609	Tallaganda	Jaglust, plot 366
24	32093	Barren Mountain	Jessievale, plot 68	77	37612	Tallaganda	Jaglust, plot 144
25	32094	Barren Mountain	Jessievale, plot 12	78	37613	Tallaganda	Jaglust, plot 124
26	32094	Barren Mountain	Jaglust, plot 130	79	37615	Badja	Jaglust, plot 278
27	32095	Barren Mountain	Jessievale, plot 326	80	37620	Badja	Jaglust, plot 143
28	32095	Barren Mountain	Jaglust, plot 137	81	37622	Glenbog	Jaglust, plot 348
29	32096	Barren Mountain	Jessievale, plot 120	82	37627	Glenbog	Jaglust, plot 210
30	32096	Barren Mountain	Jaglust, plot 226	83	37628	Glenbog	Jaglust, plot 170
31	32097	Barren Mountain	Jessievale, plot 58	84	37634	Bendoc	Jaglust, plot 410
32	32097	Barren Mountain	Jaglust, plot 30	85	37644	Ebor	Jaglust, plot 9
33	32098	Barren Mountain	Jessievale, plot 408	86	37649	Ebor	Jaglust, plot 193
34	32098	Barren Mountain	Jaglust, plot 56	87	37651	Ebor	Jaglust, plot 140
35	32099	Barren Mountain	Jessievale, plot 37	88	37652	Ebor	Jaglust, plot 209
36	32100	Barren Mountain	Jessievale, plot 62	89	37653	Ebor	Jaglust, plot 87
37	32100	Barren Mountain	Jaglust, plot 186	90	37654	Ebor	Jaglust, plot 155
38	32101	Barren Mountain	Jessievale, plot 125	91	37655	Ebor	Jaglust, plot 12
39	32102	Barren Mountain	Jessievale, plot 69	92	37656	Ebor	Jaglust, plot 32
40	32102	Barren Mountain	Jaglust, plot 63				
41	32119	Woodbush, SA	Jessievale, plot 343				
42	34831	Barrington Tops	Helvetia, plot 1				
43	34832	Barrington Tops	Jaglust, plot 206				
44	34833	Barrington Tops	Helvetia, plot 3				
45	34835	Barrington Tops	Helvetia, plot 50				

Table 4.2 (cont)

Controls: F2 treatment no.	Name	Origin
46	*E. grandis x E. nitens* hybrid	*GxN* ex SA[2] (only included at In de Diepte)
70	*E. nitens* F1	Average-performing F1 family ex Australia - 37263
93	*E. nitens* F2 bulk	F2 bulk ex Jaglust BSO[1], SA (top 70 % families)
94	*E. nitens* F2 bulk	F2 bulk ex Jessievale BSO, SA (top 70 % families)
95	*E. nitens* F1 bulk	Bulk of F1 families ex Australian parents
96	Select A: *E. nitens* F1	F1 select ex Kalmoesfontein, SA
97	Commercial A: *E. nitens* F1	Commercial – F1 ex Dorsbult SO[4], SA
98	Select B: *E. nitens* F1	F1 select ex Mpumalanga , SA
99	*E. nitens* bulk	New Zealand improved SO Barrington Tops ex P Davey
100	*E. nitens* bulk	New Zealand improved SO Errinundra ex P Davey

[1] Breeding Seed Orchard,
[2] South Africa,
[3] Number,
[4] Seed Orchard

Data collection and statistical analysis

Diameter at breast height (dbh) measurements were carried out in both trials at 87 months after trial establishment. Stem form and disease assessments were not done because these traits were already at a desired level of improvement (Swain et al. 1998). Replicate 2 in the In de Diepte trial was excluded from the analysis due to generally poor survival and growth, this replicate having been planted on a different slope to the rest of the trial, and which became more water-logged over time. Statistical analysis was conducted using SAS® Institute Inc. Software 9.2 (2002-2008a).

The following mixed models were used for the genetic parameter analysis for dbh for the individual sites as follows:

$$y_{hijk} = \mu + rep_i + block_j\,(rep_i) + fam_k + (rep*fam)_{ik} + \varepsilon_{hijk}, \text{ and}$$

$$y_{hijkl} = \mu + rep_i + block_j\,(rep_i) + prov_l + fam_k(prov_l) + (rep*fam(prov_l))_{ik} + \varepsilon_{hijkl}$$

where;

y_{hijk} / y_{hijkl} = mean for the trait of the h^{th} tree in the j^{th} block of the i^{th} rep and k^{th} family / k^{th} family within l^{th} provenance

μ = overall mean

rep = i^{th} replication effect, i = 1, …4 (fixed)

block = j^{th} block effect, j = 1,…10 (fixed)

prov = l^{th} provenance effect , l = 1,…9 (fixed)

fam = k^{th} family effect, k = 1,…90 (random)

*rep*fam* = interaction between the i^{th} rep and k^{th} family (random plot effect)

$rep*fam(prov)$ = interaction between the i^{th} rep and k^{th} family within l^{th} provenance (random plot effect)

ε_{hijk} / ε_{hijkl} = random error effects where ε_{hijk} / ε_{hijkl} ~ iid $(0,\sigma^2)$.

The following models were used for the across site analysis for dbh:

$$y_{hijk} = \mu + site_k + rep_i + fam_j + (site_k*fam_j) + \varepsilon_{hijk}, \text{ and}$$

$$y_{hijkl} = \mu + site_k + rep_i + prov_l + fam_j(prov_l) + (site*fam(prov_l))_{ik} + \varepsilon_{hijkl}$$

where;

y_{hijk} / y_{hijkl} = mean for the trait of the h^{th} tree in the i^{th} rep and j^{th} family / j^{th} family within l^{th} provenance at the k^{th} site

μ = overall mean

$site$ = k^{th} site effect, k = 1,2 (fixed)

rep = i^{th} replication effect, i = 1, …4 (fixed)

$prov$ = l^{th} provenance effect , l = 1,…9 (fixed)

fam = j^{th} family effect, j = 1,…90 (random)

$site*fam$ = interaction between the k^{th} site and j^{th} family (random)

$site*fam(prov)$ = interaction between the k^{th} site and j^{th} family within l^{th} provenance (random)

ε_{hijk} / ε_{hijkl} = random error effects where ε_{hijk} / ε_{hijkl} ~ iid $(0,\sigma^2)$.

To test for normality for traits, residuals were plotted against fitted values. There were no detectable trends or patterns and it was therefore assumed that the conditions: $\varepsilon_{hijk\,/\,hijkl}$ ~ iid $(0,\sigma^2)$, have been met for these data, and the standard ANOVA assumptions are valid. Analysis of variance for dbh was carried out for both sites, as well as across sites, and *F* statistics calculated to test for significance among treatments. Proc GLM was used to calculate family means, as this procedure is used for unbalanced designs (SAS® 2002-2008b), and significant replicate effects were tested for, the data being corrected for these effects. At each site, comparisons were made for differences between entries using Fisher's test for least significance differences for α = 0.05. Due to the highly significant effect of provenance in the F1 trials (Swain et al. 2013b), entries were grouped for i) families nested within provenance (grand-maternal effect), ii) families nested within F1 maternal family, and |iii) families nested within South African BSO, i.e., Jessievale, Jaglust and Helvetia. Comparisons were made for individual sites and across sites, using pairwise *t* tests for α = 0.05 to determine whether provenance and maternal effects were significant.

Realised gains and heritability estimates

The realised genetic gains were estimated by comparing the performance of the average of a range of improved BSO seed bulks originating from each of four ICFR BSOs, i.e., Amsterdam, Helvetia, Jaglust and Jessievale, with an unimproved general bulk from Australia (entry 22) in a series of genetic gain trials described by Swain et al. (2013a). The predicted gains estimated by Swain et al. (2013b) for the improved BSOs ranged from 0.29 cm (1.7%) to 3.17 cm (20.7%) increase in dbh, and were based on parameters estimated for F1 families nested within provenance. Once realised genetic gains were estimated, realised heritabilities were computed, because realised heritability estimates are a good measure of the effectiveness of selection in the previous generation. The following formula was used (Falconer and Mackay 1996):

$$h^2_r = G/S,$$

where;

h^2_r = realised heritability estimate

G = realised gain

S = selection differential.

In the estimation of heritabilities for the F1 and the predicted gains from the F1, heritability estimates and predicted gains were calculated for each bulk from a specific BSO, using relevant selection intensities, and then averaged to represent the heritability estimate or predicted gain of that BSO (Swain et al. 2013b). In a similar manner, realised heritability estimates were calculated for each bulk from a specific BSO, and then averaged for the realised heritability estimate to represent each BSO. Due to the phased selection applied to the BSOs, computation of the selection intensities for each bulk was also done in an incremental or phased manner, using the actual flowering numbers from the year before the seed was collected from each orchard:

$$SI_{fam} = 0.5\ SI_f + 0.5\ SI_m,$$

where $SI_f = SI_1 + SI_2$

(SI_1 = selection intensity between/among female families, i.e., number of families remaining in BSO after roguing of poor families out of the total original number of families,

SI_2 = selection intensity within female families, i.e., number of families used to make up seed orchard bulk treatment out of those remaining in BSO after roguing, and

SI_m = selection intensity within male families, i.e., the proportion of flowering in the BSO multiplied by the number of families remaining after roguing out of the total number of families in the BSO prior to roguing, also multiplied by the proportion of flowering in the BSO).

Becker's (1975) standardised selection tables were used to determine the relevant selection intensities.

Genetic parameters for F2

Breeding values are an estimation of the genetic worth of an individual, and are a useful means for improving selections within a population. To accomplish Breeding Value (BV) calculations and predictions, it is necessary to estimate family effects and genetic parameters. Since the trial was unbalanced due to the number of surviving trees and since fixed effects were included in the models, the SAS procedure MIXED with the REML method was employed to estimate variance components. Proc MIXED (REML) does not allow the variance component estimates to be negative and produces best linear unbiased predictions (Littell et al. 1996).

Family variance was estimated as $\sigma^2_f = 0.33\sigma^2_A$, where σ^2_A is the additive genetic variance. Additive variance was calculated as three times the family variance, considering the possible effect of family relationship and the presence of full-sibs within the open-pollinated families (Squillace 1974). Narrow sense individual heritability (h^2) and within-family heritability (h^2_{wf}) estimates were calculated for each site, using the formulae found in Squillace (1974) and Falconer and Mackay (1996). Standard errors of observed and genetic variance components were estimated according to Becker (1975).

Genotype by environment interaction (GEI)

Type B correlations were then estimated for each trait, to give a clearer indication of any potential genotype by environment interaction (GEI). Where two traits are measured on different individuals within genetic groups, for example a correlation between trees of the same family grown in different environments, the correlation can be designated a Type B genetic correlation (Burdon 1977). The ratio of family variance over the family and environment × family variance is equivalent to a Type B correlation from a paired site

analysis. Type B correlations at the family level (r_{Bg}) were estimated for the two sites as follows:

$$r_{Bg} = \frac{\sigma_f^2}{\sigma_f^2 + \sigma_{site*f}^2}$$

Due to the strong provenance effect in the F1 trials, r_{Bg} was also estimated for family nested within provenance.

Results

Analysis of variance

Table 4.3 presents the analysis of variance for F2 families, Australian provenance (grand-maternal effect), F1 family and South African F1 BSO origin for both individual and across sites. There were significant differences ($p < 0.001$) between the F2 families for dbh for all analyses, and both provenance and F1 family effects were significant ($p < 0.0001$) at both sites and for the across-site analysis. Although the South African BSO effect was not significant for any of the analyses ($p > 0.05$), the family nested within BSO effect was significant at Mt Gilboa. This is supported by the BSO treatment analysis, which showed significant differences between Jessievale and Jaglust BSOs in the Mt Gilboa F2 trial and in the across-site analysis (**Table 4.6**). For families-nested-within grouping, the family within provenance and family within BSO effects were significant, but the F1 family effect was only significant ($p < 0.05$) at In de Diepte.

Table 4.3 Analysis of variance for diameter at breast height for F2 family, Australian provenance (grand-maternal effect), maternal parent family (F1) and South African F1 Breeding Seed Orchard (BSO), for two *Eucalyptus nitens* progeny trials in South Africa

Treatment grouping	Source of variation	Families					Families nested within grouping				
		d.f.[1]	Mean Square	F Value	*P*-value		d.f.	Mean Square	F Value	*P*-value	
In de Diepte:											
F2 family	Rep[2]	2[3]	351.849	48.33	<.0001	***	-	-	-	-	
	Family	89[4]	17.021	2.34	<.0001	***	-	-	-	-	
	Rep x family	150	10.553	1.45	0.0007	***	-	-	-	-	
	Error	1071	7.281				-	-			
Grand-maternal (provenance) effect	Rep	2[3]	221.719	25.54	<.0001	***	2	196.851	23.45	<.0001	***
	Provenance	8	71.233	7.89	<.0001	***	5	100.707	12.00	<.0001	***
	Family (prov)	-	-	-	-		71	15.657	1.87	<.0001	***
	Error	1206	9.033				1086	8.393			
F1 family	Rep	2[3]	222.194	25.38	<.0001	***	2	196.851	23.45	<.0001	***
	F1 parent	70	20.644	2.36	<.0001	***	67	20.342	2.42	<.0001	***
	Family (F1 parent)	-	-	-	-		9	26.250	3.13	0.0010	**
	Error	1204	8.755				1086	8.393			
F1 BSO	Rep	2[3]	233.978	24.78	<.0001	***	2	221.983	25.77	<.0001	***
	BSO	2	11.905	1.26	0.2838		2	16.794	1.95	0.1428	
	Family (BSO)	-	-	-	-		77	21.530	2.50	<.0001	***
	Error	1202	9.443				1125	8.615			
Mt Gilboa:											
F2 family	Rep	3	40.087	2.63	0.0490	*	-	-	-	-	
	Family	88[4]	44.927	2.94	<.0001	***	-	-	-	-	
	Rep x family	259	23.278	1.53	<.0001	***	-	-	-	-	
	Error	1424	15.264				-	-			
Grand-maternal (provenance) effect	Rep	3	44.048	2.50	0.0581		3	44.632	2.73	0.0426	*
	Provenance	8	64.248	3.64	0.0003	***	5	93.493	5.72	<.0001	***
	Family (prov)	-	-	-	-		71	40.887	2.50	<.0001	***
	Error	1634	17.631				1503	16.348			
F1 parent family	Rep	3	43.466	2.62	0.0491	*	3	42.113	2.57	0.0525	*
	F1 family	71	46.510	2.81	<.0001	***	68	47.718	2.92	<.0001	***
	Family (F1 family)	-	-	-			9	13.461	0.82	0.5949	
	Error	1571	16.563				1502	16.355			
F1 BSO	Rep	3	44.795	2.51	0.0569		3	46.050	2.78	0.0399	*
	F1 BSO	2	47.492	2.66	0.0699		2	60.758	3.67	0.0258	*
	Family (F1 BSO)	-	-	-	-		77	43.153	2.60	<.0001	***
	Error	1640	17.822				1563	16.574			
Across site analysis:											
F2 family	Site	1	4500.097	316.95	<.0001	***	-	-	-	-	
	Family	91[4]	41.384	2.91	<.0001	***	-	-	-	-	
	Site x family	86	25.516	1.80	<.0001	***	-	-			
	Error	3162	14.198				-	-			
Grand-maternal (provenance) effect	Site	1	4735.942	324.89	<.0001	***	1	4991.220	358.69	<.0001	***
	Provenance	5	171.851	11.79	<.0001	***	5	155.101	11.15	<.0001	***
	Family (prov)	-	-	-	-		74	27.556	1.98	<.0001	***
	Site x family (prov)	-	-	-	-		74	25.885	1.86	<.0001	***
	Site x prov	5	33.554	2.30	0.0424	*	-	-	-	-	
	Error	2928	14.578				2785	13.915			
F1 parent family	Site	1	4692.560	327.82	<.0001	***	1	4991.220	358.68	<.0001	***
	F1 family	73	40.604	2.84	<.0001	***	70	37.077	2.66	<.0001	***
	Family (F1 family)	-	-	-	-		10	19.614	1.41	0.1694	
	Site x fam[5] (F1 fam)	-	-	-	-		74	25.884	1.86	<.0001	***
	Site x F1 family	68	25.507	1.78	0.0001	***	-	-	-	-	
	Error	2915	14.315				2784	13.916			
F1 BSO	Site	1	1889.592	124.17	<.0001	***	1	4971.362	348.75	<.0001	***
	F1 BSO	2	41.389	2.72	0.0660		2	35.202	2.47	0.0848	
	Family (F1 BSO)	-	-	-	-		80	38.427	2.70	<.0001	***
	Site x fam (F1 BSO)	-	-	-	-		77	25.785	1.81	<.0001	***
	Site x F1 BSO	2	19.455	1.28	0.2786		-	-	-	-	
	Error	3052	15.217				2897	14.255			

[1] degrees of freedom,
[2] Replicate,
[3] Rep 2 excluded from analysis due to poor survival, thus only three replicates included,
[4] More than one F2 family has been derived from the same F1 family over the three BSOs
[5] Family; *, **, *** = significant at 5%, 1% and 0.1% levels of probability, respectively

Comparison of means

Table 4.4 presents the 87 month dbh means for the top 15 families and controls at In de Diepte and Mt Gilboa respectively, ranked for family BV. Growth was better at Mt Gilboa than at In de Diepte (trial means for dbh of 15.29 and 12.30 cm, respectively). At both sites, the majority of F2 families were not significantly different from each other ($p > 0.05$), with the exception of the bottom five or six families at each site.

Table 4.4 Ranking of top 15 F2 *Eucalyptus nitens* families according to Breeding Value (BV) for diameter at breast height (Dbh) at 87 months, with controls, in two progeny trials in South Africa

In de Diepte					Mt Gilboa				
F2 number	**F1 BSO**	**Grand-maternal (provenance) effect**	**BV[1]**	**Dbh87 (cm)**	**F2 number**	**F1 BSO**	**Grand-maternal (provenance) effect**	**BV**	**Dbh87 (cm)**
Top families and top controls:									
28	Jaglust	Barren Mountain	0.613	14.68*	60	Jaglust	Badja	4.367	18.75*
36	Jessievale	Barren Mountain	0.605	14.53	42	Helvetia	Barrington Tops	3.962	18.48
26	Jaglust	Barren Mountain	0.594	14.50	16	Jessievale	Badja	3.571	18.08
31	Jessievale	Tallaganda	0.538	13.71	18	Jessievale	Badja	3.506	18.12
32	Jaglust	Barren Mountain	0.523	14.47	25	Jessievale	Barren Mountain	3.358	17.91
1	Helvetia	Nelshoogte SA[2]	0.507	14.14	31	Jessievale	Barren Mountain	2.833	17.54
67	Jaglust	Tallaganda	0.490	14.14	26	Jaglust	Barren Mountain	2.518	17.23
77	Jaglust	Barren Mountain	0.477	13.77	89	Jaglust	Ebor	2.428	17.23
43	Jaglust	Barrington Tops	0.450	13.97	29	Jessievale	Barren Mountain	2.021	16.90
30	Jessievale	Barren Mountain	0.428	14.01	56	Jaglust	Glenbog	1.847	16.79
38	Jessievale	Barren Mountain	0.387	13.84	1	Helvetia	Nelshoogte SA	1.602	16.57
5	Jessievale	Tallaganda	0.369	13.86	92	Jaglust	Ebor	1.561	16.54
23	Jaglust	Barren Mountain	0.354	13.93	44	Helvetia	Barrington Tops	1.524	16.51
94	-	F2 Jessievale bulk	-	13.71	27	Jessievale	Barren Mountain	1.469	16.48
93	-	F2 Jaglust bulk	-	13.61	32	Jaglust	Barren Mountain	1.405	16.44
92	Jaglust	Ebor	0.326	13.52					
42	Helvetia	Barrington Tops	0.324	13.51					
Controls:									
96	-	Select A	-	12.78	94	-	F2 Jessievale bulk	-	16.07
95	-	Australian bulk	-	12.44	93	-	F2 Jaglust bulk	-	15.69
98	-	Select B	-	11.90	97	-	Commercial A	-	15.21
97	-	Commercial A	-	11.06	41	-	Woodbush SA	-	14.59
100	-	NZ SO Errinundra	-	10.68	96	-	Select A	-	14.39
99	-	NZ SO Barrington Tops	-	10.63	98	-	Select B	-	13.74
46	-	*GxN*[3]	-	10.27	95	-	Australian bulk	-	13.24
70	-	Australian av.[4] family	-	9.70	70	-	Australian av. family	-	12.65
					100	-	NZ SO Errinundra	-	10.79
					99	-	NZ SO Barrington Tops	-	7.73
Mean				12.30					15.29
SD[5]				2.955					3.908

[1] Breeding Value,
[2] South Africa,
[3] *E. grandis x nitens* hybrid,
[4] average,
[5] Standard deviation,
* no significant differences between the top 15 families for dbh at either site

At both sites the F1 controls performed below average and worse than the majority of F2 families. The exception to this was the Australian bulk (Treatment 95) at In de Diepte, which performed at the trial mean (12.44 cm) and was ranked higher than both the Select B and Commercial A controls (Treatments 98 and 97, respectively), although not significantly so ($p > 0.05$). The control seedlot from Nelshoogte (South Africa, Treatment 1) performed in the top 11 treatments at both sites, with the Woodbush (South Africa) control (Treatment 41) performing in the top 20 treatments at Mt Gilboa only. Both ICFR F2 bulks (Treatments 94 and 93) performed above average at both sites and better than the South African Select (Treatments 96 and 98) and Commercial (Treatment 97) controls, and any F1 material. Although there was no significant difference between the ICFR Jessievale and Jaglust F2 bulks (Treatments 94 and 93, respectively) ($p > 0.05$), with both being in the top 20% of treatments at In de Diepte, the Jessievale bulk performed better than the Jaglust bulk at Mt Gilboa. The ranking of the South African Select and Commercial bulks changed across site, although not significantly so ($p > 0.05$).

The *E. nitens* controls from New Zealand, i.e., Barrington Tops Seed Orchard and Errinundra Seed Orchard (Treatments 99 and 100, respectively) performed very poorly at both sites, with less than15% final stocking at Mt Gilboa. The average-performing F1 family (37263 ex Tallaganda, Treatment 70) performed in the bottom seven treatments at both sites, as did the *GxN* control (Treatment 46) at In de Diepte.

Figure 4.1 presents a regression of the family mean dbh for each site, as an indication of how the families have performed at the different sites and whether ranking changes have occurred.

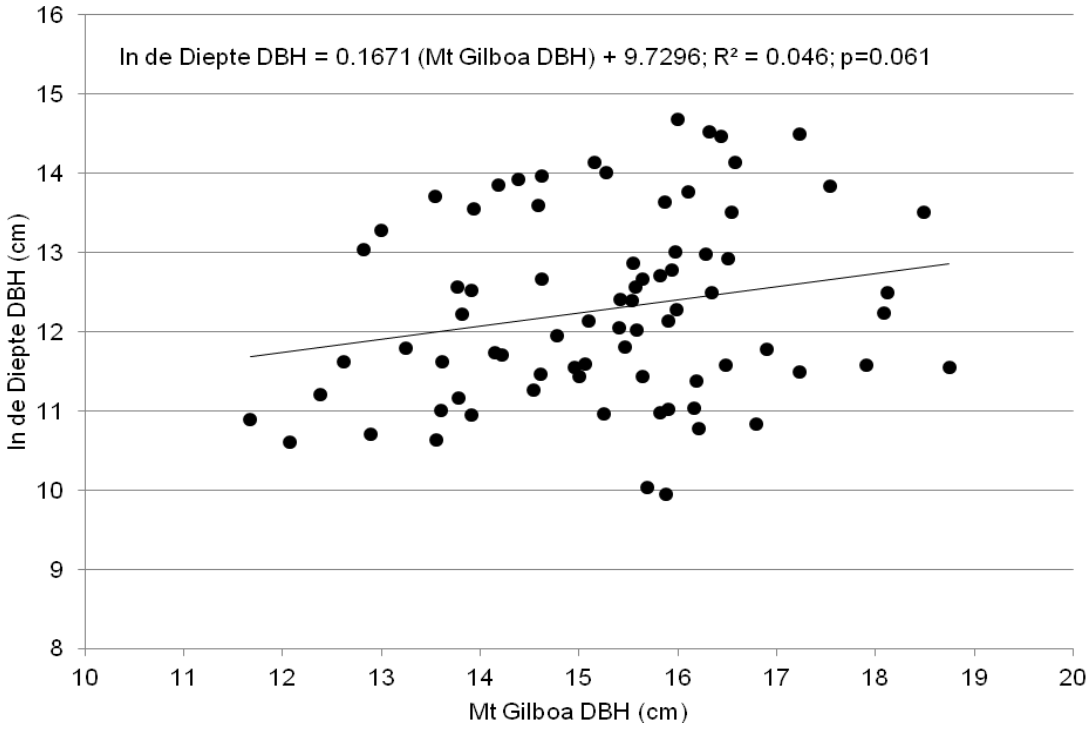

Figure 4.1 Regression of F2 family means for diameter at breast height (DBH) at 87 months in two *Eucalyptus nitens* progeny trials in South Africa

Provenance effects from the maternal grandparent were still significant in the progeny trials (**Tables 4.3** and **4.5**), with the northern NSW provenances of Barren Mountain and Barrington Tops outperforming ($p < 0.05$) the more central and southern NSW provenances of Badja, Tallaganda and Glenbog for dbh at In de Diepte. A slightly different trend was found at Mt Gilboa, where only material originating from Glenbog and Tallaganda performed significantly worse ($p < 0.05$) than the two northern NSW provenances of Barren Mountain and Barrington Tops. The provenances of Badja and Ebor were not significantly different ($p > 0.05$) from these top Australian provenances, however. The same trend was found in the across-site analysis.

The effect of the South African BSO was significant at Mt Gilboa and in the across-site analysis, with progeny from Jessievale BSO performing significantly better ($p < 0.05$) than progeny from Jaglust (**Table 4.6**). This latter finding supported the individual treatment comparisons (**Table 4.4**), where the Jessievale bulk produced larger dbhs than the Jaglust bulk at Mt Gilboa only.

Table 4.5 Final mean diameter at breast height (Dbh), in and across two *Eucalyptus nitens* F2 progeny trials in South Africa, for F2 families grouped by original Australian provenance (grand-maternal effect). Treatment means which do not differ significantly from each other bear the same letter of the alphabet

In de Diepte			Mt Gilboa			Across site		
Grand-maternal (provenance) effect	**No.[1] of seedlots**	**Dbh87 (cm)**	**Grand-maternal (provenance) effect**	**No. of seedlots**	**Dbh87 (cm)**	**Grand-maternal (provenance) effect**	**No. of seedlots**	**Dbh87 (cm)**
Barren Mountain	19	13.2 a	Barrington Tops	5	16.0 a	Barren Mountain	19	14.8 a
Barrington Tops	5	13.0 a	Badja	12	15.9 a	Barrington Tops	5	14.7 a
Badja	14	12.1 b	Barren Mountain	19	15.9 a	Badja	12	14.3 ab
Tallaganda	18	12.0 b	Ebor	8	15.2 ab	Ebor	8	13.9 bc
Ebor	8	11.7 bc	Glenbog	14	14.9 b	Tallaganda	18	13.6 c
Glenbog	14	11.4 c	Tallaganda	21	14.7 b	Glenbog	14	13.5 c
Mean		12.30			15.36			14.09
SD[2]		2.95			4.20			3.82

[1] Number,
[2] Standard deviation

Table 4.6 Final mean diameter at breast height (Dbh), in and across two *Eucalyptus nitens* F2 progeny trials in South Africa, for F2 families grouped by South African F1 Breeding Seed Orchard (BSO) origin. Treatment means which do not differ significantly from each other bear the same letter of the alphabet

In de Diepte			Mt Gilboa			Across site		
F1 BSO[1]	**No.[2] of seedlots**	**Dbh87 (cm)**	**F1 BSO**	**No. of seedlots**	**Dbh87 (cm)**	**F1 BSO**	**No. of seedlots**	**Dbh87 (cm)**
Helvetia	4	12.9 a	Jessievale	27	15.7 a	Helvetia	4	14.7 a
Jaglust	48	12.3 a	Helvetia	4	15.5 ab	Jessievale	27	14.3 ab
Jessievale	28	12.2 a	Jaglust	49	15.1 b	Jaglust	48	14.0 c
Mean		12.30			15.36			14.14
SD[3]		2.95			4.07			3.90

[1] Breeding Seed Orchard,
[2] Number,
[3] Standard deviation

Realised gains and heritability estimates

The gains in dbh achieved in the F2 at 87 months are presented in **Table 4.7**, and compared to the gains predicted from the F1 (Swain et al. 2013b). Gains varied according to site, but were generally closer to the predictions using heritability estimates based on the family nested within provenance variance components, the family across provenance predictions markedly over-predicting gain in most cases. Comparison of realised gains with family nested within provenance predictions showed a range of under and over-estimations, as well as some estimations similar to the gains that were predicted from the F1. The two largest digressions from the predicted gains were Helvetia and Babanango, the former greatly under-predicting gain, and the latter markedly over-predicting gain. No realised gains were

available for the E88/07 trial series of Goedehoop and Arthur's Seat, because no seed had been produced at the related seed orchard at Pinewoods, nor the converted progeny trial at Goedehoop, by the time the F2 trials were established. It should be noted that gains in dbh (cm) may be biased downwards due to a lack of competition as a result of poor survival in the unimproved control, and thus gains are better represented by the improvement in total volume per hectare over the unimproved Australian bulk, which takes survival into account (**Table 4.7**). The volume gains were notable, ranging from 43.9 to 63.5% over seed orchards. Realised heritabilities (h^2_r) are also included in this table, with estimated heritabilities from the F1 trials in brackets, for comparison purposes. Realised heritabilities were in the same range of the estimated heritabilities, with the exception of Helvetia and Babanango. **Appendix 2** provides detail of the realised gain and heritability estimates for each seed orchard bulk that made up the calculation for the respective seed orchards in **Table 4.7**.

Table 4.7 Predicted gains for i) family nested within provenance (*fam (prov)*) and ii) families across provenance (*fam*) compared to realised gain in dbh of averaged F2 *Eucalyptus nitens* bulk treatments at 87 months over an unimproved F1 bulk, per orchard, and realised narrow sense heritabilities (h^2), as estimated from genetic gain trials (Swain et al. 2013a)

Trial series & site name (age in months at final measurement[1])	Gains in dbh (cm) predicted from F1: *i) fam (prov) (%)* *ii) fam*	Realised gain in cm dbh (%)	Between family SI[2] per BSO[3] bulk (bulk entry number[4])	Within family SI[2] per BSO bulk	Realised $h^2_{fam(prov)}$ (estimated $h^2_{fam\ (prov)}$ ± SE)	Realised improve-ment in total volume (m^3ha^{-1}) (%)
E88/01 Jessievale (101)	i) 0.99 (6.2%) ii) 1.72	**1.02 (7.3)**	1.087 (1, 2,4) 0.863 (7) 1.077 (9)	1.539	0.10 [0.05 ± 0.022]	**63.4 (43.9)**
E88/01 Amsterdam (101)	i) 1.25 (7.4%) ii) 1.76	**1.90 (13.7)**	0.119 (5)	1.539	0.21 [0.14 ± 0.055]	**82.3 (57.0)**
E88/03 Daspoort (113)	i) 2.05 (11.4%) ii) 6.43	**Helvetia bulks[5]:** **1.80 (12.9)**	1.159 (3) 0.917 (8)	1.267	0.14 [0.13 ± 0.045]	-
E88/03 Helvetia (94)	i) 0.29 (1.7%) ii) 1.89	**1.80 (12.9)**	1.159 (3) 0.917 (8)	1.267	0.15 [0.01 ± 0.026]	**91.7 (63.5)**
E88/05 Babanango (112)	i) 2.52 (14.0%) ii) 3.19	**Jaglust bulk[6]:** **1.35 (9.7)**	1.299 (6) 1.422 (10)	1.267	0.12 [0.18 ± 0.040]	**71.7 (49.7)**
E88/06 Woodstock (76)	i) 1.55 (7.0%) ii) 1.61	**Jaglust bulk[6]:** **1.35 (9.7)**	1.299 (6) 1.422 (10)	1.267	0.17 [0.14 ± 0.032]	**71.7 (49.7)**
E88/07 Goedehoop (113)	i) 3.07 (17.1%) ii) 2.99	-[7]	-	-	- [0.22 ± 0.055]	-
E88/07 Arthur's Seat (113)	i) 3.17 (20.7%) ii) 3.50	-[7]	-	-	- [0.27 ± 0.060]	-

SE = Standard error of h^2,
[1] Different ages should be noted,
[2] Selection Intensity,
[3] Breeding Seed Orchard
[4] As described in Swain et al. (2013a),
[5] As no seed had been produced from this BSO by the time progeny trials were established, parameters from the related Helvetia BSO were used,
[6] Jaglust is a BSO representing the material in the Babanango and Woodstock trials,
[7] No seed had been produced from any of these trials or related BSOs by the time the progeny trials were established

Genotype by environment interaction (GEI)

The Type B genetic correlation (r_{Bg}) between the two sites was 0.61 for dbh and the phenotypic correlation, as estimated using Pearson's correlation coefficient, was 0.32. As this was contrary to what was found in the F1 trials (Swain et al. 2013b), the r_{Bg} was also estimated using REML in Proc MIXED in SAS®, producing an estimate of r_{Bg} = 0.50.

Genetic parameters for F2

Variance components and individual heritability estimates for dbh in the progeny trials are presented in **Table 4.8**. The individual heritability coefficients (h^2) were 0.06 and 0.17 for dbh at In de Diepte and Mt Gilboa, respectively, with standard errors for h^2 being larger than the estimated h^2 at the former site. Family variance for dbh was higher at Mt Gilboa than at In de Diepte.

Table 4.8 Variance components and genetic parameters with standard errors for diameter at breast height (dbh) at 87 months in two F2 *Eucalyptus nitens* trials in South Africa

Site	σ^2_A	σ^2_f	σ^2_e	σ_p	σ_{wf}	h^2	h^2_{wf}
In de Diepte:							
dbh	0.54 ± 0.792	0.18 ± 0.261	6.80 ± 0.312	3.03	2.22	0.06 ± 0.086	0.04
height	2.19 ±1.398	0.72 ± 0.461	3.44 ± 0.158	2.99	1.94	0.24 ± 0.156	0.18
volume	336.64 ± 382.515	111.09 ± 126.230	2142.489 ± 98.105	59.17	40.76	0.10 ± 0.109	0.07
Mt Gilboa:							
dbh	2.97 ± 1.080	0.98 ± 0.356	15.16 ± 0.591	4.20	3.49	0.17 ± 0.061	0.12

σ^2_A = additive variance,
σ^2_f = family variance,
σ^2_e = error variance,
σ_p = phenotypic standard deviation,
σ_{wf} = standard deviation within families,
h^2 = heritability of individual values (narrow-sense),
h^2_{wf} = within family heritability

Discussion

Genotype by environment interaction in F2

The Type B genetic correlation (r_{Bg}) between the two sites was 0.61 and 0.50 using SAS® procedures VARCOMP and MIXED respectively, both of which are lower than the point at which the GEI variance represents 50% of the total additive variance (0.67), and where it is postulated that GEI variance may be a cause for concern in tree breeding (Shelbourne 1972). The moderate r_{Bg} estimated for the site pair in this study thus indicates the possibility of GEI for dbh for the *E. nitens* genotypes tested over these two sites. This was also supported by the ranking of family mean performance differing markedly between the two

sites, as indicated by the low regression coefficient (R^2 = 0.046) in **Figure 4.1**. This was unexpected, because r_{Bg} were very high in the F1 of this population (Swain et al. 2013b), indicating very little GEI. The r_{Bg} estimation for family nested within provenance was 0.49 using Proc MIXED, indicating that GEI might be a problem at both the family and provenance level. It is possible that the two sites in these progeny trials were so different that GEI was able to be expressed. Mt Gilboa, although at a markedly lower altitude than In de Diepte, is a colder site than the latter, most likely due to the more southern latitude, and this may have accounted for the resultant GEI. The majority of the F1 trials were situated on the Highveld in central Mpumalanga, and understandably had high r_{Bg} for these site pairs. However, two F1 trials were situated on different site types, i.e., Helvetia (1 ° latitude further north on the Mpumalanga escarpment) and Babanango (2 ° further south and 1 ° further west in KwaZulu-Natal, at a markedly lower altitude than the other trials). Both these sites were colder and drier than the other F1 trial sites, yet despite this, both trials had high r_{Bg} for those pairwise site comparisons that had enough treatments in common to be valid (Swain et al. 2013b), which is in contrast to what was found between the F2 sites.

Alternatively, the low heritability of one of the sites in the F2 trials (see later in document) may have negatively affected the genetic correlation between sites. Johnson (1997) found that sites with lower heritabilities gave poorer estimates of family values in Coastal Douglas-fir and, as the family values became more random, the less they were likely to correlate with family values from other sites. In addition, the F2 are likely to have a narrower range of variation than the F1, and the poorer families included in the F1 may thus have masked GEI in the F1.

The possibility of GEI in the F2 population presents a challenge for breeders with regards to production of improved seed for the South African forestry industry, because sites for commercial establishment of *E. nitens* range from the north eastern Cape in the south (-33 °S), up through eastern KwaZulu-Natal to the Highveld in central Mpumalanga in the north (-4 °S). Options for managing the GEI would be to either; a) use the stable families which perform well over a range of environments, and remove the unstable families from the F2 seed orchards, or b) breed separate populations of the species for different site types in South Africa (Raymond and Namkoong 1990). The latter, however, would require further testing over a range of sites to properly characterise the different environments, because only two site types or environments were covered in this progeny trial series (Matheson and Cotterill 1990).

Comparison of treatments in F2

Comparison of individual treatments at the two sites showed that both of the F2 South African bulks performed above average and better than any other F1 material. There was no significant difference between the Jessievale and Jaglust bulks (Treatments 94 and 93, respectively) ($p > 0.05$), both being in the top 20% of treatments at In de Diepte, although the Jessievale bulk performed better than the Jaglust bulk at Mt Gilboa. This lack of significance is comparable to the results found in *E. nitens* genetic gain trials testing the South African populations (Swain et al. 2013a). Final stocking of the improved F2 material was notably better than that of both the improved material from overseas, and the unimproved controls. The *GxN* control did not perform well at In de Diepte, ranking in the bottom five treatments of that trial, this despite the final stocking of the clone being not too poor at 67%. Further investigation into the background of the clone showed it to be a poor performer which was in the early stages of testing at the time of the F2 trial establishment, and which was later removed from the clonal programme.

The poor performance of the improved *E. nitens* controls from New Zealand showed the risk of large-scale planting of material from winter rainfall areas into summer rainfall regions without prior testing. Both of these provenances originate from Australia, with native Australian Barrington Tops (ex northern NSW) performing well in the summer rainfall regions of South Africa (Swain et al. 1998, Swain et al. 2013b). Material from Errinundra is relatively unknown in South Africa, due to origins in the more winter rainfall distribution of Victoria. Improved seed of both these provenances was established in seed orchards in New Zealand, and it is progeny from these orchards that was included in the ICFR F2 trials. Material from both New Zealand Seed Orchards performed very poorly at both sites, with less than15% final stocking at Mt Gilboa. This poor growth and survival was already apparent at the 30 month measurement at Mt Gilboa, primarily due to susceptibility to *Mycosphaerella* leaf blotch disease (unpublished data). The New Zealand material had, in all likelihood, adapted to the winter rainfall and climate of that country, and was not suited to growth under the summer rainfall conditions in South Africa. Errinundra provenance from Australia has been found to have poor growth and survival (Tibbits and Hodge 2003) and frost tolerance (Tibbits and Reid 1987) in other trials. Landrace material from other winter rainfall countries, such as Chile, has also suffered severely from leaf spot when planted in South Africa (Swain and Gardner 2003, Komakech et al. 2009). It is therefore advisable to plant only local,

improved material when such seed is available, or to use provenances identified in trials within the summer rainfall region.

Realised versus predicted gain

Realised gains were generally closer to the family nested within provenance predictions, the family across provenance predictions markedly over-predicting gain in most cases. Thus further discussion will be confined to the realised gains and family nested within provenance predictions. The gains in dbh and volume realised in the F2 compared well with those achieved in other *E. nitens* breeding programmes in South Africa, these having average tree volumes of 0.168 m^3 (van den Berg and Stanger 2007). When compared to gains achieved in Chile, although the percentage increases in gain were much higher from the ICFR, the actual increase in volume in Chile was greater i.e., a 5.3% increase in volume (683 m^3ha^{-1}) at one site in Chile over the F1 (Velilla et al. 2007).

The gains in dbh achieved in the F2 varied according to site and, when compared to the gains predicted from the F1 using family nested within provenance parameters, a 30% roguing scenario and the formula by Verryn et al. (2000), showed these predictions to range from under- to over-predictions, with a majority showing similar estimates. The two most notable differences between the gains predicted (G_P) and those realised (G_R) were the marked under-prediction from Helvetia (G_P = 0.29 cm dbh, G_R = 1.80 cm dbh) and the over-prediction from Babanango (G_P = 2.52 cm dbh, G_R = 1.35 cm dbh) (**Table 4.7**). The realised heritabilities (h^2_r), which were derived using the realised gain and the actual selection intensities used in the seed orchards, reflected the differences between predicted and realised gain. In the case of Helvetia, the h^2_r was markedly higher than the h^2_p (estimated heritability), due to the higher realised gains (h^2_p = 0.01, h^2_r = 0.15). The h^2_p at Helvetia was very low, but interrogation of the F1 data did not provide any clear answers as to why this was so. Conversely for Babanango, the h^2_r was notably lower than the h^2_p (h^2_p = 0.18, h^2_r = 0.12), reflecting the lower realised gains. Realised heritabilities are a good measure of the effectiveness of selection (Hettasch et al. 2007), and these h^2_r fall into the intermediate range of narrow-sense heritabilities, where intermediate to good gains can be achieved by selection (Namkoong 1979, Cotterill and Dean 1990). This is supported by the intermediate to high h^2_r for Amsterdam (h^2_r = 0.21), with related marked increase in dbh (1.9 cm, or 13.7%) (**Table 4.7**).

With reference to the five seed orchard bulks that made up the estimation of realised gain and heritability for Jessievale BSO (**Appendix 2**), it is interesting to note that two of the bulks had markedly higher h^2_r than the other three (Treatments 2 and 9, respectively, details of which can be found in Swain et al. (2013a)). Both of these bulks originated from the BSO in years when flowering was 40% or greater, compared to the other three bulks, where flowering was 15%. This supports the recommendation that seed should be collected, where possible, from seed orchards where 40% or more flowering was observed in the previous year (Swain et al. 2013a). The seed orchard bulk from Amsterdam represented 20% flowering, and these gains were above average, this anomaly noted by Swain et al. (2013a) (Chapter 3).

There are several assumptions made in the predictions of genetic gains which, if incorrect, may be reasons for the inaccuracy in predictions using this deterministic methodology, i.e., relying on quantitative genetic theory to predict the genetic gains (Chapter 5 or Swain et al. 2013c):

1) The assumption of *cr* = 0.33 to allow for the presence of inbreeding within the open-pollinated families;
2) The assumption that flowering and mating is random, and that panmictic pollination is occurring, i.e., assuming equal contributions to the pollen pool from each tree (Hodge and White 1993);
3) The assumption that there is no correlation between flowering and growth, i.e., flowering individuals would not be selected against due to their growth, and *vice versa*;
4) The assumption of the absence of non-additive effects such as heterosis or inbreeding (negative heterosis), the former as a result of the mixing of provenances by outcrossing of all families in the F1 (Hodge et al. 1996, Hamilton et al. 2008).

The first three points will be further investigated (Chapter 5 or Swain et al. 2013c), and the provenance effect (point 4) is discussed later in this document/Chapter.

Actual selection intensity may also have a role to play in differences between predicted versus realised gains. In reality, with flowering of individual trees in ICFR BSOs ranging from 15 to <50% (Swain et al. 2013a), stratified selection based on flowering could have taken place, with up to ten trees contributing seed from some families and with only one (or no)

tree contributing from other families, i.e., the selection intensity in the orchard would have been different to the figures used in the predictions, resulting in lower realised genetic gains. Thus, in reality, the selection intensities should be revised, implying that even greater gains can be expected in future, if flowering can be increased.

There was some concern, at the time of establishment of these progeny trials, that gains might be suppressed due to the poor flowering and lack of resultant seed from a wide range of selected individuals. However, selection under these conditions still seems to have been effective, with the exception of the 15% flowering bulks from Jessievale BSO. Thus production from commercial plantations established with improved seed from the F1 BOSs can be expected to provide gains in the same region.

Provenance (grand-maternal) and F1 family effects in F2

Provenance effects were still significant in the progeny trials, despite top F1 families having come from a mixed provenance breeding environment. This is similar to what was found by Gea et al. (1997) in F2 trials in New Zealand. These significant grand-maternal effects indicate that a diluted provenance identity is still being expressed through the maternal influence in the F2, which would be expected unless strong heterosis has occurred. In addition, the ranking of provenances in the F2 was unchanged from the F1 trials (Swain et al. 2013b), suggesting a strong additive effect, and very little likelihood of heterosis occurring. Due to the indications that heterosis has not occurred within this population, it is unlikely that it will be necessary to keep provenances separate to harness such an effect in current and future ICFR seed orchards. However, such a decision could be supported by formal molecular marker studies to determine levels of outcrossing and to ensure that inter-provenance crossing did actually occur. The strong effect of provenance suggests that additional selections could be made from the top-performing provenances of Barren Mountain and Barrington Tops in the F1 BSOs, trials or related clonal seed orchards to further increase the gain from the F1. However, to prevent narrowing of the genetic base, selections from other provenances should still be retained in the production population.

The F1 family effect was also significant at both sites. This effect appears to be confounded with the grand-maternal provenance effect, because there was strong representation of the top F1 families in the best performing provenances of Barren Mountain and Barrington Tops in the F2. This supports the suggestion above that the provenance effect/identity is still being

expressed through the maternal/female line. Once again, this is an indication that heterosis, as a result of inter-provenance crosses, is unlikely to have occurred in the F2.

With regards to the assumptions underlying predictions of genetic gain, as listed above, the stability, or greater influence, of female effects in reproductive success in some eucalypt species has been noted by several authors (Tibbits 1989, Leal and Cotterill 1997, Suitor et al. 2009), as well as the suggestion that only a few effective males may contribute to pollination of particular trees in a seed orchard (Hodge et al. 1996, Suitor et al. 2009). Once again, it will be necessary to do a genotyping study to determine the role of female and male parents in the seed orchards. This study should be extended to controlled crosses, because this will assist in identifying parents with good Specific and General Combining Ability, which will improve future seed orchard management and increase gains.

Seed orchard effect in F2

The effect of the South African BSO from which seed was collected was significant at Mt Gilboa, with progeny from Jaglust BSO performing significantly worse ($p < 0.05$) than those from Jessievale BSO. Investigation into whether this performance may have been as a result of the flowering percentages in the BSOs the year before the seed was collected, showed that this was not the case, because Jessievale with the better performing progeny, had 15% total flowering during 1997, compared to Jaglust with 47% total flowering in the same year. Once selection intensity (SI) was scrutinised, however, it emerged that the Jessievale BSO had 51 trees out of a possible 341 from which to collect seed (SI = 1.5 (Becker 1975)), whilst there were 245 out of a possible 521 in Jaglust BSO (SI = 0.86 (Becker 1975)), and this higher selection intensity at Jessievale may have resulted in higher gains from the grouped Jessievale treatments. The significant difference in BSOs is in contrast to what was found in the related ICFR *E. nitens* genetic gain trials (Swain et al. 2013a), where no significant differences were found between BSO treatments. However, in the latter study, BSOs were represented by only one to five treatments, which may not have been sufficiently representative for differences to be expressed.

Genetic parameters for F2

The heritability estimates for dbh in this study (0. 06 and 0.17) compared well with those found in 2nd generation material in New Zealand (Gea et al. 1997), but were lower than those for volume in two F2 *E. nitens* trials in South Africa (0.21 and 0.22) (van den Berg and

Stanger 2007). Narrow-sense heritability estimates ranging from 0.10 to 0.30 are considered low to intermediate, this being the range most commonly found in eucalypts (Namkoong 1979, Cotterill and Dean 1990). These heritability estimates indicate that moderate genetic gains can be expected from individual tree selection in the Mt Gilboa trial, and lower gains from the In de Diepte trial (Cotterill and Dean 1990). These heritabilities are generally lower than those estimated in the F1 of the *E. nitens* breeding population (Swain et al. 2013b), which is common as a result of selection (Snedden 2001). Gea et al. (1997) found that the use of different coefficients of relationship for different generations of *E. nitens* proved an efficient tool for making heritabilities comparable between the F1 and F2 generations. Although coefficients of relationship could change over generation, this should not be of a high magnitude unless selection intensity was high and population size small. This study indicates that a coefficient of relationship of 0.33 appears to be acceptable for the F1 of this *E. nitens* population. Therefore, an increase in relatedness in the F2 would mean moving towards a coefficient of relationship of 0.4 for estimations of heritability in this generation. There is no evidence to suggest that the level of relatedness is increasing at such a rate, although this could be further investigated through a genotyping study.

The lower the heritability estimate, the more the emphasis should be placed on family selection, rather than on individual-within-family selection (Hettasch et al. 2007). Thus, in the case of the F2 trial at In de Diepte (h^2 = 0.06), family mean information could normally be used to inform family selection at Mt Gilboa (h^2 = 0.17). However, the low Type B correlations between the two sites make even this risky. It is thus proposed that the family mean information from In de Diepte, in conjunction with Mt Gilboa family means, be used to identify stable genotypes across both sites. Selections should be made in the top 10 common families at Mt Gilboa and In de Diepte at a ratio of 2:1, to account for the low heritability at In de Diepte. Additional selections should be made from families ranked below 10th at Mt Gilboa in order to maintain a sufficiently broad genetic base. The within-family selections should be constrained to prevent the highest ranking individuals coming from only a few families (Tibbits and Hodge 1998).

In planning a breeding and selection strategy for continued improvement in this population, the amount of flowering and outcrossing should be taken into account, to ensure that at least a modest level of flowering (> 40%) is taking place. Thus, individuals and families should be selected for flowering and reproductive traits, in addition to growth traits.

Conclusions

Analysis of the F2 trials has resulted in the comparison of realised versus predicted gains, and related genetic parameters, the understanding of which is highly useful to continued tree improvement in this species. The role that provenance is still playing in the population, two generations on from the P_0 population, has also been highlighted. Although the F2 trials were not designed to detect heterosis/non-additive effects, it is unlikely that these exist in this population, due to the still-significant provenance effect and the lack of grandparent-based provenance rank changes in the F2, indicating the presence of a strong additive effect. Therefore indications are that it is not necessary to keep provenances separate in current and future ICFR seed orchards, which will make practical management of orchards easier.

The average gains per BSO achieved in this study range from increases of 43.9 to 63.5% in total volume per hectare, and can be utilised commercially by using an F1 *E. nitens* bulk instead of using commercial or unimproved seed. At present, the F1 bulks comprise seed from the top 70% of families, and indications are that future commercial bulks made up of the top 20% of families, for example, would have even greater gains over the base population material. Survival of the progeny plays a marked role in improvement over the unimproved controls.

The low to intermediate heritabilities estimated from the F2 trials indicate that moderate genetic gains can still be expected in the next generation from individual tree selection. However, the possibility of GEI in the F2 population presents a challenge for production of improved seed, because sites for commercial establishment of *E. nitens* differ widely. Two options for managing the GEI are to either; a) use only stable families which perform well over a range of environments or, b) breed separate populations of the species for different site types in South Africa. It is likely that the former will be used for this Tree Improvement programme, as it is the most practical and cost-effective method, and would maximise gain potential.

These results will assist with developing an advanced generation breeding strategy to maximise gains in *E. nitens*. The Breeding Values predicted from this analysis will enable the more effective re-selection of parents in the F1 BSOs, in which additional trees have now produced seed for a second series of progeny trials. In addition, the information obtained from this forward selection trial series can facilitate further selections in current trials for the

establishment of F3 trials and seed orchards. Gains can be predicted for both forward and backward scenarios, taking time and cost effectiveness into account, to determine which will be the most suitable option for the breeding programme.

Acknowledgments

Many thanks to the foresters and research staff at Mondi Ltd., Sappi Forests and York Timbers Ltd. for their assistance with the establishment and maintenance of these trials and seed orchards.

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CHAPTER 5

An investigation of assumptions made in estimating genetic parameters and predicting genetic gain in a *Eucalyptus nitens* breeding programme in South Africa

T-L Swain[1]*, SD Verryn[2], MD Laing[3]

[1] *Institute for Commercial Forestry Research, P.O. Box 100281, Scottsville, Pietermaritzburg 3209, South Africa.*

Tammy.Swain@icfr.ukzn.ac.za

Ph: 27-33-3862314 Fax: 27-33-3868905

[2] *Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa and Creation Breeding Innovations, 75 Kafue St, Lynnwood Glen, 0081, South Africa.*

[3] *School of Agricultural Earth and Environmental Sciences (SAEES), University of KwaZulu-Natal, PO Box X01, Scottsville, 3209, South Africa.*

* Author for correspondence: T-L Swain

Telephone: +27 33 386 2314

Fax: +27 33 386 8905

Tammy.Swain@icfr.ukzn.ac.za

[**Submitted to** "New Forests", October 2013]

Summary

It is important to have an understanding of the population genetics and validity of the pertinent underlying assumptions of a species in order to design an effective breeding strategy. In a South African breeding population of *Eucalyptus nitens*, various scenarios investigating a range of assumptions were developed and used to predict genetic gain in the F2 populations. These were compared with realised gains achieved in a series of genetic gain trials. The variance components based on a model with family nested within provenance, predicted gains closer to the realised gain than those where provenance effects were removed from the model. In the two scenarios using firstly, actual flowering for family nested within provenance and, secondly, estimated flowering after 30% roguing of poor families, a coefficient of relationship of 0.33 predicted gains closest to realised gain. The statistical information suggested that outcrossing in the seed orchards was greater than 80%. Indications were that the effects were additive, and that very little or no heterosis had occurred, due to the still significant provenance effects and the lack of provenance rank changes in the F2 populations.

The custom of assuming a degree of inbreeding (and using a coefficient of relationship of 0.33) and of including provenance effects in the models resulted in genetic gain predictions which were very similar to the realised genetic gains in this population of *Eucalyptus nitens*.

Keywords

E. nitens, genetic parameters, coefficient of relationship, outcrossing, provenance effects

Introduction

A good understanding of the population genetics and underlying assumptions of a species are important for designing breeding strategies (Tibbits and Hodge 1998). *Eucalyptus nitens* is an important commercial cold tolerant eucalypt (CTE) species, grown primarily for pulp and paper production, in the summer rainfall regions of South Africa. Currently there are no suitable alternative commercial eucalypt species to *E. nitens* in South Africa for sites prone to moderate frost and heavy snowfalls (Gardner and Swain 1996). There is considerable variation for several growth, reproductive and wood property traits within the species in South Africa, which is based primarily on Australian provenance origin (Swain et al. 2013c). This variation provides potential for genetic improvement, and it is important that an appropriate advanced generation breeding strategy be developed for improvement of *E. nitens*.

Several studies have been done to estimate genetic parameters in *E. nitens* (King and Wilcox 1988, Whiteman et al. 1992, Johnson 1996, Gea et al. 1997, Tibbits and Hodge 1998), with some research into the factors affecting these estimates, i.e., panmixis (Tibbits 1989, Grosser et al. 2010), outcrossing rates (Moncur et al. 1995, Gea et al. 1997, Grosser et al. 2010), self-incompatibility and inbreeding (Hardner and Tibbits 1998, Pound et al. 2003). However, there is little known about the underlying assumptions in the South African populations of *E. nitens*, and there is some concern about the impact that poor and erratic flowering, and subsequent seed production, of the species in South Africa (Gardner 2003) may have on genetic gain. In addition, the poor flowering may violate some of the basic assumptions in parameter estimation and genetic gain prediction, such as occurrence of panmixis, absence of non-additive effects, and no correlation between flowering and growth. Some of these may affect the coefficient of relationship used.

Statistical analysis of several F1 and F2 *E. nitens* trials run by the Institute for Commercial Forestry Research (ICFR) in South Africa has led to the development of estimates for genetic parameters and of juvenile-mature and trait-trait genetic correlations, as well as an indication of the presence/absence of genotype by environment interaction (Swain et al. 2013b, Swain et al. 2013c).These have been invaluable in determining the breeding potential of the *E. nitens* population, selection of superior families and individuals, as well as informing the breeding strategy for the species. Genetic gain trials allowed for comparison of realised gain in the F2 populations with predicted genetic gain from the F1 (Swain et al. 2013c), and this

provides an opportunity to assess the performance of the quantitative predictions using underlying assumptions.

There are several assumptions made in the predictions of genetic gains which, if incorrect, may cause inaccuracy in predictions using deterministic methodology (Verryn et al. 2000):

1) The assumption of the coefficient of relationship (*cr)* = 0.33 to allow for the presence of full-sibs within the open-pollinated families, and that at least some inbreeding occurs (Squillace 1974, Snedden et al. 2007);

2) The assumption of > 70% outcrossing between eucalypt seed orchard trees; Moncur et al. (1995) estimated that outcrossing was at a level of 75% in an *E. nitens* seed orchard, and Pound et al. (2003) found that levels of self-incompatibility in *E. nitens* ranged from 25.8 to 93.6%. The species demonstrates preferential outcrossing and appears to have a late-acting self-incompatibility system operating to reduce the production of selfed seed (Tibbits 1989, Pound et al. 2003). The assumption of > 70% outcrossing in eucalypts made by many authors (Moran et al. 1989, Moncur et al. 1995, Hodge et al. 1996, Butcher and Williams 2002, Grosser et al. 2010), may not be true of these F1 *E. nitens* seed orchards, due to poor flowering.

3) The assumption that flowering and mating is random, and that panmictic pollination is occurring, i.e., that all individuals have equal opportunities to mate with any other individual (Hodge and White 1993);
   i) Flowering has been shown to be heritable in several eucalypt species (Hodge et al. 1996; Chambers et al. 1997, Gardner 2003, Varghese et al. 2009) and asynchronous flowering exists between provenances (Tibbits 1989, Volker et al. 1990, Moncur and Boland 2000, Jones 2002), indicating that this assumption may not be correct. However, these flowering windows do overlap (Jones 2002, Gardner 2003), which may allow for panmictic pollination some of the time. In addition to asynchronicity, flowering in *E. nitens* is subject to seasonal influences (Moncur and Hasan 1994, Tibbits 1989, Jones 2002, Gardner and Bertling 2005) and, as the stability, or greater influence, of female effects in reproductive success in some eucalypts has been noted (Tibbits 1989, Leal and Cotterill 1997, Suitor et al. 2009), it is possible that only a few effective males may contribute to pollination of all the other trees in a seed orchard (Hodge et al. 1996, Suitor et al. 2009).

Conversely, pollen from a few heavy-flowering individuals may have too large a genetic influence on adjacent female trees (Moncur and Boland 2000). Grosser et al. (2010) also found that the parental contribution to progeny varied amongst clones in an *E. nitens* clonal seed orchard, suggesting that panmictic pollination was not occurring.

ii) Actual selection intensity - in reality, stratified or phased selection based on flowering could have taken place in the ICFR seed orchards, as flowering of individual trees ranged from 15 to <50% in the year prior to seed collections (Swain et al. 2013a). Trees which do not flower are effectively removed from the population, thereby reducing the population size from which selections can be made. In the case of populations less than 400, the selection intensity decreases with decreasing population size (Becker 1975), thereby decreasing the genetic gain.

4) The assumption that there is no correlation between flowering and growth, i.e., flowering individuals would not be selected against due to their growth, and *vice versa;*

Varghese et al. (2009) found contrasting trends in their studies on *E. camaldulensis* and *E. tereticornis* in India. These authors found a negative genetic correlation between flowering and outstanding growth performance in unimproved provenances in *E. camaldulensis*, but no such correlation was found in an improved seed orchard of *E. teretecornis*. Although there are currently no published results on such studies on *E. nitens* in South Africa, Gardner (pers comm[1]) did not find any correlations between flowering and growth in flowering studies in *E. nitens*, nor has Jones (pers comm[2]) found any strong correlations in *E. nitens* seed orchards.

5) The assumption of the absence of non-additive effects such as inbreeding or heterosis, the latter as a result of mixing of provenances by outcrossing of all families in the F1;

It has been assumed that variance is additive in this *E. nitens* breeding population, with negligible non-additive effects, as has been found in other studies on the species

[1] Gardner RAW. 2012. Institute for Commercial Forestry Research, Pietermaritzburg, PO Box 100281, Scottsville, 3209, SOUTH AFRICA

[2] Jones W. 2013. Shaw Research Centre, Tweedie, PO Box 473, Howick, 3290, SOUTH AFRICA

(Hamilton et al. 2008, Hodge et al. 1996), but if non-additive effects were playing a role, the predictions of genetic gain would be biased.

Therefore, to determine which assumptions best fit the ICFR *E. nitens* populations for the estimation of genetic parameters, several scenarios of predicted genetic gain were developed and compared with the gains realised from genetic gain trials. This study will also indicate whether there are important digressions from the assumptions made in the F1 study (Swain et al. 2013b), and whether the *E. nitens* breeding strategy needs to be adapted to take these into account.

Material and methods

Eight F1 provenance-progeny trials comprising *E. nitens* seedlots imported from Australia were established in the summer rainfall region of South Africa during the 1980s and 1990s. Details of these F1 trials, statistical analysis thereof, estimation of genetic parameters for the F1 and predicted gain for the F2 can be found in Swain et al. (2013b). Six of these trials were subsequently thinned to seed orchards, based on the results of the final measurements, and seed collected over several years to establish progeny trials (F2) of this material (Swain et al. 2013c). In addition, three genetic gain trials were established on temperate forestry sites in South Africa early in 2001 to test the improved material (Swain et al. 2013a). The genetic gain trials also allowed for comparison of realised gain with predicted genetic gain, as well as the calculation of realised heritabilities in the F1 (Swain et al. 2013c).

The realised genetic gains in the F2 were estimated by comparing the performance of the average of a range of improved bulks originating from each of four ICFR seed orchards, i.e., Amsterdam, Helvetia, Jaglust and Jessievale, with an F1 bulk (Treatment 22) in the genetic gain trials described by Swain et al. (2013a). These realised gains were then compared with the gains predicted from a range of scenarios, testing provenance effects, coefficients of relationship and selection intensities, which is the focus of this paper. Three scenarios were investigated.

1. Provenance effect

Two scenarios for variance component estimation were investigated, i.e., families nested within provenance and families across provenance. The F1 genetic parameters estimated for family nested within provenance, to remove provenance effects (Tibbits and Hodge 1998),

differed markedly from those obtained ignoring provenance effects (Swain et al. 2013b). Although the significant F1 provenance differences may be as a result of non-panmictic effects, they may also hint at the presence of additive effects, which will have implications on the design of the breeding and production strategies.

2. Coefficient of relationship and outcrossing rate

Following the approach of Squillace (1974), and extending his calculations, it has been shown that 30% selfing gives an average coefficient of relationship among open-pollinated (OP) progeny of 0.4 (Griffin and Cotterill 1988). Snedden et al. (2007) assumed a coefficient of relationship of 0.33 in an *E. grandis* study where approximately 20% inbreeding was found. Extending this approach, the assumed inbreeding of 20% may be too low, and the assumed coefficient of relationship of 0.33 may be too high in seed orchards where flowering is poor and pollen trees may be isolated from maternal parents. Although levels of relatedness and inbreeding are not available from ICFR seed orchards until DNA genotyping/genetic marker studies have been completed, flowering percentages have ranged from 15 to 47% over orchard and year, in terms of the number of individual trees flowering (Swain et al. 2013a), which could decrease genetic gain achieved. Thus this scenario considered an additional higher level of selfing of 25% (and, by extension, outcrossing rate of ≈ 75%) for both the family within provenance and across provenance parameters estimated above, using a coefficient of relationship of 0.4 as a surrogate for the selfing rate. An assumed selfing rate of 0% (surrogate *cr* = 0.25; assumed outcrossing 100%) was also included for comparative purposes.

3. Selection intensity

Should flowering be poorer in reality, i.e., 40% flowering, this would decrease the population size from which selections are made, given that the population size was less than 400 (Becker 1975), resulting in lower genetic gains.This scenario is represented by actual flowering that occurred in the ICFR seed orchards the year before seed was collected for the ICFR genetic gain trials, and compared with an assumed 100% flowering of the remaining 70% of families after roguing. Due to the incremental selection applied to the seed orchards, incorporating roguing, thinning and bulking of selected seedlots, computation of the selection intensities was also done in an incremental or phased manner. This scenario was run for both the family nested within provenance and across provenance models estimated above, as well as for the different outcrossing scenarios.

Thus, there were 12 different scenarios to be compared, as summarised in **Table 5.1**.

Table 5.1 Predicted gain scenarios investigated to determine which assumptions are most suitable for the estimation of genetic parameters in a *Eucalyptus nitens* population in South Africa

Co-efficient of relationship (*cr*) [estimated outcrossing %]	Scenario Number (Selection intensity type)			
	Family (provenance)		Family across provenance	
cr = 0.25 [≈100%]	1 - Roguing	4 - Actual flowering	7 - Roguing	10 - Actual flowering
cr = 0.33 [80-85%]	2 - Roguing	5 - Actual flowering	8 - Roguing	11 - Actual flowering
cr = 0.40 [≈75%]	3 - Roguing	6 - Actual flowering	9 - Roguing	12 - Actual flowering

Genetic gains were predicted from the following formula (Verryn et al. 2000, adapted) for the rogued scenarios:

$$\Delta G = 0.5(\Delta G_f) + 0.5(\Delta G_m), \text{ where;}$$

$$\Delta G_f = \left[\left(SI_1 \times cr \times \frac{\sigma^2{}_A}{\sigma_{fm}} + SI_{wf} \times \frac{(t-1)}{t} \times (1-cr) \times \frac{\sigma^2{}_A}{\sigma_{wf}}\right) + \left(SI_2 \times cr \times \frac{\sigma^2{}_A}{\sigma_{fm}} + SI_{wf} \times \frac{(t-1)}{t} \times (1-cr) \times \frac{\sigma^2{}_A}{\sigma_{wf}}\right)\right]$$

$$\text{and } \Delta G_m = \left[SI_1 \times cr \times \frac{\sigma^2{}_A}{\sigma_{fm}} + SI_{wf} \times \frac{(t-1)}{t} \times (1-cr) \times \frac{\sigma^2{}_A}{\sigma_{wf}}\right],$$

and the predictions are the predicted genetic gains from female and male selection in a population, respectively.

(SI_1 = selection intensity between/among female or male families, respectively, i.e., number of families remaining in seed orchard after roguing of poor families out of the total original number of families,

SI_2 = selection intensity within female families, i.e., number of families used to make up bulk treatment out of those remaining in seed orchard after roguing,

cr = coefficient of relationship,

$\sigma^2{}_A$ = additive genetic variance,

σ_{fm} = standard deviation between/among families,

SI_{wf} = selection intensity within female or male families, respectively, within plots,

t = number of trees per plot, and

σ_{wf} = standard deviation within families).

The selection intensity for male and females differed as, in addition to roguing and thinning, the top 8 to 16 families were selected to make up bulked seed orchard treatments. Male and female selection intensities between/among families, and within families within plots, were determined using the standardised selection intensity tables of Becker (1975).

The genetic gain equations used for the actual flowering scenarios are similar to those above, except that both the ΔG_f and ΔG_m equations now incorporate the actual number of families flowering, as follows:

$$\Delta G_f = \left[\left(SI_1 \times cr \times \frac{\sigma^2{}_A}{\sigma_{fm}} + SI_{wf} \times \frac{(t-1)}{t} \times (1-cr) \times \frac{\sigma^2{}_A}{\sigma_{wf}} \right) + \left(SI_3 \times cr \times \frac{\sigma^2{}_A}{\sigma_{fm}} + SI_{wf} \times \frac{(t-1)}{t} \times (1-cr) \times \frac{\sigma^2{}_A}{\sigma_{wf}} \right) \right]$$

$$\text{and } \Delta G_m = \left[SI_4 \times cr \times \frac{\sigma^2{}_A}{\sigma_{fm}} + SI_{wf} \times \frac{(t-1)}{t} \times (1-cr) \times \frac{\sigma^2{}_A}{\sigma_{wf}} \right],$$

where;

SI_3 = selection intensity within female families, i.e., number of families used to make up bulk treatment out of those flowering in seed orchard after roguing,

SI_4 = selection intensity within male families, i.e., the proportion of flowering in the seed orchard multiplied by the number of families remaining after roguing out of the total number of families in the orchard prior to roguing, also multiplied by the proportion of flowering in the seed orchard.

The remainder of the variables are defined as for ΔG_f and ΔG_m in the roguing scenario above.

Results and discussion

Comparisons of the predicted gains from the various scenarios to investigate the underlying assumptions used in estimating genetic parameters for the F1 are summarised in **Figure 5.1**, and presented in more detail with the realised gain in **Table 5.2.** There were a few "outliers" in the predicted gains when compared to the realised gains. With regards to the family across provenance scenario for Daspoort, the predicted gains were on average, about three-fold greater than the realised gains. This was due to the particularly high narrow sense heritability (h^2) estimated for this site in the F1 trials (h^2 = 0.58 at 113 months) (Swain et al. 2013b). Similarly, the low predicted gains at Helvetia for the family within provenance scenario differed notably from the higher realised gains, due to a particularly low heritability estimate in the F1 (h^2 = 0.14 at 94 months).

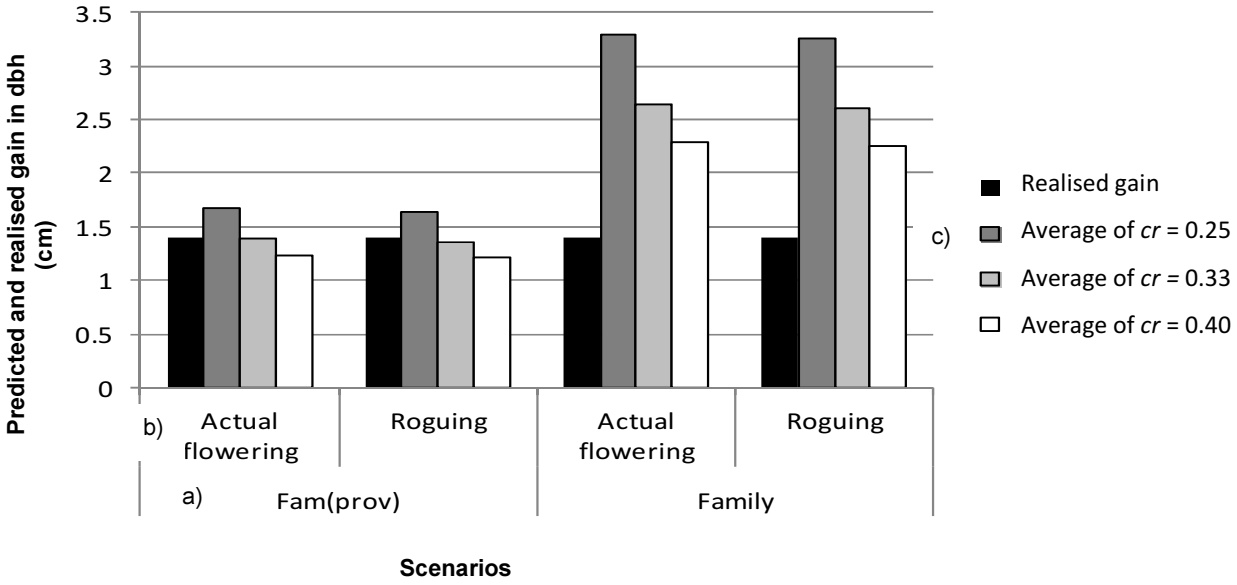

Figure 5.1 Comparison of mean realised gain in diameter at breast height (dbh) (cm) in three *Eucalyptus nitens* genetic gain trials in South Africa with mean gains predicted from six sites for different scenarios, including; a) variance components for family nested within provenance (Fam (prov)) and family across provenance (Family) models, b) Selection Intensity, c) Coefficient of relationship (*cr*)

1. Provenance effect

The gains predicted using family nested within provenance variance component estimates were closer to the realised gains than those predicted using family across provenance estimates. In further discussion, the family across provenance predictions are disregarded, as these scenarios all greatly over-predicted gain (**Figure 5.1**).

Progeny trials are not generally designed to detect heterosis/non-additive effects (Vaillancourt et al. 1995), yet it is unlikely that these effects exist in this population, due to the still significant provenance effect and the lack of provenance rank changes in the F2 (Swain et al. 2013a). Although low levels of dominance effects have been found in selfed seedlots of *E. nitens* (Hardner and Tibbits 1998), Hodge et al. (1996) found negligible levels of non-additive effects in their *E. nitens* study and an absence of inbreeding depression (1%). These authors also found lower levels of non-additive genetic variation in *E. nitens* than in

Table 5.2 Predicted gains in diameter at breast height (dbh) for various scenarios in F1, compared to realised gains of F2 *Eucalyptus nitens* bulk treatments at 87 months over unimproved F1 bulk, from six seed orchards, as estimated from genetic gain trials (Swain et al. 2013a)

		Trial series & site name (age in months at final measurement[1])							
		E88/01 Jessievale at 101 mths (cm) (%)	**E88/01 Amsterdam at 101 mths (cm) (%)**	**E88/03 Daspoort at 113 mths (cm) (%)**	**E88/03 Helvetia at 94 mths (cm) (%)**	**E88/05 Babanango at 112 mths (cm) (%)**	**E88/06 Woodstock at 76 mths (cm) (%)**	**E88/07 Goedehoop at 113 mths (cm) (%)**	**E88/07 Arthur's Seat at 113 mths (cm) (%)**
Realised gain in cm dbh (%)		**1.02 (7.3)**	**1.90 (13.7)**	**Helvetia bulk [2] 1.80 (12.9)**	**1.80 (12.9)**	**Jaglust bulk[3]: 1.40 (10.1)**	**Jaglust bulk[3]: 1.35 (9.7)**	**-[4]**	**-[4]**
Family (provenance)	**Scenarios**	**Predicted gain in cm dbh (% gain)**							
Roguing:	**1 – *cr = 0.25***	1.17 (8.4)	1.84 (13.2)	2.47 (17.7)	0.35 (2.5)	3.00 (21.5)	1.84 (13.2)	3.84 (27.5)	4.00 (28.6)
	2 – *cr = 0.33*	0.99 (7.1)	1.25 (8.9)	2.05 (14.7)	0.32 (2.3)	2.52 (18.0)	1.55 (11.1)	3.07 (22.0)	3.17 (22.7)
	3 – *cr = 0.40*	0.89 (6.4)	0.94 (6.7)	1.82 (13.0)	0.31 (2.2)	2.26 (16.2)	1.40 (10.0)	2.65 (19.0)	2.72 (19.5)
Actual flowering:	**4 – *cr = 0.25***	1.18 (8.5)	1.94 (13.9)	2.49 (17.8)	0.35 (2.5)	3.06 (21.9)	1.87 (13.4)	2.38 (17.1)	2.55 (18.3)
	5 – *cr = 0.33*	1.00 (7.1)	1.36 (9.7)	2.07 (14.8)	0.32 (2.3)	2.58 (18.4)	1.59 (11.3)	1.61 (11.5)	1.73 (12.4)
	6 – *cr = 0.40*	0.90 (6.4)	1.04 (7.4)	1.83 (13.1)	0.31 (2.2)	2.31 (16.6)	1.43 (10.2)	1.19 (8.5)	1.28 (9.1)
Family across provenance									
Roguing:	**7 – *cr = 0.25***	2.11 (15.1)	2.58 (18.5)	8.24 (58.9)	2.29 (16.4)	3.85 (27.5)	1.90 (13.6)	3.74 (26.8)	4.43 (31.7)
	8 – *cr = 0.33*	1.72 (12.3)	1.76 (12.6)	6.43 (46.0)	1.89 (13.5)	3.19 (22.8)	1.61 (11.5)	2.99 (21.4)	3.50 (25.0)
	9 – *cr = 0.40*	1.50 (10.8)	1.31 (9.4)	5.44 (38.9)	1.68 (12.0)	2.83 (20.2)	1.45 (10.3)	2.59 (18.5)	2.99 (21.4)
Actual flowering:	**10 – *cr = 0.25***	2.13 (15.2)	2.71 (19.4)	8.28 (29.2)	2.31 (16.5)	3.91 (28.0)	1.93 (13.8)	2.31 (16.5)	2.88 (20.6)
	11 – *cr =0.33*	1.74 (12.4)	1.88 (13.5)	6.48 (46.3)	1.91 (13.7)	3.25 (23.2)	1.64 (11.7)	1.56 (11.2)	1.95 (14.0)
	12 – *cr = 0.40*	1.52 (10.9)	1.44 (10.3)	5.49 (39.3)	1.69 (12.1)	2.89 (20.7)	1.48 (10.6)	1.15 (8.3)	1.44 (10.3)

[1] Different ages should be noted,
[2] As no seed had been produced from this seed orchard by time of F2 trial establishment, parameters from the related Helvetia orchard were used,
[3] Jaglust is a seed orchard representing the material in the Babanango and Woodstock trials,
[4] No seed had been produced from either of these trials, or related seed orchards, by the time the progeny trials were established

E. globulus, as well as lower levels of deleterious abnormalities (Hodge et al. 1996). Many authors assume negligible or zero non-additive effects in *E. nitens* (Hamilton et al. 2008).

The argument for negligible non-additive effects in this population is strong. The significant provenance effect could be as a result of asynchronous flowering between provenances and resultant lack of panmixis. Asynchronous flowering between provenances in the F1 BSOs would violate the assumption that pollination occurs randomly and that pollen parents are equivalent (Shelbourne et al. 2007), and would result in traits and grouping of genes by provenance in the F2. The lack of panmixis would have an effect on the variance components and heritability estimates, with a possible underestimation of family variance components and resultant heritability, if the parents were actually correlated by provenance (Squillace 1974).

2. Coefficient of relationship and outcrossing rate

With regards to the scenarios using actual flowering for family nested within provenance, which in theory, should be the most accurate predictor of gain, *cr* = 0.33 was closest to realised gain (**Table 5.2**), indicating the presence of some full sibs within the open-pollinated F1 families. This was probably mainly as a result of selfing. This coefficient of relationship was also found to produce predictions closest to realised values for the roguing scenarios. These coefficients of relationship support earlier work by various authors (Griffin et al. 1987, Griffin and Cotterill 1988, Verryn 1993, Hodge et al. 1996), where it was suggested that seed orchard OP families are not true half-sib families, and may contain sufficient full-sib families to increase the coefficient of relationship from 0.25. A coefficient of relationship of 0.33 has been commonly used by many authors for OP eucalypt populations (Borralho et al. 1992), particularly in South Africa (Louw 2006, van den Berg and Stanger 2007, Ndlovu 2008, van Deventer 2009, Verryn et al. 2009). This coefficient of relationship could be used for future prediction of gains in roguing scenarios in *E. nitens*, based on our knowledge and the data presented here.

In addition, the statistical information suggests that outcrossing was >80% in the ICFR seed orchards. This is higher than expected by these authors, given the general sparse flowering in the species in South Africa (Jones 2002, Gardner and Bertling 2005). Hodge et al. (1996) found that estimates of outcrossing in *E. nitens* are higher in seed orchards than in native stands and, following microsatellite studies, Gea et al. (2007) estimated an outcrossing rate

of 0.87 in an OP *E. nitens* clonal seed orchard in New Zealand. Grosser et al. (2010) determined an average outcrossing rate of 85% in a similar orchard in Australia. Thus these suggestions of higher than expected outcrossing in the ICFR seed orchards are not unreasonable, although such indications would need to be verified with molecular marker studies in this *E. nitens* population. It is thought that outcrossing is high in *E. nitens* due to successful late-acting self-incompatibility mechanisms in the species (Hodge and White 1993, Pound et al. 2003).

As variation in the outcrossing rate between families and individuals may obscure differences in breeding values between parents when estimated with OP families (Burgess et al. 1996), it may be worth using family/individual outcrossing rates to better predict breeding values. Hodge et al. (1996), however, found that OP tests predicted breeding values well for *E. nitens,* and better than for *E. globulus*.

3. Selection intensity

The actual flowering scenarios had similar predicted gains to the roguing scenarios. This was initially surprising due to flowering commonly being poor in *E. nitens* in South Africa (Jones 2002, Gardner 2003, Swain and Gardner 2003, Gardner and Bertling 2005), with possible subsequent poor outcrossing and genetic gain. Further investigation, however, showed that although the number of individuals flowering in the ICFR orchards was relatively low in the year prior to the seed collections for the genetic gain trials (15 to 47% (Swain et al. 2013a)), the total number of families flowering in each seed orchard was generally high (59 to 91%). As genetic gains are most sensitive to the family selection intensity (as opposed to within family selection) (Shelbourne 1992), this, together with the self-incompatibility mechanisms that exist in *E. nitens*, may explain the similar predicted gains for the actual flowering and roguing scenarios.

The next logical consideration, i.e., that increased flowering in seed orchards may increase gains due to an increase in population size from which selections are made, bears further consideration. A decrease in outcrossing rates has been linked to a decrease in progeny growth in eucalypts (*E. nitens*: Hardner and Tibbits 1998; *E. globulus*: Hardner and Potts 1995, Patterson et al. 2004; *E. regnans*: Griffin and Cotterill 1988), and it has been shown that flowering above a certain low level may result in increased gains (Varghese et al. 2009, Swain et al. 2013a). It is unclear, however, whether additional flowering above this level will

confer any further benefit. The flowering levels in the seed orchard bulk entries in the genetic gain trials represent the number of individuals flowering in the seed orchard, irrespective of the number of trees flowering per family, which may be as little as one. A future increase in flowering is most likely to result from more trees per family flowering, rather than a marked increase in the number of families flowering. This is because the number of families flowering in the orchards the year prior to making up the bulked entries for the genetic gain trials was relatively high (59 to 91%), compared to the total trees flowering (15-47%) (Swain et al. 2013a). An increase in the number of trees per family flowering would lead to an increase in gain if the best trees per family flowering were selected. Should an increase in the numbers of families flowering be possible in those orchards with less families flowering (e.g., 59% at Helvetia and 67% at Jaglust), this, together with selection of the best trees per family flowering, would lead to even greater gains.. It is likely that outcrossing would also be improved with increased flowering.

The numbers of families used to make up the bulked entries in the genetic gain trials were small and ranged from 8 to 16 families per bulk (Swain et al. 2013a). Although these bulks could certainly be used for establishment of high productivity plantations, it is more likely that, due to the shortage of improved *E. nitens* seed in South Africa, commercial bulks would comprise at least 16 families. By contrast then, this would mean a decrease in the selection intensity in the seed orchards, with a resultant slight decrease in genetic gain.

Although superior individuals can be grafted from tree improvement trials into clonal seed orchards (CSOs) for capture of maximum genetic gain, problems associated with grafting of *E. nitens* (de Little et al. 1992, Moncur 1998) can cause delays in production from CSOs, making the South African forestry industry reliant on production of improved seed from breeding seed orchards (BSOs). It is therefore important to construct BSOs with both sufficient families and individuals per family, as well as adequate numbers of families flowering simultaneously (or with periods of overlapping flowering), to ensure sufficient outcrossing and to actually realise potential gains. Flowering and seed orchard research that results in technologies to improve and stabilise flowering (Gardner et al. 2011, Gardner 2012, Gardner and Germishuizen 2012, Germishuizen and Gardner 2013) will help to make this possible.

Although it is difficult to test whether panmixis occurred in these populations without a molecular study, Hodge and White (1993) argued that deviations from panmixis should have little or no effect on the average genetic value of an orchard crop. They suggested that crosses that were absent, or present in unequal frequencies, would only negatively affect overall genetic quality of a seed crop if: a) Specific Combining Ability (SCA) effects are large relative to General Combining Ability (GCA) effects; b) a specific clone crosses more with one or a few clones than with others; and c) the SCA effects of crosses are negative due to the occurrence of a rare event. However, other researchers differ on this point (Squillace and Goddard 1982, Moncur and Boland 2000, Grosser et al. 2010), and maintain that deviations from panmixis can affect the genetic worth of seed.

The prediction of gain using various scenarios was onerous without the use of a modelling programme, and future modelling of gains in ICFR trials will be done with the assistance of such.

Conclusions

Parameter estimations using family nested within provenance components in the F1 *E. nitens* population resulted in predicted gains closest to the realised gains, whilst those estimated from family across provenance overestimated gain. This is similar to what has been found in many other eucalypt species. A coefficient of relationship of 0.33 appears to fit well in parameter estimation and gain predictions for this population of *E. nitens*. Levels of selfing appear to be low (<20%), and indications are that levels of outcrossing may be over 80%, despite poor flowering of the species in South Africa. It is clear that molecular studies in the seed orchards and resultant progeny would provide an effective tool to monitor outcrossing rates and the role of male and female parents in the orchards, as well as to determine whether panmixis is occurring. This information would allow for refinement of the models over time if necessary.

The stratified or phased selection which is likely taking place within the flowering trees in these seed orchards, may actually be decreasing gains and thus, in reality, the selection intensities used here should be revised downwards. This implies that even greater gains are possible if flowering can be increased. It is therefore important firstly, to construct seed orchards with sufficient numbers of families, sufficient individuals per family and adequate numbers of synchronously flowering families; and secondly, to apply technologies to increase

flowering, not only to support outcrossing, but also to decrease selection intensity, such that maximum potential gains can be realised.

Although the progeny trials were not designed to detect heterosis/non-additive effects, it is unlikely that these exist in this population, due to the still significant provenance effects and the lack of provenance rank changes in the F2. It is suggested, therefore, that it is not necessary to keep provenances separate in current and future ICFR seed orchards, which will make practical management of seed orchards easier.

This study has provided an objective and quantitative assessment of the underlying assumptions used for estimating genetic parameters and predicting gain in this population of *E. nitens.* It can be concluded that the assumptions used in the F1 study were correct and no adjustments are necessary to that step in the breeding programme.

Acknowledgments

Masonite Ltd., Mondi Ltd, NCT Forestry Co-Operative Ltd., PG Bison, TWK Ltd. and York Timbers Ltd. are gratefully acknowledged as funders of this project.

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CHAPTER 6[1]

Genetic gain as a function of breeding and production strategies in *Eucalyptus nitens*

T-L Swain[2]*, SD Verryn[3], MD Laing[4]

[2] *Institute for Commercial Forestry Research, PO Box 100281, Scottsville, Pietermaritzburg 3209, South Africa.*

Tammy.Swain@icfr.ukzn.ac.za

Ph: 27-33-3862314 Fax: 27-33-3868905

[3] *Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa and Creation Breeding Innovations, 75 Kafue St, Lynnwood Glen, 0081, South Africa.*

[4] *School of Agricultural Earth and Environmental Sciences (SAEES), University of KwaZulu-Natal, PO Box X01, Scottsville, 3209, South Africa.*

* Author for correspondence: T-L Swain

Telephone: +27 33 386 2314

Fax: +27 33 386 8905

Tammy.Swain@icfr.ukzn.ac.za

[1 **Published as:**
Swain T-L, Verrryn SD, Laing MD. Genetic gain as a function of breeding and production strategies in *Eucalyptus nitens*. *ICFR Bulletin Series* No. 13/2013, Institute for Commercial Forestry Research, Pietermaritzburg.]

Summary

Two generations of breeding in *Eucalyptus nitens* in South Africa have resulted in a more thorough understanding of the factors necessary for extending an advanced generation breeding strategy for the species. Modelling of predicted genetic gain, using the algorithm *G-Assist (Version 4.0)* for various breeding strategy scenarios, played an important role in developing the advanced generation breeding strategy. In addition, the modelling exercise highlighted various management options which can be used to increase gains in the existing production populations or orchards in the short term. Indications are that additional roguing of; 1) existing clonal seed orchards based on results of F2 trials, and 2) F1 breeding seed orchards based on stricter provenance selection, will markedly increase the quality of the seed produced from these orchards within one season, i.e., 21.7% increase in diameter at breast height for stricter roguing of clonal seed orchards versus 16.3% for the current rogued situation. This is important, as establishment and management of improved material in seed orchards to ensure a sustainable supply of improved seed to the South African forestry industry is a key objective of the Institute for Commercial Forestry Research *Eucalyptus nitens* breeding programme. The study also highlighted the importance of shortening the breeding cycle in *Eucalyptus nitens*, particularly in view of the delays caused by reticent flowering and seed production in the species.

A proposal has been made for an advanced generation breeding strategy in *E. nitens*, using parental reconstruction of open-pollinated progeny to secure pedigree information of forward selections. A simulation using *G-Assist (Vs 4.0)* predicted gains of 7.3% in the breeding population, with an additional 26.4% in the proposed bi-clonal production population for diameter at breast height, or approximately 2.8% per year over 12 years. This strategy thus combines the benefits of significantly increased genetic gain with a shortened breeding cycle.

Keywords

E. nitens, predicted gain, deterministic methodology, modelling, breeding strategy

Introduction

A breeding programme for *Eucalyptus nitens* has been in place at the Institute for Commercial Forestry Research (ICFR) for over two decades. *Eucalyptus nitens* is an important commercial cold tolerant species in the summer rainfall region of South Africa and is considered to be the most cold and snow tolerant of the eucalypt species grown in this country. Although grown primarily for pulp and paper production in South Africa, *E. nitens* has a range of alternate end product uses such as mining timber, veneer and solid wood products (Cele et al. 2012). The potential area for optimum growth of the species within the current summer rainfall forestry growing region is 119 304 ha (National Land Cover 2000, Smith et al. 2005), which is 8% of the summer rainfall plantation area in South Africa. Currently, approximately 46 000 ha of *E. nitens* are grown commercially in South Africa (Germishuizen pers comm[1]).

Early species and provenance/progeny trials showed that significant variation exists between the Australian provenances grown in South Africa for a range of traits (Darrow 1996, Swain et al. 1998, Gardner 2001, Swain et al. 2013b), which points to potential for improvement for a wide range of sites and end products. The ICFR *E. nitens* breeding programme, comprising a series of F1 and F2 trials, seedling and clonal (grafted) seed orchards (BSOs and CSOs, respectively) and genetic gain trials, has shown that significant improvement in growth has been realised in the F2 over the F1 (Swain et al. 2013a).

Tree improvement in *E. nitens* has its difficulties, however, because the species is a reticent and inconsistent flowerer (Gardner 2003, Pound et al. 2003), causing delays in the breeding programme and restricted and variable mating of genotypes; flower buds are small (Boland et al. 1992), making emasculation and controlled-pollination tedious, time-consuming and expensive (Hamilton et al. 2008); the species does not coppice reliably (Little and Gardner 2003) for vegetative propagation; and micro- and macro-propagation cutting techniques typically have a low rooting success (de Little et al. 1992, Griffin 2001). However, grafting of selections can be done, allowing for the capture of superior genotypes in grafted CSOs. In South Africa, production of seed for both breeding and commercial purposes is heavily reliant on the siting of seed orchards for cumulative cold prior to appearance of flower buds, and the application of the plant growth retardant paclobutrazol (Gardner and Bertling 2005).

[1] Germishuizen I. 2012. Institute for Commercial Forestry Research, Pietermaritzburg, PO Box 100821, Scottsville, 3209, SOUTH AFRICA

Research and operational experience have shown that application of these and other seed orchard management techniques, such as topping of grafts from an early age, spacing of seedling trees and canopy management, can increase both flowering and subsequent seed production (Reid et al. 1995, Moncur and Boland 2000, Gardner and Bertling 2005). Despite extensive research into alternative deployment strategies for *E. nitens,* improved genotypes are still almost universally deployed as seedlings derived from open-pollinated (OP) seed orchards (Hamilton et al. 2008).

In addition to improvement of growth, one of the main objectives of the ICFR breeding programme in recent years has been to develop a more formal advanced generation breeding strategy for *E. nitens*. This includes research into:

- The role of genotype by environment interaction (GEI);
- Determining the role that original Australian provenances play in gains achieved in advanced generations, and how to manage this;
- Estimating genetic parameters for the species and investigating the assumptions which underlie the estimation of these parameters;
- Realised versus predicted gain, and factors affecting actual gain;
- The impact that flowering in *E. nitens* has on realised gain.

Once an understanding of these areas has been achieved, a management plan for the current ICFR trials and seed orchards can be developed and implemented, whilst simultaneously expanding the breeding strategy.

There are many options which must be considered when developing a breeding and production strategy, and predicting the optimal option or combination of options, requires a large number of calculations involving many variables. Thus modelling the options or scenarios using computer programmes/software is more efficient, less time-consuming and allows less room for error, than doing so manually. Programmes or algorithms have been developed to estimate genetic gains using different methodologies (Mullin and Park 1995, Rezende and Olivieria 1997, Verryn et al. 2000, McRae et al. 2004), and these enable comparison of a range of scenarios to determine which strategy is best suited to a breeder's purpose in terms of genetic gain, length of breeding cycle, population size, for example.

A range of scenarios were modelled for the ICFR *E. nitens* breeding and production populations to assist with decisions as to which strategy or management actions will deliver the most genetic gains per unit time with the given biological, logistical and budgetary constraints. A strategy that is based on open pollination (due to difficulties with controlled pollination (CP) in the species), but with the advantages of CP in terms of increased gains, and allowing for infusion of new or additional genetic material as necessary, may be best suited for this species.

This paper summarises the current understanding in the five research areas, investigates various strategies and management scenarios, and proposes a plan for managing current and future ICFR breeding and production populations to optimise genetic gain and breeding cycle length.

Materials and methods

Breeding and production population scenarios

Diameter at breast height (dbh) was measured at 87 months in two *E. nitens* F2 field trials in Mpumalanga (In de Diepte) and KwaZulu-Natal (Mt Gilboa) in South Africa (Swain et al. 2013c). Genetic parameters were estimated for the F2 population and Breeding Values (BV) predicted for dbh. As Type B genetic correlations of 0.61 indicated the presence of GEI for the two F2 sites (Swain et al. 2013c), the two trials are being managed separately in terms of selections and future seed production. The individual heritability estimates were 0.06 and 0.17 for dbh at In de Diepte and Mt Gilboa, respectively, with standard errors for heritability being larger than the estimated heritability at the former site (Swain et al. 2013c). For this reason, only the Mt Gilboa site, with relevant genetic parameters, was considered for the rest of this study.

Several scenarios were investigated to determine which is most likely to provide the greatest gains in an optimal time period from the current F2 trials and F1 and F2 seed orchards (or production populations). The genetic gains were largely calculated using the deterministic modelling algorithm *G-Assist (Version 4.0)* (Verryn and Snedden 2007), which facilitates the comparison of predicted gains for different tree breeding strategies and relies on quantitative genetic theory to predict the parameters of interest. The algorithm is based on modified and combined forms of the genetic gain equations of Shelbourne (1992) and Gea (1997), and

uses the selection intensity tables developed by Becker (1984). *G-Assist (Vs 4.0)* is able to serve generically for the six breeding and 11 production strategies described by Shelbourne (1992). Each scenario requires information that describes the breeding population and the selection strategy to be followed in both the breeding and production populations. In addition, the production population scenarios also require information about the progeny test, such as number of progeny tested, heritability estimates and phenotypic standard deviations (Verryn et al. 2000, Hettasch et al. 2007). Although the programme predicts percentage gains from the various scenarios, the primary use of the gains in this study was to offer guidance on different strategies by providing relative gains, rather than absolute figures. The numbering system used hereafter is that used by Verryn et al. (2000) to refer to Shelbourne's scenarios when using the algorithm *G-Assist (Vs 4.0)*.

Appropriate OP breeding strategies were selected, from those described by Shelbourne (1992), for genetic gain predictions. Only two breeding population options involving OP seed were considered due to the difficulty of i) cloning sufficient numbers of *E. nitens* selections for trials and ii) making controlled crosses to obtain full sibs. These were:

i) B1.2 – Breeding population of open-pollinated progenies, where the best families are selected and, following thinning and roguing, seed is collected from the best individuals within each family to establish the next generation breeding population. Both between- and within-family selection are taken into account here.
ii) B1.3 – Breeding population of open-pollinated families ex archive, where selected parents (the best individuals within the best families) are grafted and planted into a clonal archive to produce improved seed for the establishment of the next generation. There is better control of the pollen source in this seed, and both between- and within-family selection are taken into account.

Production populations included options of clonal and seedling seed orchards, clone or seed production, and roguing, thinning or no treatment of orchards. Forward selection (FS - selection of the best individuals within the best families, based on information gathered from the individual and its family (siblings)) and backward selection (BS – selection of parents based on the performance of their progeny) (Shelbourne et al. 1989) were additional options for the production populations. The production population options tested were:

i) P2.1 – Half pedigreed open-pollinated seedling orchard/ forward selection (FS) from current F2 trials. The OP progeny test (F2) is converted to a seedling seed orchard once

the progeny test role has been completed, this achieved by thinning to the best tree/plot and roguing the poorest 30% of families. This incorporates both between- and within-family selection.

ii) P2.9 – Seed orchard from FS/ FS from current F2 BSOs. The best families and the best trees/family are selected in a BSO that was established at the same time as the breeding population. As with P2.1, the BSO is thinned to the best tree per plot. A modification to the scenario described by Shelbourne (1992) and Verryn et al. (2000) is that in the case of this *E. nitens* population, the 30% poorest families were rogued from the seed orchard, thus incorporating both beween- and within-family selection.

iii) P2.4 – CSO from FS/ FS using CSOs. This CSO is similar to P2.9, but with a higher family selection intensity when individuals are grafted from top families into a CSO. Both between- and within-family selection are included.

iv) P2.6 – CSO from FS and roguing/ backward selection (BS) using FS CSOs. The CSO from P2.4 is thinned (rogued), based on the performance of the progeny of the clones in the new OP breeding population/progeny test.

The breeding and production population options are presented in **Table 6.1**. The current ICFR breeding programme and applications of these scenarios and combinations thereof, are summarised in **Figure 6.1**. Each breeding population option was modelled with each production population where appropriate, resulting in five scenarios being tested. Modelling the breeding population of OP families ex archive (B1.3) was only effective with production population P2.6 (representing CSOs from FS and roguing (BS)). In the cases of production populations P2.4 and P2.9, predicted gains would be the same as for breeding population of OP families (B1.2).

The parameters used in the modelling of the scenarios were established by Swain et al. (2013b, 2013c, 2013d), and are presented in **Table 6.1**. Modelling was done using parameters for only one site, as the objective was to identify those strategies with the highest gains, not to provide actual predictions of gain per site. The narrow sense heritability estimate (h^2) for dbh was highest at the F2 Mt Gilboa site (h^2 = 0.17), and thus the parameters for this site were used, as a higher heritability estimate is more reliable and will slightly favour FS options. It was assumed that non-additive effects were not significant. Gains were predicted on the basis of two-stage between- and within-family selection, and were calculated separately for male and female parents so that different selection intensities

could be applied to each. In a few of the scenarios, it was not possible to predict genetic gains in one iteration of the programme, i.e., where male and female selection intensity differed between selection within a plot and over the whole family, respectively. In these cases, the algorithm was run more than once to accurately capture the gains attributed to male and female selection, respectively.

The genetic gain equations used to capture the different scenarios are presented in **Appendix 3**. In terms of breeding cycle length, the use of juvenile-mature genetic correlations for mid-rotation selections to shorten the breeding cycle was assumed (Swain et al. 2013b). In addition, the use of seed orchard management techniques such as consecutive thinning of trees within plot in the BSO from age 2, the application of paclobutrazol after final thinning in BSOs at age 5 or 5.5 years, and topping and application of paclobutrazol in CSOs to promote early flowering and seed production has been assumed in all scenarios. Thus sufficient seed production after thinning was assumed at age 9 in BSOs, and seed production after grafting of selections and establishment of grafts in CSOs was assumed three to five years after establishment of grafts infield.

The modelling of the F2 predicted gains also provided insight into the optimal management of current F1 CSOs.

Selections

The predicted genetic worth of families and individuals within families was used to rank and select individual trees, in combination with a rigorous phenotypic evaluation for stem form and pathogen incidence.

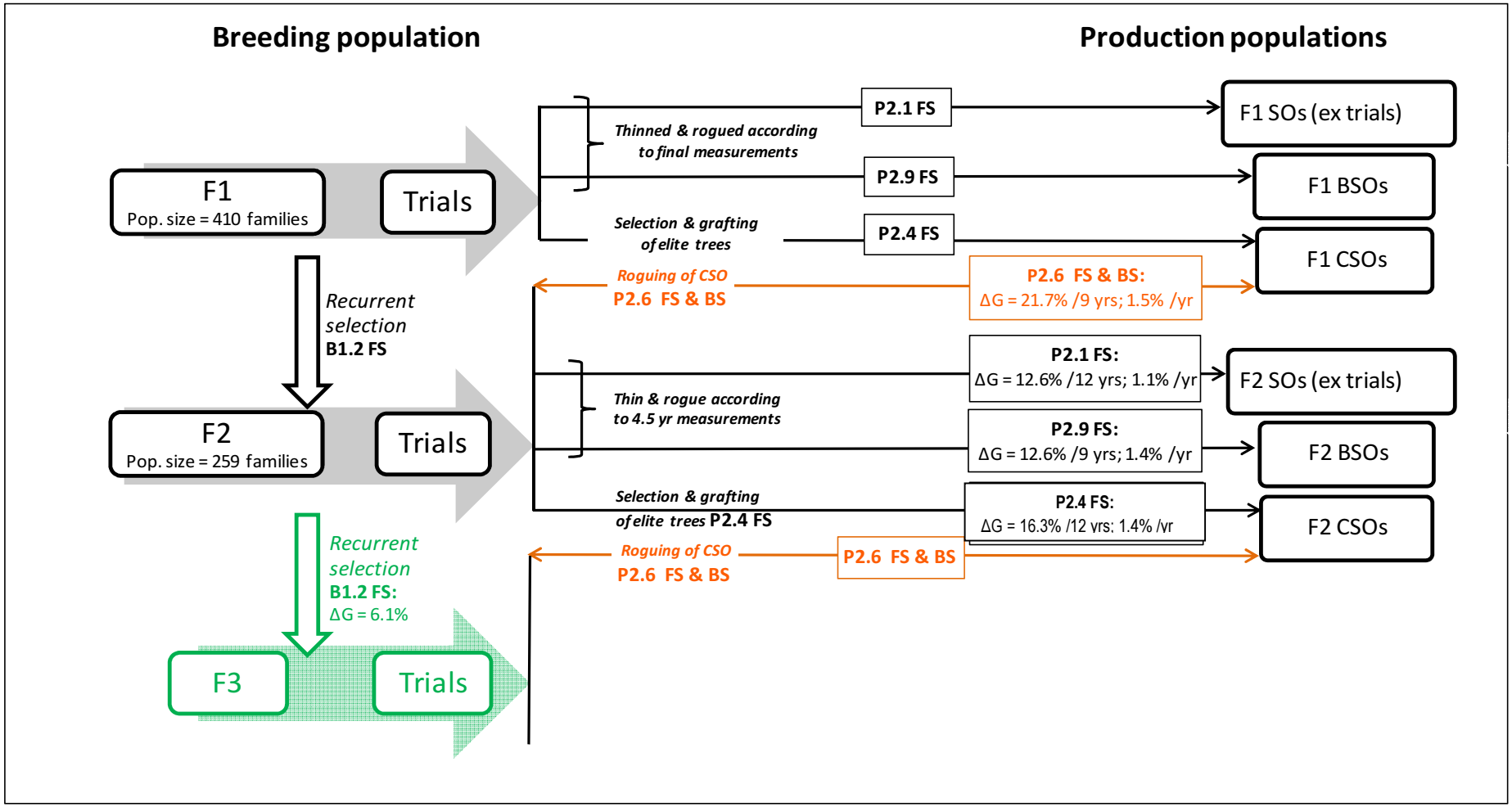
Table 6.1 Parameter inputs for prediction of percentage gains in diameter at breast height (dbh) of *Eucalyptus nitens* for a range of breeding and production population scenarios

Generic population parameters across scenarios	Values	Generic population parameters across scenarios	Values
Breeding population size	80 families	Effective population size (assuming 75% of families flowering) (Swain et al. 2013d)	60 families
Mean of current breeding population	15.36 cm (Swain et al. 2013c)	Mean of previous production population	14.46 cm (Swain et al. 2013b)
Narrow sense heritability (h^2) ± SE[a] for dbh (F2)	0.17 ± 0.067 (Swain et al. 2013c)	Phenotypic standard deviation (F2)	4.2 (Swain et al. 2013c)
Assumed h^2 estimate for dbh (progeny tests) [1]	0.15	Assumed phenotypic standard deviation (progeny tests) [1]	3.8
Coefficient of relationship (*cr*)	0.33 (Swain et al. 2013d)	Assumed inbreeding [2]	0.20 (Swain et al. 2013d)
Number of trees per family	24	Number of trees/ plot (number of replications)	6 (4)
Roguing of poor families	30% = 56	Thinning per plot (within family)	1 in 6 retained

Selection intensities for breeding & production population scenarios (with scenario numbering as per Verryn et al. (2000))[3]					
Production population scenarios	**FS[4] from current F2 trials (P2.1)**	**FS from current F2 BSOs[5] (P2.9)**	**FS using CSOs[6] (P2.4)**	**FS using CSOs and roguing (BS[7]) (P2.6)**	**FS using CSOs and roguing (BS) (P2.6)**
Breeding population of OP[8] families (B1.2):					***Breeding population of OP families ex archive (B1.3):***
Female among / within family selection	42:60 / 2:24 [9]	42:60 / 2:24 [9]	42:60 / 2:24 [9]	42:60 / 2:24 [9]	40:60 / 2:24 [9]
Male among / within family selection	42:60 / 1:5 [10]	42:60 / 1:5 [10]	42:60 / 1:5 [10]	42:60 / 1:5 [10]	40:60 / 2:18 [11]
Production population:					
Female among / within family selection	42:60 / 3:3 [12]	42:60 / 3:3 [12]	30:60 / 2:24	30:60 / 2:24	30:60 / 2:24
Male among / within family selection	42:60 / 3:3	42:60 / 3:3	30:60 / 2:24	30:60 / 2:24	30:60 / 2:24
Additional thinning/roguing (taking 75% flowering into account)	1:6 (1:5)	1:6 (1:5)	-	30 /42	20/30
Total breeding & production cycle length	12	9	12	15	18

[a] Standard error of heritability estimate
[1] These were assumed for the next generation, using the F2 values less 10%, allowing for reduction in heritability over generations,
[2] Although this has not yet been established using molecular studies in the ICFR's *E. nitens* breeding population, indications are that outcrossing in these populations is high, i.e., ≥80%,
[3] Number of families/trees/clones selected : effective population size considering flowering where relevant,
[4] Forward selection,
[5] Seedling Seed Orchard,
[6] Clonal Seed Orchard,
[7] Backward selection,
[8] Open-pollinated,
[9] Number of trees/ family (24), as family means based on all 24 trees,
[10] Number of trees/plot (6), decreased to take 75% flowering into account,
[11] Number of trees/ family (24), decreased to take 75% flowering into account,
[12] 4 trees/family remaining after thinning, decreased to take 75% flowering into account

Figure 6.1 Summary of the current ICFR breeding programme with applications of various scenarios and predicted gains from four production populations, as described by Shelbourne (1992). Numbering system is that used by Verryn et al. (2000) to refer to Shelbourne's scenarios when using the algorithm *G-Assist (Vs 4.0)* to model the scenarios



'B' refers to Breeding Population,
SO – seedling orchards,
yr - year

'P' refers to Production Population,
BSO – breeding seed orchard,

FS – forward selection,
CSO – clonal seed orchard,

BS – backward selection,
ΔG – total gain in breeding cycle,

Results and discussion

Breeding and production population scenarios

Predicted gains in dbh, as measured by both total percentage increase over the whole breeding cycle, and percentage increase/year, are presented for the five tested scenarios in **Table 6.2**. The gains are presented as an increase over the previous production population (F1). The breeding population of OP families (B1.2) provided a shorter breeding cycle than the breeding population of OP families ex a clonal archive (B1.3), and thus gains per year were generally better in the former scenario. The gains predicted with the breeding population of OP families (B1.2) are also included in **Figure 6.1**. Modelling the breeding population of OP families (B1.2) with the production population P2.6 (representing CSOs from both FS and roguing (BS)) predicted the highest percentage gains over the whole breeding cycle (21.7%). This was followed by production population P2.4 (CSOs from FS) (16.3%). Gains would be expected to be higher in a clonal production population, and the use of BS (roguing) increased gains still further. However, when breeding cycle length was taken into account, the BSO from FS with thinning and roguing (P2.9) predicted similar gains per year to the two clonal scenarios, due to the decrease in breeding cycle length. Although selection intensity in the CSO was far greater than in the BSO (30:60 compared to 42:60) and grafts flower before seedlings due to the maturity of the scion (Moncur and Boland 2000), it still takes a few years to capture the majority of selections successfully into a CSO. This time lag allows seedlings in a trial or BSO situation, which have been thinned from an early age and treated with chemicals such as paclobutrazol, to flower before the grafted selections, thus increasing gain per unit time. The complete breeding cycle lengths from the OP breeding population ranged from nine to 15 years (**Table 6.2**).

With regards to the breeding population of OP families ex archive (B1.3), the CSO from FS and roguing (P2.6) predicted good gains of 21.2%, but due to the longer breeding cycle length of 18 years, this only provided 1.2% gains/year (**Table 6.2**).

Estimates of gain can be inaccurate unless all major components are included, and accuracy is improved by accumulating gain at each stage of selection (Namkoong et al. 1966). The equations developed by Shelbourne (1992) and Gea (1997), and used as the basis for *G-Assist (Vs 4.0),* incorporate both these aspects. The gains also rely on the accuracy of the components utilised in the equations which, in this case, were the heritability estimates and phenotypic standard deviation of the population under discussion (**Table 6.1**).

Table 6.2 Percentage gains in diameter at breast height for *Eucalyptus nitens*, as predicted by a range of breeding and production population scenarios

Scenario (with scenario numbering as per Verryn et al. (2000))	Breeding cycle length[1] (years)	Total predicted increase in dbh over previous production population (%)	Predicted increase per year of breeding cycle (%)	Advantages and disadvantages
Breeding population of OP[2] families (B1.2)		6.1		
With production populations:				
FS[3] from current F2 trials (P2.1)	12	12.6	1.1	Minimal costs, low gain/unit time.
FS from current F2 BSOs[4] (P2.9)	9	12.6	1.4	Faster immediate gains, costs average.
FS using CSOs[5] (P2.4)	12	16.3	1.4	Good gains, intermediate time. Costs higher due to grafting.
BS[6] using FS CSOs and roguing (P2.6)	15	21.7	1.5	Slower gains, but large when realised. Costs higher due to grafting.
Breeding population of OP families ex archive (B1.3)		8.5		
With production population:				
BS using FS CSOs and roguing (P2.6)	18	21.2	1.2	Large gains, but slow and thus lower gains/unit time. Costs higher due to grafting in both populations.

[1] Breeding cycle length = breeding cycle + production cycle,
[2] Open-pollinated,
[3] Forward selection,
[4] Seedling Seed Orchard,
[5] Clonal Seed Orchard,
[6] Backward selection

Additional increases in timber gain can be achieved in the production populations involving backward selection by further decreasing the number of clones or families remaining in the CSOs (production populations) after backward selection (P2.6). This could also be done in the FS CSOs, however, as relatedness is not known in the FS scenarios, this is risky. The use of DNA genotyping to establish relatedness would be useful in these cases.

Current F1 seed orchards/production populations

The OP breeding population scenario most closely resembles the ICFR's current *E. nitens* OP breeding population, and the production populations P2.1 (half-pedigreed OP seedling orchard), P2.4 (CSO from forward selection) and P2.9 (seedling orchard from forward selection with thinning and roguing) most resemble the production populations. Although future production populations can be developed for deployment of maximum gains in South Africa, the reality is that there are currently insufficient quantities of improved *E. nitens* seed available to the Forestry Industry. This means that F1 seedling orchards are likely to continue supplementing seed supplies for the future decade, as will lower producing orchards (in terms of genetic gain), i.e., trials converted to BSOs, etc. This is common globally, although the seed produced from individual seedling seed orchards is thought to have improved over time in terms of genetic quality, due to roguing and/or selective harvest of genotypes (Hamilton et al. 2008).

With this in mind, various management options are proposed to increase gains in the existing production populations:

1. Current F1 FS CSOs should be rogued, based on the results of the F2 progeny trials. This would upgrade the CSOs from "CSOs from forward selection (P2.4)" to "CSOs from forward selection and roguing (B2.6)", with subsequent increases in gain by an estimated 5%. Although the delay caused by waiting for measurement of the F2 trials is generally considered a disadvantage of production option P2.6, this would not be the case in *E. nitens*, where (i) the juvenile-mature genetic correlations of dbh at four and a half years with dbh at eight or nine years are high ($r_g \geq 0.91$) (Swain et al. 2013b), thus allowing roguing of CSOs to be done based on four-year F2 trial measurements, (ii) the number of years required to successfully graft sufficient numbers of selections into the CSOs would probably be three years, and (iii) flowering in the CSOs, even using flowering-enhancement techniques, is unlikely to occur before three years of age.

2. Current F1 FS CSOs (as described in point 1.) could be utilised to establish CSOs from BS.

   The best parents from the F1, based on measurements of the F2 trials, should be grafted from the F1 CSOs into new BS CSOs. This will result in higher gains from the new BS CSO, as selection has been based on two generations of improvement, although the gains will be delayed somewhat due to grafting and establishment of this CSO. An alternative to this is to rogue the poorer parents from the existing CSOs, allowing a rapid increase in gain in the next seed crop produced, as the current ICFR CSOs are well stocked and are already producing FS seed. This is not always possible in all CSOs, if there are not sufficient trees to withstand a roguing.
3. Over the past two years, one of the F1 FS CSOs burnt down (Blyfstaanhoogte), and a second will be abandoned due to poor flowering and very low seed numbers per capsule, despite application of flowering-enhancement technologies (Goedehoop A). The selections in the Blyfstaanhoogte CSO will be re-accessed where possible, from old F1 trials (now converted to seed orchards); and the selections in the Goedehoop A CSO are being grafted onto a site with higher levels of cumulative winter chill, which may be more suitable for flowering (Gardner and Bertling 2005). Both these operations, although costly, provide the opportunity for BS and roguing of poor parents from the CSOs by re-grafting only those F1 parents that have performed well in the progeny tests.
4. With regards to existing F1 seed orchards, genetic parameter estimations have indicated which of the F1 trials (now converted to seed orchards) have the highest heritability estimate, implying that the most gain will be made by making selections and producing seed from these orchards. In most cases, this has already been done, with the exception of two seed orchards from the E88/07 trial series, Goedehoop and Arthur's Seat. These trials had moderate to high heritability estimates for dbh at 113 months (h^2 = 0.21 and 0.30, respectively) and resultant high predicted gains, but seed had not been produced from these trials or related seed orchards by the time of establishment of the F2 trials (Swain et al. 2012b). This was due to poor (or no) flowering in the seed orchard and accidental felling of the Goedehoop trial before selections could be made and grafted. It is unfortunate that these two trials had the highest predicted gains of all the F1 trials. Since then however, the felled Goedehoop trial has been coppiced and converted to a seed orchard, with the first heavy flower bud production occurring early in 2013. Although it is difficult to make selections at

this stage, as the original trees are no longer standing for phenotypic inspection, this will at least allow for utilisation of FS seed in progeny trials. The Arthur's Seat trial is also being rehabilitated and it is hoped that late selections will be possible, for capturing by grafting.

5. Australian provenance effects were significant in the F1 (Swain et al. 2013b), and still played a significant role in the F2 as the grand-maternal effect (Swain et al. 2013c). This suggests that additional selections could be made from the top-performing provenances of Barren Mountain and Barrington Tops in the F1 trials (now seed orchards), to further increase the gain from the F1. Perusal of the F1 data indicates that an additional 10 to 15 selections can be made, should those trees still remain standing. However, to prevent narrowing of the genetic base, selections from other provenances should still be retained in the production population.
6. Existing F1 BSOs still retain a high stocking of families across all provenances (≈ 70%), as it was uncertain whether BSOs would have to be managed for keeping families separate by provenance. Due to the indications that heterosis has not occurred within this population (Swain et al. 2013c), it is unlikely that it will be necessary to keep provenances separate in current and future ICFR seed orchards. Such a decision should be supported by formal studies to determine the absence or non-significance of non-additive provenance effects and a DNA genotyping study to determine outcrossing levels and to ensure that inter-provenance crossing actually did occur. Until then, however, the F1 BSOs can be retained with their current provenance diversity, but should undergo further roguing or thinning in certain BSOs with high stocking. This applies particularly to Daspoort (E88/03) and Jaglust (BSO representing trial series E88/06 and E88/07).

F2 and F3 – moving forwards

Breeding cycle length

It is possible to achieve establishment, selection and mating cycles of approximately 10 to12 years in *E. nitens* (de Little et al. 1992, Griffin 2001). However, biological constraints associated with flowering and seed production have resulted in delays in generation turnover in breeding strategies where breeding populations are maintained in discrete generations (Hamilton et al. 2008). This has lengthened breeding cycles beyond optimal time-frames in most breeding programmes, particularly so in the ICFR's breeding programme, and in this case, has resulted in disjunct generations of trials. In breeding programmes with generation

intervals ranging from five to 15 years, a delay of even one year represents a 5 to 20% decrease in gain per unit time (Borralho and Dutkowsi 1998). It has become apparent that shortening the breeding cycle plays a key role in increasing gain in *E. nitens*. Several ways this can be addressed are:

1. Utilising juvenile-mature genetic correlations so that individual tree and family selections can be made as early as five years. High genetic correlations of greater than 0.90 between dbh measurements at 52 to 62 months after establishment, and dbh measurements at 94 to 113 months, were found in the F1 of this *E. nitens* breeding programme (Swain et al. 2012b). This suggests that the breeding cycle could be decreased by at least three to four years, with selections based on growth traits being made for seed or vegetative production after the mid-rotation measurement. This would increase gains/year from 1.0% to 1.4%. In addition, time will also be gained by timely thinning of seed orchards to promote early flowering and subsequent seed production. Earlier juvenile-mature correlations of three year dbh measurements with full-rotation measurements are encouraging, and should be investigated further (Swain et al. 2012b).
2. Improving the grafting success of superior individuals so that all selections can be captured in CSOs within two years. This improvement is anticipated with the upgrade of the ICFR grafting tunnel with a fogging system, which will hold the relative humidity stable during the hot, dry winds that can be experienced in Pietermaritzburg during August and September, the two months following grafting of *E. nitens*.
3. The use of new flowering-enhancement technologies to encourage early flowering in seed orchards and to improve seed production. This includes more appropriate siting of seed orchards to achieve winter chilling (Gardner and Germishuizen 2012, Germishuizen and Gardner 2013) and the use of precocious rootstock when grafting elite selections (Adejumo et al. 2012). Placing of the breeding population/progeny trials on sites which are conducive to flowering will also improve flowering if/when those trials are converted to seed orchards at full rotation.

F2 selections

The predicted genetic worth of families and individuals within families was used to rank and select individual trees at Mt Gilboa and In de Diepte. Sixty-eight and 75 superior *E. nitens* individuals were selected at Mt Gilboa and In de Diepte, respectively. The number of individuals selected per family was dependent on the individual BLUP rank and the family

BLUP rank, with more selections allowed for the families with a higher BLUP ranking. This was combined with a rigorous phenotypic evaluation for stem form and incidence of damage by pests or pathogens in field. Due to indications that GEI exists in this F2 population (Swain et al. 2013c), only stable families that performed well at both sites should be used in the production populations (Raymond and Namkoong 1990). However, as the heritability for dbh was much lower at In de Diepte than at Mt Gilboa (h^2 = 0.06 and 0.17, respectively), selections should be accessed from the top 10 common families at Mt Gilboa and In de Diepte in the ratio of 2:1. Additional selections should be made from families ranked below 10th at Mt Gilboa to maintain genetic diversity. Care should also be taken to infuse the F3 breeding population with more material, if possible, to increase genetic diversity.

These selections will be grafted into FS CSOs to produce elite seed for the establishment of advanced generation breeding populations in the species, as well as for commercial seed production. The selected trees will also remain in a BSO format once the poorer trees have been thinned from the existing trials, providing opportunities for rapid seed production. Two related BSOs were established with the F2 trials and have undergone regular thinning to best tree/plot, as well as roguing of the bottom 30% of families at six years of age. These BSOs have already started producing seed which can be used for commercial production, as well as inclusion in the next generation of trials for progeny testing.

Second series F2 trials

The F2 trials that were established in 1999, and which have been described above and extensively by Swain et al. (2013b, 2013c), included only seed from those 80 individuals that had flowered and produced seed by mid-1998. Since then, extensive seed collections have been completed in six ICFR F1 *E. nitens* BSOs and one CSO, and a subsequent F2 trial series (2nd series) established on sites in Mpumalanga and KwaZulu-Natal early in 2008. This series comprises 169 new seedlots collected from the F1 seed orchards and 13 controls in common with the F2 trials established in 1999. Mid-rotation measurements in these trials have recently been completed. Analysis of these trial data will allow a direct comparison of FS with BS, as well as determining whether GEI is present in the population when established over a wider range of sites than the 1st series F2 trials. Selections in these trials will enable the introduction of further genetic diversity into the F3 breeding population.

Seed orchards

The establishment and management of improved *E. nitens* material in seed orchards is critical to ensure a sustainable supply of improved seed from the 1st series F2 selections, future selections from the 2nd series F2 trials, and future selections in further generations of breeding. The importance of keeping breeding and production populations/orchards separate has been emphasised by several authors (White 1987, Varghese et al. 2009). Production orchards can comprise relatively few outstanding individuals to maximise gains, whereas orchards for continuation of the breeding programme must have adequate diversity and high relative population size to capture genetic variation and to prevent inbreeding in successive generations of breeding (Varghese et al. 2009). This is often a challenge with reticent flowering species.

With regards to establishment and management of seed orchards in the *E. nitens* breeding programme, several factors need to be considered for maximum genetic gain.

a) *Flowering levels*

In the series of *E. nitens* genetic gain trials, significant differences in progeny growth were found for levels of flowering, with higher levels of flowering (≥40%) producing substantially greater progeny growth than lower flowering levels (≤20%) (Swain et al. 2013a). It is unclear, however, whether additional flowering above this level will confer any further benefit. Flowering precocity, as well as fecundity (pollen and seed contributions from an individual), has been found to be heritable in some eucalypts (Chambers et al. 1997, Varghese et al. 2009), and the latter authors also found that fecundity greatly increased in *E. camaldulensis* and *E. tereticornis* after one generation of domestication. Thus, as *E. nitens* is a reticent flowering species, and it has been possible to collect seed only from those families and individuals that have flowered within a certain time-frame, it can be assumed that some level of selection for precocious flowering has occurred in this breeding programme, together with selection for improved growth, stem form, etc. Flowering, if heritable in *E. nitens*, should thus increase in the F2. Assessments have been made in F2 BSOs, and although early, indications suggest that more seed is available at an earlier age in these orchards (unpublished data). However, this may be confounded by the better siting of F2 orchards on sites more likely to meet the chilling requirement for flowering. Assessments in F2 orchards need to continue and to be compared with historic assessments in F1 orchards. Suitor et al. (2009) argue that the selection of genetically fecund females will reduce costs of manual

pollination for breeding and, in the case of *E. nitens*, can replace CP.

b) *Outcrossing*

Indications are that outcrossing was high (>80%) in the F1 population (Swain et al. 2013c), although this should be confirmed with molecular genetics studies. However, as only one or two individuals may have represented a family in the seed orchard bulks made up from the F1 BSOs, due to poor flowering and resultant poor seed production, it is possible that only a few male parents may have contributed as pollen parents and that pollen from heavy flowering individuals may have had a large genetic influence on adjacent female trees (Hodge et al. 1996, Moncur and Boland 2001, Grosser et al. 2010). This is a cause for concern in that high frequencies of near-neighbour pollinations with just a few male parents may increase the number of full siblings formed in OP families (White 1996). It is unlikely that this has caused a problem in terms of inbreeding or loss of genetic diversity in the F1 BSOs, as shown by the good progeny performance, however, the outcrossing rate and relative influence of male contributors should be investigated with a DNA genotyping study. This will enable management of activities to prevent loss of diversity in further generations.

Diversity in flowering times can also affect outcrossing. Tibbits (1989) and Jones and van Staden (2001) found a good correlation in *E. nitens* flowering times from one year to the next, suggesting strong genetic control of flowering. In addition, the ICFR F1 *E. nitens* families were able to be categorised into “early” and “late” flowerers, in terms of months of the year, based on their flowering times in F1 seed orchards over several years (unpublished data). Assuming that there is some genetic control of time of year of flowering, mixing of families from a range of provenances in the F1 BSOs, with a range of flowering times represented, should result in a converging of flowering times, to varying degrees. This will aid in synchronising pollination amongst trees in the orchards. This will also be investigated in the assessments of the F2 orchards.

c) *Selection intensity*

The number of families remaining in both BSOs and CSOs after roguing or backward selection will impact on genetic gain. With regard to the breeding population/orchard, it is important to retain a large number of families to preserve genetic diversity, but for the production population/orchard, decreasing the number of genoytpes to a few elite

genotypes will increase gains. Ideally, ICFR commercial seed bulks should comprise at least 16 families from the smaller F1 BSOs, as used in bulks in the *E. nitens* genetic gain trials (Swain et al. 2013c), or up to 35 families from some of the larger orchards which comprise over 100 families. However, due to the shortage of improved *E. nitens* seed in South Africa, seed bulks might need to comprise even more families to be able to produce adequate quantities of seed for the South African forestry industry, i.e., up to 25 or 55 families from the small and large orchards, respectively. This would mean a decrease in the selection intensity in the seed orchards, with a resultant decrease in genetic gain, relative to the bulks compared in the *E. nitens* genetic gain trials. Should an increase in the numbers of families flowering after selection be possible in those orchards with less families flowering, however (e.g., 59% at Helvetia, 67% at Jaglust), the gains could be expected to be greater.

The three points discussed above could be managed, to a large degree, by ensuring that seed orchards are constructed with both sufficient families and individuals per family, as well as adequate numbers of families flowering simultaneously (or with periods of overlapping flowering), to ensure sufficient outcrossing and to realise potential gains. Information on relatedness of clones would be very useful in CSOs, as this would decrease the risk of including only a few high value clones in a CSO for increased gains, as inbreeding would be controlled.

d) Pollen contamination

Historically, contamination of *E. nitens* seed orchards from surrounding plantations of lower genetic worth has not been a large consideration in the South African forestry landscape. Although *E. nitens* is pollinated by insects and its pollen is normally deposited within short distances, Barbour et al. (2005) found that it has the potential to move long distances. However, in the South African situation, *E. nitens* BSOs were not considered to be at high risk of contamination, as any surrounding plantations were generally felled at approximately nine years of age, at which stage flowering would not have occurred. In addition, isolation of orchards with several buffer rows of improved *E. nitens* is a common practice. More recently, with the specific siting of *E. nitens* orchards on sites more suitable for flowering, the surrounding plantations are often various *Pinus* species, thus reducing the risk of contamination further. However, as precocious flowering is bred into *E. nitens*, and with the possibility of inter-specific

hybridisation with more precocious species in adjacent commercial stands, this may become a consideration in the future.

A new breeding strategy

Breeding programmes based on OP management of the breeding population, at least in the 1st generation of breeding, have been opted for in many Tree Improvement progammes, based on; a) theoretical genetic gains calculations (Cotterill 1986), b) realised genetic gains from field trials in breeding programmes (Rockwood et al. 1989, Hodge et al. 1996), and c) practical and logistical ease (Brawner and Elizaul 2007, Griffin 2011). To date, the ICFR breeding programme has only tested OP families, as it is difficult and expensive to produce *E. nitens* CP seed (Hamilton et al. 2008). Although techniques are available for CP in *E. nitens*, there is limited success with such intra-species controlled crosses in South Africa (Venter and Sivlal 2007, Louw pers comm[2]). Such crosses should be more successful in South Africa (Mphahlele pers comm[3]), but the use of CP to produce full sibs for the ICFR breeding programme is unlikely in the near future.

Therefore a strategy that is based on OP, but with the advantages of CP, and which allows for infusion of new or additional genetic material as necessary, would be best suited for this species. Although the most important current application of genomic analysis in tree improvement is for verification of identity and pedigree of genotypes (Jain and Minocha 2000), such analysis also has an application in seed orchard management. This is in estimation of selfing and outcrossing rates, determination of mating patterns, and estimating levels of pollen contamination (Gea et al. 2007, Grosser et al. 2010). Gea and co-authors (2007) provided a useful description of the validity and use of different genotyping techniques, as well as the first use of parental analysis using a polymix progeny as an alternative to full-sib breeding in *Pinus taeda* L. (Lambeth et al. 2001). Gea et al. (2007) also investigated the feasibility of using parental reconstruction of *E. nitens* OP progeny to estimate SCA of parents, make FS from field tests and advance the *E. nitens* breeding programme with little compromise to genetic gain, whilst securing pedigree information of the FS.

[2] Louw A. 2013. Sappi Shaw Research Centre, Tweedie, PO Box 473, Howick, 3290, SOUTH AFRICA
[3] Mphahlele M. 2013. Mondi Trahar Technology Centre, Hilton, PO Box 12, Pietermaritzburg, Hilton, 3245, SOUTH AFRICA

This strategy has the advantage of accumulating additional gain in each generation by incorporating pedigree reconstruction in the FS, providing information on inbreeding and outcrossing levels in the orchards and allowing monitoring and management thereof, as well as monitoring and management of pollen contamination (Gea et al. 2007). At the same time, such use of genotyping analysis in this strategy avoids the cost of CP and provides the pedigree information that is lacking in OP programmes (Hamilton et al. 2008). Such a strategy would be well suited to the ICFR *E. nitens* breeding programme for these reasons, and could be implemented immediately, as F2 progeny are available from both OP BSOs and CSOs, with the majority of F1 female (and possible male) parents still standing in F1 BSOs and CSOs.

Additionally, in an effort to capture large genetic gains rapidly for commercial deployment, whilst still maintaining a broad genetic base breeding population, the use of bi-clonal seed orchards (BCOs) is proposed in the *E. nitens* breeding strategy. Twenty top individuals can be selected from the progeny trials and grafted. The DNA genotyping of these individuals and their parents, which has been proposed above, will provide information on relatedness of these clones, thus facilitating the assignment of two pairs of clones to BCOs. This will result in 10 BCOs, which will then produce highly improved seed to be bulked for deployment by the South African forestry industry. It is, theoretically, not necessary to progeny test the BCOs as the clones have been genotyped, but due to the novelty of the exercise and for risk management, the seed from the BCOs will be tested. This will also allow for BS of the BCOs, and roguing of 30 to 50% of the “poorer” BCOs. A simulation using *G-Assist (Vs 4.0)* predicted gains in the region of 33.7% for dbh, or 2.8% per year over 12 years (**Table 6.3**). These predicted gains are in the region of those predicted for clonal breeding programmes (Snedden and Verryn 2004).

Thus, this approach has the following advantages; provides pedigree information for relatedness which can then be used to manage inbreeding in both the breeding and production populations, provides information on both SCA of individuals and GCA of families, overcomes the physical difficulties associated with CP in *E. nitens* whilst still providing the same information, and provides information on the number of males involved in recombination in ICFR seed orchards, and levels of outcrossing.

Table 6.3 Predicted percentage gains in diameter at breast height for bi-clonal seed orchards of *Eucalyptus nitens*, the selected clones originating from a strategy using parental reconstruction of an open-pollinated population

Individual parameters[1]	Parental reconstruction scenario		
	Selection intensities[2] for breeding & production population scenarios	Total predicted increase in dbh over previous production population (%)	Predicted increase per year of breeding cycle (%)
Co-efficient of relationship (*cr*)	*cr* = 0.5		
Breeding population		7.3	-
Female among / within family selection	42:60 /2:24		
Male among / within family selection[3]	42:60 /2:24		
Production population (bi-clonal seed orchards)		26.4	2.8
Female among / within family selection	20[4]:60 / 1:24		
Male among / within family selection	20:60 / 1:24		
Additional roguing	10:20		
Total breeding & production cycle length	12	**TOTAL**: 33.7	2.8

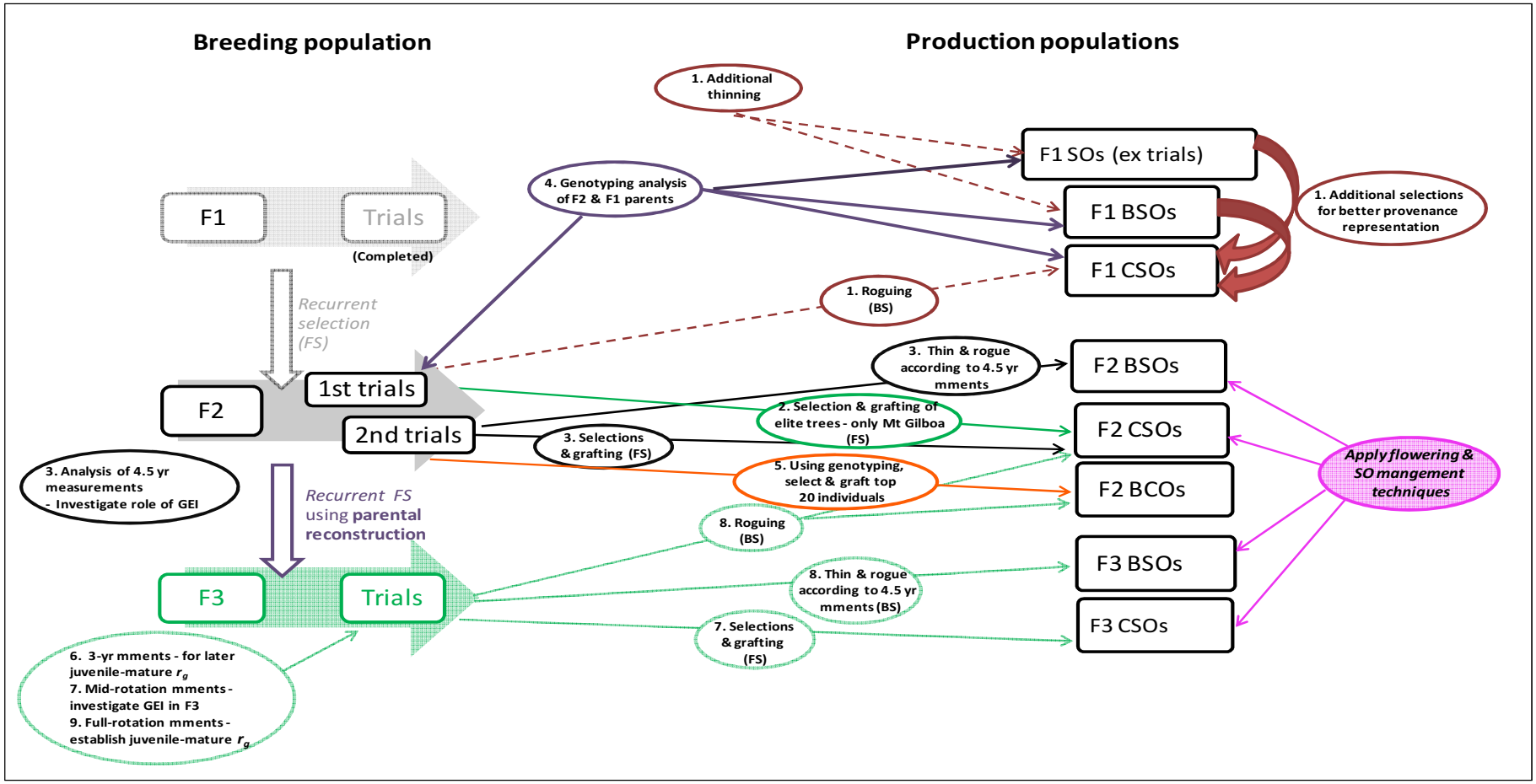
[1] Generic parameters for the population were those described in **Table 6.1**,
[2] Number of families /clones selected : effective population size considering flowering where relevant,
[3] Panmixis is assumed, i.e., that all males contribute equally to mating,
[4] 20 clones to make up 10 pairs for 10 bi-clonal orchards, which can be rogued to five or more eventually, after progeny testing

With reference to enhancing the current ICFR *E. nitens* breeding and production populations, the parental recombination and bi-clonal strategies could be implemented as follows (**Figure 6.2**, where action points below compare with actions in the figure):

1. Complete outstanding roguing, thinning, selections and grafting in F1 as proposed above.
2. Graft selections from 1st series F2 into F2 CSO, as suggested earlier. This would comprise mainly selections from Mt Gilboa. Apply flowering and seed orchard management technologies.
3. Complete analysis of mid-rotation 2nd series F2 trials (4.5 years), make selections and graft into F2 CSOs. Investigate role of GEI in this population. Apply flowering and seed orchard management technologies.
4. Genotyping study on F2 FS progeny (both series) and parents in F1 BSOs/CSOs. This provides the full pedigree of parents for future seed orchards and breeding populations. This study will also provide useful information on outcrossing, inbreeding and influence of male parents/numbers of full sibs in the ICFR orchards. The expertise of a molecular geneticist will be accessed for this.

The first four points are independent of each other and can run concurrently.

Figure 6.2 Summary of proposed open-pollinated breeding strategy for ICFR *Eucalyptus nitens*, using parental reconstruction to establish pedigree and a combination of forward and backward selection



FS – forward selection, BS – backward selection, SO – seedling orchards, BSO – breeding seed orchard, CSO – clonal seed orchard,
BCO - bi-clonal orchard, yr – year, mment – measurement, r_g – genetic correlation, GEI – genotype by environment interaction

5. Select the top 10 individuals from F2, based on growth traits and precocious flowering. Using pedigree information, design 10 BCOs. Graft sufficient numbers of each clone for establishment of BCOs. Establish BCOs for seed production. (Based on producing 5 kg of elite BCO seed/annum, 75% flowering in the BCOs and an average production of 45 g clean seed/tree canopy, 150 grafts are required in total. Due to selection, flowering may be higher than 75% in reality).
6. Establish the F3 generation and related seedling seed orchards, using results of genotyping analysis. Infuse new material by including 2nd series F2 selections.
7. Do three-year measurements for use in establishing earlier juvenile-mature genetic correlations.
8. Following the mid-rotation measurements (4.5 years) of F3, do BS in F2 CSOs and BSOs. Investigate the role of GEI in F3 population.
9. Complete the full-rotation measurements in F3 and establish juvenile-mature genetic correlations with three-year measurements.
10. Should there be an additional generation, the maternal identity will be certain, but paternal identity will be limited to grandparent information unless there is funding to genotype the complete progeny test.

Such a strategy should accomplish greater genetic gains than the current OP breeding strategy being implemented at the ICFR for *E. nitens*, without compromising on breeding cycle length.

Low success with vegetative propagation of *E. nitens* through micro- and macro-propagation has limited clonal production of superior selections to grafting. Although commercial deployment by such methods is not viable, use of these techniques to duplicate elite selections should not be ignored, as only small numbers per clone are needed to establish production populations.

There have been many references throughout this paper to the need for a molecular genetics study in the *E. nitens* population. The statistical analysis of the growth data from two generations of field trials has provided indications on levels of selfing and outcrossing, and molecular genetics studies would verify these results, as well as perhaps refine these further. The coefficient of relationship could be verified or adjusted accordingly. Such studies would show whether inter-provenance crossing did actually occur, and could then support or

disprove the theory that there is enough overlap between provenance flowering for provenance outcrossing to occur, and that provenance effects in the F2 signify a lack of non-additive or heterotic effects. In addition, the relative contribution of male parents to the population will be measured and it can be determined whether the assumption of panmixis in the seed orchards is correct or not. The pedigree information generated by a genotyping study will allow for control of inbreeding, which then makes it possible to safely include fewer elite selections in seed orchards, with resultant increases in genetic gain. Thus the support for such studies is strong and yet, no formal molecular studies have been done in this programme to date. Although costs of such fingerprinting and genomic analysis studies have decreased dramatically over the past few years, budgetary constraints in this breeding programme have been a major factor in limiting the use of molecular techniques. The cost of DNA primers is still high in South Africa, and the number of samples needed to identify male parentage of F2 offspring from F1 BSOs is large. However, recent progress at the ICFR with regards to development of an in-house molecular genetics laboratory is extremely positive. The findings of the statistical analyses and modelling exercises reported here will be utilised to motivate for funding to do such molecular studies as discussed here, on the premise that genetic potential will be reached by doing so.

Conclusions

A more thorough understanding of the factors necessary for extending an advanced generation breeding strategy for *E. nitens* has resulted from two generations of breeding in the species.

Estimation of genetic parameters in both the F1 and F2 enabled the prediction of individual tree and family BVs which has allowed for selections of superior genotypes to be made in the breeding population. The investigation of the assumptions underlying the estimations of these parameters has afforded confidence in these and future estimates in the *E. nitens* breeding population. The role of GEI has been established in both the F1 and F2, which provides guidelines on how to produce improved seed of this species for different regions in South Africa. This should be confirmed in each new trials series and for each subsequent generation of breeding.

A comparison of realised gains in the F2 with predicted gains from the F1 has quantified the genetic gain made after one generation of breeding, and has shown that improvement in

survival of the advanced generation material plays an important role in the gains in total volume per hectare achieved. The role that flowering and seed orchard factors play in realising predicted gain was investigated and showed that there were significant differences in progeny growth between the levels of flowering, with higher levels of flowering (≥40%) producing substantially better progeny growth than lower flowering levels (≤20%). The seed orchard had no effect on progeny growth in genetic gain trials, suggesting that seed collected from any of the four seed orchards tested will produce trees with significant improvement in growth.

Provenance (grand-maternal) effects were still significant in the F2 trials, despite top F1 families having come from a mixed provenance breeding environment. These indicate that a diluted provenance identity is still being expressed through the maternal influence in the F2, which would be expected unless strong heterosis has occurred. As there are indications that heterosis has not occurred within this population, it is unlikely that it will be necessary to keep provenances separate to harness such an effect in current and future ICFR seed orchards.

Information from, and an understanding of, these areas enabled the investigation of various management options to increase gains in the existing production populations. Indications are that additional roguing of 1) existing CSOs based on results of F2 trials (BS), and 2) F1 BSOs based on stricter provenance selection, will markedly increase the quality of the seed produced from these orchards within one season, i.e., 21.7% increase in dbh for roguing of CSOs versus 16.3% for the current unrogued situation. This study also highlighted the importance of shortening the breeding cycle in *E. nitens*, particularly in view of the delays caused by reticent flowering and seed production in the species.

A proposal has been made for an advanced generation breeding strategy in *E. nitens*, using parental reconstruction of OP progeny to secure pedigree information of forward selections. A simulation using *G-Assist (Vs 4.0)* predicted gains of 7.3% in the breeding population, with an additional 26.4% in the proposed bi-clonal production population for dbh, or approximately 2.8% per year over 12 years. This strategy thus combines the benefits of significantly increased genetic gain with a shortened breeding cycle.

Acknowledgements

Masonite Ltd., Mondi Ltd, NCT Forestry Co-Operative Ltd., PG Bison, TWK Ltd. and York Timbers Ltd. are gratefully acknowledged as funders of this project.

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THESIS OVERVIEW

The objective of this study was to ultimately develop an advanced generation breeding strategy for *Eucalyptus nitens*. Improvement in the species was slow, and there was an open question of how much genetic gain was being affected by poor flowering in seed orchards. The author had the perception that a substantially better breeding strategy might emerge from the study, which would dramatically solve the existing problems associated with breeding *E. nitens*.

Eucalyptus nitens is an important cold tolerant eucalypt species grown commercially in South Africa, and is the only eucalypt that can be grown on sites with a risk of snow. These facts, together with *E. nitens* being a significant hybridising partner with *Eucalyptus grandis*, make this an important species with which to continue breeding. The significant provenance variation that exists for a range of growth, reproductive and wood property traits makes this species suited to improvement and able to meet the requirements of the diverse forestry sites and end products in South Africa. The potential area for optimum growth of *E. nitens* within the current summer rainfall forestry growing region is 119 304 hectares (ha) (National Land Cover 2000; Smith et al., 2005), which is 8% of the summer rainfall plantation area in South Africa, although currently approximately only 46 000 ha have been planted to the species (Germishuizen pers comm[1]).

There are problems, however, associated with breeding *E. nitens*. The main problem is the poor and erratic flowering of the species, resulting in delays in generation turnover in the breeding programme, and in production of improved seed for commercial deployment (Gardner 2003, Pound et al. 2003). A further issue was whether the poor flowering was negatively impacting on genetic gain due to poor outcrossing (Pound et al. 2003) and stratified selection. In addition, the small flower buds of the species (Boland et al. 1982) make emasculation and controlled-pollination programmes difficult (Tibbits 1989), and clonal propagation of superior individuals through micro- or macro-propagation using cuttings has a low success rate (de Little et al. 1992, Griffin 2001).

[1] Germishuizen I. 2012. Institute for Commercial Forestry Research, Pietermaritzburg, PO Box 100821, Scottsville, 3209, SOUTH AFRICA

Even with these biological constraints, it became necessary to develop an advanced generation breeding strategy for *E. nitens* at the ICFR, to ensure genetic gain was captured within a reasonable time frame for commercial deployment. To develop such a strategy, the following areas of understanding were needed, and subsequently comprised focus areas of the thesis:

- The population genetics and underlying assumptions of the species;
- The role that poor and inconsistent flowering in seed orchards plays in realised genetic gain in *E. nitens*;
- The role that the different Australian provenances play in the breeding population, in order to decide whether to keep seed orchards separate by provenance or to allow provenance mixing;
- The factors affecting genetic gain and ways to optimise these without increasing breeding cycle length.

This study has analysed performance results from two generations of tree improvement trials to develop an understanding of the four areas above, and then synthesised the information to develop a suitable breeding strategy for *E. nitens*.

Analysis of eight F1 trials enabled characterisation of the ICFR's breeding population in the following way:

- High Type B genetic correlations for all sites pairs, except one comparison, ranged from 0.75 to 0.99 for diameter at breast height (dbh) at 76 to 113 months, indicating little, or no, genotype by environment interaction (GEI) for dbh for the genotypes tested. Thus the F1 production populations can be managed jointly for most sites where *E. nitens* is grown in the summer rainfall region of South Africa.
- High genetic correlations of greater than 0.90 between dbh measurements at 52 to 62 months after establishment and dbh measurements at 94 or 113 months were found, indicating that selections can be reliably made at five years.
- Diameter measurements at both 60 months and full rotation were highly correlated with the final height measurements in these trial series (r_g > 0.71 and > 0.83, respectively), demonstrating that selections made for dbh would include selection for height.
- Narrow sense heritability coefficients ranged from 0.01 to 0.34, indicating that the species generally exhibits sufficient breeding opportunities for improvement of diameter growth.

- Provenance testing showed significant differences between Australian provenances planted in South Africa; and that the more northerly New South Wales provenances of Barren Mountain and Barrington Tops are distinctly better suited to growth in South Africa than the southern New South Wales provenances and the Victorian provenances.

Estimates of genetic parameters allowed for genetic gains to be predicted in the F2. The measurement of three genetic gain/seed orchard variable trials, and analysis thereof, has shown that significant improvements were made over the first generation of selection in the ICFR *E. nitens* breeding programme, with genetic gains ranging from increases of 23.2 to164.8 m^3ha^{-1} over the unimproved commercial seed. It should be noted that improvement in survival of the advanced-generation material plays an important role in the gains in total volume per hectare achieved. It is therefore recommended that seed from any of the ICFR improved bulks be accessed for commercial deployment when available, rather than using unimproved or land-race material from Australia and South Africa, respectively.

The genetic gain trials were also useful in testing which genetic assumptions best fit the ICFR *E. nitens* F1 population for the estimation of genetic parameters. Several scenarios of predicted genetic gain were developed and compared with the gains realised from the genetic gain trials. These comparisons showed that the family nested within provenance scenarios proved to be closer to realised gains than the family across provenance predictions. In the scenarios using firstly, actual flowering for family nested within provenance and secondly, estimated flowering for 30% roguing of poor families, a coefficient of relationship of 0.33 predicted gains closest to realised gains. Indications were that the effects were additive, and that very little or no heterosis had occurred. Realised gains achieved in the F2 genetic gain trials were then used to calculate realised heritabilities. These only differed markedly from the estimated heritabilities at two sites where an under-prediction and over-prediction of genetic gains had occurred, respectively. This study thus provided an objective and quantitative assessment of the underlying assumptions used for estimating genetic parameters in *Eucalyptus nitens.* It also showed that there were no important digressions from the assumptions made in the F1 study, and no adjustments need to be made to the breeding strategy with regards to these assumptions.

Outcrossing levels appear to be higher in the ICFR seed orchards than were expected, considering the slow and poor flowering of the species. The statistical information suggested that outcrossing in the seed orchards was more than 80%. DNA genotyping studies will provide a useful tool to monitor outcrossing rates, which will then allow for refinement of the models over time, as necessary.

A secondary but important objective of the genetic gain trials was to establish whether a number of seed source and orchard variables influence the performance of the progeny. Indications from this study are that the levels of flowering have an impact on progeny growth, as seed orchards with 15% flowering resulted in poorer genetic gains in the progeny than those with ≥40% flowering. However, this was not consistent and it is thus difficult to draw any definite conclusions in this regard. Indications are that flowering above a certain low level may result in increased gains in a population, but that additional flowering above this level may confer very little, if any benefit. Further investigation of flowering levels should be carried out with larger numbers of observations per flowering level. Until then, it is recommended that seed should be collected, where possible, from seed orchards where flowering of 40% or more was observed in the previous year. This was supported by substantial improvements in total volume of the progeny, there generally being a more than 20% increase in volume at these higher levels of flowering.

In terms of family composition of the seed orchard bulks, one bulk type differed significantly from the other four, but the reasons for this were unclear, despite further exploring flowering and family-within-bulk performance. The F1 seed orchard from which seed originated had no effect on progeny growth, and this suggests that seed collected from any of the four seed orchards tested will produce trees with significant improvement in growth over the unimproved and commercial material. It should, however, be noted that certain combinations of seedling seed orchard and bulk composition, particularly at the lower levels of flowering, produced much better progeny growth than others, even if this difference was not statistically significant. It is thus recommended that such higher yielding bulk and seedling seed orchard combinations should be used for commercial deployment. This will impact on management of ICFR seed orchards and future seed bulk composition.

Once again, DNA genotyping of the *E. nitens* seed orchards will provide a better understanding of the relative levels of selfing, outcrossing and relatedness in this breeding

population. This, in turn, will allow for manipulation of current and future seed orchards to ensure that maximum gains are captured in the seed for commercial deployment.

Results from the F2 trials enabled estimation of genetic parameters for the F2, and prediction of breeding values for both families and individuals. Using these, selection of elite individuals was made in F2 trials. Narrow sense heritability estimates for dbh at 87 months were 0.17 and 0.06 in the two F2 trials, respectively. Given that the latter is very low, it is recommended that selections should be accessed from the top 10 common families at Mt Gilboa and In de Diepte in the ratio of 2:1. Additional selections should be made from families ranked below 10th at Mt Gilboa. There is obviously some risk of narrowing the genetic base of the breeding population by doing this (Eldridge et al. 1993), but infusion of selections from the later, 2nd series of F2 trials should prevent this. The intermediate heritability estimate of 0.17 in the one F2 trial indicates that further improvement is possible in this population of *E. nitens*. Type B genetic correlations of 0.61 indicated the possible presence of GEI for the two F2 sites, which presents a challenge for production of improved seed. Two options for managing the GEI are to either a) use the stable families which perform well over both sites, or b) breed separate populations of the species for different site types (Raymond and Namkoong 1979) in South Africa. However, as the indication of GEI may be biased due to this material only being tested over two sites, it is likely that the exploitation of stable families will be used for this Tree Improvement programme until more reliable estimates can be obtained from the multi-sited 2nd series F2 trials.

Provenance (grand-maternal) effects were still significant in the F2 trials, despite the top F1 families having come from a mixed-provenance breeding environment. These indicate that a diluted provenance identity is still being expressed through the maternal influence in the F2, which would be expected unless strong heterosis had occurred. Thus, although the progeny trials were not designed to detect heterosis/non-additive effects, it is unlikely that these exist in this population, due to the still significant provenance effects and the lack of provenance rank changes in the F2. It is therefore unlikely that it will be necessary to keep provenances separate to harness such an effect in current and future ICFR seed orchards. Current seed orchards can then undergo their final roguing, as necessary, to allow for provenance mixing.

In a shy-flowering species such as *E. nitens,* there is often a compromise between genetic gain and breeding-cycle length (Hamilton et al. 2008), with the highest possible gains

frequently taking longer to realise than more moderate gains. Optimising the relationship or balance between these two conflicting factors was a serious consideration during the development of the breeding strategy. Modelling of predicted genetic gain by means of various breeding strategy scenarios was used as a tool to determine which strategy or management plan would deliver the most genetic gains per unit time. This process utilised the understanding developed in the four focus areas of the thesis, as well as the parameters established in the study. In addition to indicating which options would optimise gain with breeding-cycle length, management actions for improvement of the existing production populations became apparent, specifically; additional roguing of existing clonal seed orchards based on results of F2 trials, and roguing of F1 breeding seed orchards based on stricter provenance selection. These actions will rapidly and markedly increase the quality of the seed produced from these orchards within one season.

The advanced generation breeding strategy that has been developed for *E. nitens* uses parental reconstruction of open-pollinated (OP) progeny to secure pedigree information of forward selections. The strategy is based on one developed by Gea et al. (2007) for *E. nitens* in New Zealand, where parental reconstruction of OP progeny was successfully used to estimate General Combining Ability of parents, to make forward selections from field tests and to advance the *E. nitens* breeding programme with little compromise to genetic gain, whilst securing pedigree information of the forward selections. In addition, such a strategy provides information on inbreeding and outcrossing levels in the orchards, allows monitoring and management thereof; and also allows for monitoring and management of pollen contamination. At the same time, such use of genotyping analysis in this strategy avoids the cost of controlled pollination, alleviates the problem associated with OP in that genetic information is only available for the maternal parent (Hamilton et al. 2008), and combines the benefits of increased genetic gain with a shortened breeding cycle. Gains in dbh predicted for the next generation of improvement using this strategy are 7.2% in the breeding population, and up to an additional 26% in the production population, depending on the population deployed.

A key focus in the next phase of the breeding programme, therefore, will be to characterise the material in the breeding programme genomically. Not only will this include DNA fingerprinting for the purpose of pedigree information, but also for determining levels of selfing and outcrossing, and fertility or influence of pollen parents. Although the intention at

the start of this PhD study was to use conventional breeding methodology, it would have been useful to incorporate molecular genetics at certain stages in the research, as a means of supporting the quantitative genetics. However, budgetary constraints have prevented the use of molecular techniques in the breeding programme to date. The recent development of an in-house molecular genetics laboratory at the ICFR, together with the findings from this PhD study to motivate for additional funding, may make it possible to proceed with this focus area. This research should be undertaken by an expert in the field, rather than a plant breeder.

Cotterill and Dean stated, in 1990, that "maximising gains from advanced generation breeding is largely a matter of efficient selection". For *E. nitens*, this appears to be the first step. Thereafter, the plant breeding challenges revolve around getting the selections to flower quickly, outcross and produce seed. With this thought in mind, and considering other constraints in the breeding of *E. nitens*, several areas of research, not all of them new, could assist with increasing genetic gain and decreasing breeding cycle length in the species.

- Seed orchard studies to determine whether outcrossing varies depending on position of the flowers in the canopy. Patterson et al. (2004) concluded that seed collectors should confine collections to mid- to upper-third of the crown to ensure acceptable levels of outcrossing of the seed in *E. globulus*. However, seed orchard managers would prefer to collect from more easily accessible lower branches and thus reduce collection costs, if adequate outcrossing is shown to occur.
- Estimation of fecundity (fertility) in seed orchards and sibling coefficient to quantify the fecundity difference between orchard genotypes. This will provide information to support the DNA genotyping analysis on the proportion of fertile trees in an orchard and how many of these trees effectively contribute to seed production (Varghese et al. 2009). Suitor et al. (2009) argue that the selection of genetically fecund females will reduce costs of manual pollination for breeding and, in the case of *E. nitens*, could replace CP.
- Continued research into technologies that will improve the level of flowering, as well as inducing early flowering in orchard trees. Managing large flower crops on orchard trees without subsequent abortion, under South Africa conditions, will also be a consideration.
- Development of a successful, inexpensive and time-efficient technique for controlled pollinations in *E. nitens* in South Africa. The technique itself may be available, but

may be limited by insufficient numbers of flowers on small, accessible trees or potted orchards.

- Propagation of selections through rooted cuttings. Low levels of successful rooting of vegetative cuttings of *E. nitens* through both micro- and macro-propagation in South Africa have limited clonal production of superior selections to grafting. Although commercial deployment by such methods is currently not viable in this country, use of these techniques to duplicate elite selections should not be ignored, as only small numbers per clone are needed to establish production populations.
- Adaption and application of early selection tools in the nursery for traits such as cold and frost hardiness (Tibbits and Reid 1987, Tibbits and Hodge 2003), bearing in mind their constraints, to further decrease the length of the breeding cycle. There has been ongoing selection for cold and frost tolerance in the ICFR breeding population, often measured as 'survival', which has been successful according to anecdotal reports from growers of the improved material. Early selection tools would enhance this.
- The calculation of realised heritability (h^2_r) from realised gains in this study was complex due to the incremental selection used in the breeding population. In reality, the more appropriate formula for calculating h^2_r, i.e., $\Delta G = SI \times h^2 \times \sigma_p$ (Falconer and Mackay 1996), using algebraic functions to solve components of h^2_r, could not be solved. Instead, the simpler equation, i.e., $\Delta G = h^2 \times s$ (Falconer and Mackay 1996) was used. It may not have been possible to solve the former equation, involving four levels of incremental selection, because the components of the equation are related, and therefore confound the equation. This bears further investigation.

The stratified selection which appears to be taking place in the ICFR seed orchards due to erratic flowering will result in a decrease in genetic gains and therefore, the applied selection intensities should be revised. This implies that even greater gains would be possible if flowering prolificacy could be increased. It is therefore important firstly, to construct BSOs with sufficient numbers of families, with sufficient individuals per family and with adequate numbers of synchronously flowering families; and secondly, to apply technologies to increase flowering, such that sufficient outcrossing is ensured. This will ensure that enhanced genetic gains will be realised.

Information on wood properties would add considerable value to the breeding programme. Such studies are often constrained by finances, but the availability of rapid screening and

non-destructive techniques such as Near Infra Red Spectrophotometry should make selections for wood properties an acceptable and manageable breeding objective. Heritabilities for wood properties in *E. nitens* are reportedly higher than growth traits such as dbh and volume (Tibbits and Hodge 1998, Kube et al. 2001, Hamilton and Potts 2008), and thus selections for wood properties should be included in the next level of selection in the breeding population.

This study is the first such reported comprehensive study on *E. nitens* in South Africa, and the objective of developing an advanced generation breeding strategy for the species has been met. Although the proposed strategy may not be the perfect solution originally envisaged by the author, the pedigree information will be critical to realising larger gains in future generations. These gains, together with the relatively simple improvements proposed to improve the production populations, will result in germplasm of *E. nitens* with substantially increased gains being commercially deployed within one cycle of breeding.

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APPENDICES

Appendix 1a) List of families/seedlots represented in ICFR F1 trial series. **X** in bold indicates those families that remained once the trials were rogued and thinned to seed orchards. All families/seedlots are from Australia unless otherwise specified. SA = South Africa, NSW = New South Wales

Seedlot	Provenance	E88/01 Amsterdam	E88/01 Jessievale	E88/03 Helvetia	E88/03 Daspoort	E88/05 Woodstock	E88/06 Babanango[1]	E88/07 Goedehoop	E88/07 Arthur's Seat
1	Jessievale, SA			x	x				
2	Jessievale, SA			x	x				
13	Jessievale, SA			x	x				
14	Jessievale, SA			x	x				
22	Nelshoogte, SA			x	**X**		x		
24	Nelshoogte, SA			x	**X**	**X**	x		
25	Nelshoogte, SA			**X**	x	**X**	x		
26	Nelshoogte, SA			**X**	**X**	x			
27	Nelshoogte, SA			**X**	**X**				
28	Nelshoogte, SA			x	**X**		x		
29	Nelshoogte, SA			**X**	**X**				
30	Nelshoogte, SA			**X**	**X**	**X**	x		
40	Belfast, SA			x	x				
41	Belfast, SA			x	x				
42	Belfast, SA			**X**					
43	Belfast, SA			X	x				
27832	Tallaganda		**X**						
31188	Penny Saddle	x	x	x					
31189	Penny Saddle	**X**	x	**X**					
31327	Tallaganda		**X**						
31328	Tallaganda		**X**						
31329	Tallaganda		**X**						
31330	Tallaganda		**X**						
31331	Tallaganda		**X**						
31332	Tallaganda		**X**						
31333	Tallaganda	**X**	**X**						
31334	Tallaganda	**X**	x						
31335	Tallaganda	**X**	x						
31336	Tallaganda	**X**	x						
31337	Tallaganda		**X**						
31338	Tallaganda	**X**	**X**						
31339	Tallaganda	**X**							
32076	Badja	**X**	x	**X**	**X**				
32077	Badja	**X**	**X**	x	**X**				
32078	Badja	**X**	**X**	**X**	x	**X**	x		
32079	Badja	x	x	**X**	**X**	**X**			
32080	Badja	x	x	**X**		x			
32081	Badja	x	x	x	x	**X**			
32082	Badja	**X**	**X**	**X**	**X**	**X**	x		
32083	Badja	x	**X**	x	x	**X**	x		
32084	Badja	**X**	**X**		x				
32085	Badja	x	**X**	x	x	**X**	x		
32086	Badja	x	**X**	**X**	x	**X**			
32087	Badja	**X**	**X**	**X**		**X**			
32088	Badja	x	**X**	x	**X**	x	x		
32089	Badja	**X**	x	**X**	x				
32090	Badja	x	x	**X**	x	x			
32091	Badja	**X**	x	x	**X**		x		
32092	Barren Mountain	**X**	**X**	x	**X**	**X**			
32093	Barren Mountain	**X**	x	**X**	**X**	**X**	x		
32094	Barren Mountain	**X**	**X**	x	**X**	**X**			

Seedlot	Provenance	E88/01 Amsterdam	E88/01 Jessievale	E88/03 Helvetia	E88/03 Daspoort	E88/05 Woodstock	E88/06 Babanango[1]	E88/07 Goedehoop	E88/07 Arthur's Seat
32095	Barren Mountain	X	X	X	X	X	x		
32096	Barren Mountain	X	x	X	X	X			
32097	Barren Mountain	X	X	X	X	X			
32098	Barren Mountain	X	X			X			
32099	Barren Mountain	X	X	X	X	X	x		
32100	Barren Mountain	X	X	X	X				
32101	Barren Mountain	X	X						
32102	Barren Mountain	X	X		X	X			
32119	Woodbush, SA	X	x						
34769	Northern NSW			x	X				
34831	Barrington Tops			X	X	X			
34832	Barrington Tops			X	X	X	x		
34833	Barrington Tops			X	X	X	x		
34834	Barrington Tops			X	X	x	x		
34835	Barrington Tops			X	X	X	x		
34836	Barrington Tops			X	x	X	x		
34837	Barrington Tops			X	X				
34838	Barrington Tops			X	X	X			
34839	Barrington Tops			X	X	x	x		
34840	Barrington Tops			X	X	x			

[1] Babanango trial burnt down before it could be converted to a seed orchard

Appendix 1b) List of families/seedlots represented in ICFR F1 trial series – Woodstock and Babanango continued. **X** in bold indicates those families that remained once the trials were rogued and thinned to seed orchards. All families/seedlots are from Australia unless otherwise specified. NZFRI = New Zealand Forestry Research Institute

Seedlot	Prove-nance	E88/05 Wood-stock	E88/06 Baba-nango[1]	E88/07 Goede-hoop	E88/07 Arthur's Seat	Seedlot	Prove-nance	E88/05 Wood-stock	E88/06 Baba-nango[1]	E88/07 Goede-hoop	E88/07 Arthur's Seat
37198	Glenbog	**X**	X			37257	Tallaganda		X		
37200	Glenbog	**X**	X			37259	Tallaganda	**X**	X		
37202	Glenbog	**X**	X			37261	Tallaganda	**X**			
37203	Glenbog	**X**	X			37262	Tallaganda	X	X		
37204	Glenbog	**X**	X			37263	Tallaganda	**X**			
37205	Glenbog	**X**	X			37264	Tallaganda	X			
37206	Glenbog	**X**				37265	Tallaganda	**X**			
37208	Glenbog	X	X			37268	Tallaganda	X	X		
37209	Glenbog	**X**	X	X	X	37270	Tallaganda	**X**	X		
37210	Glenbog	**X**	X			37271	Tallaganda	**X**	X		
37211	Glenbog	X	X			37272	Tallaganda	X	X		
37212	Glenbog	**X**	X			37282	Tallaganda		X		
37213	Glenbog	X	X			37283	Tallaganda		X		
37214	Glenbog	**X**				37607	Tallaganda	X			
37215	Glenbog	**X**	X			37608	Tallaganda	**X**			
37216	Glenbog	**X**	X			37610	Tallaganda	X			
37217	Glenbog	**X**	X			37611	Tallaganda	**X**			
37218	Glenbog	**X**				37612	Tallaganda	**X**			
37219	Glenbog	**X**				37613	Tallaganda	**X**			
37220	Glenbog		X			37614	Badja	**X**			
37221	Glenbog		X			37615	Badja	**X**	X		
37222	Glenbog	**X**				37617	Badja	X			
37224	Glenbog	**X**	X		X	37618	Badja	X			
37225	Glenbog		X			37619	Badja	**X**			
37226	Glenbog	**X**	X			37621	Glenbog		X		
37228	Badja	**X**				37622	Glenbog	**X**			
37229	Badja	**X**				37623	Glenbog	**X**			
37230	Badja	**X**				37624	Glenbog	**X**			
37231	Badja	**X**	X	X	X	37626	Glenbog	**X**			
37232	Badja	**X**	X			37628	Glenbog	**X**			
37233	Badja	**X**	X			37631	Bendoc	**X**			
37234	Badja	**X**				37633	Bendoc	**X**			
37235	Tallaganda	**X**	X			37634	Bendoc	**X**			
37236	Tallaganda	**X**	X			37636	Bendoc	**X**			
37237	Tallaganda	**X**	X			37637	Bendoc	**X**			
37238	Tallaganda	X	X			37641	Ebor	X			
37239	Tallaganda		X			37642	Ebor	X			
37240	Tallaganda	**X**				37643	Ebor	**X**			
37241	Tallaganda	X	X			37644	Ebor	**X**			
37243	Tallaganda	**X**	X			37645	Ebor	**X**			
37244	Tallaganda	X	X			37646	Ebor	X	X		
37245	Tallaganda	X	X			37647	Ebor	X	X		
37246	Tallaganda	**X**	X			37648	Ebor		X		
37247	Tallaganda	**X**				37649	Ebor		X		
37248	Tallaganda	**X**				37650	Ebor	X	X	X	X
37249	Tallaganda	**X**	X			37651	Ebor	X			X
37250	Tallaganda	X				37652	Ebor	X			
37252	Tallaganda		X			37653	Ebor	X			
37253	Tallaganda		X			37654	Ebor	X	X		
37254	Tallaganda	**X**	X			37655	Ebor	**X**			
37255	Tallaganda	**X**	X	X	X	37656	Ebor	X	X		
37256	Tallaganda		X								

Seedlot	Prove-nance	E88/05 Wood-stock	E88/06 Baba-nango[1]	E88/07 Goede-hoop	E88/07 Arthur's Seat	Seedlot	Prove-nance	E88/05 Wood-stock	E88/06 Baba-nango[1]	E88/07 Goede-hoop	E88/07 Arthur's Seat
37657	Barrington Tops	X				9226-9252	Barrington Tops (Kholwa Fire Trail)		X	X	
37658	Barrington Tops	X	X								
37659	Barrington Tops		X			9253-9267	Barrington Tops (Mt Carson)		X	X	
37660	Barrington Tops	X	X								
37661	Barrington Tops	**X**	X			9201-9225	Badja		X	X	
37662	Barrington Tops	X				9268-9282	NZ FRI		X	X	
37664	Barrington Tops	X	X			9283-9286	Thaba Putsoa, Lesotho		X	X	
37665	Barrington Tops	**X**	X								
37666	Barrington Tops	**X**									
37667	Barrington Tops	X	X								

[1] Babanango trial burnt down before it could be converted to a seed orchard

Appendix 2 Predicted gains for family nested within provenance (*fam(prov)*) compared to realised gains in diameter at breast height (dbh) of individual F2 *Eucalyptus nitens* bulk treatments at 87 months over an unimproved F1 bulk, and realised narrow sense heritability estimates (h^2), as estimated from genetic gain trials (Swain et al. 2013a, Chapter 3)

Trial series & site name (age in months at final measurement): Bulk treatment number[1]	Gains in dbh predicted from F1 (cm)	Realised gain in dbh (cm)	Between family SI[2] per BSO bulk	Within family SI[2] per BSO bulk	Realised $h^2_{fam(prov)}$ (estimated $h^2_{fam(prov)}$ ± SE)	Realised improvement in total volume (m^3ha^{-1}) (%)
E88/01 Jessievale (101):						
1	0.99	**0.9**	1.087	1.539	0.07	**63.4 (43.9)**
2		**1.7**	1.087		0.14	
4		**0.8**	1.087		0.07	
7		**0.1**	0.863		0.01	
9		**1.6**	1.077		0.13	
					[0.05 ± 0.022]	
E88/01 Amsterdam (101):						
5	1.25	**1.9**	0.119	1.539	0.21	**82.3 (57.0)**
					[0.14 ± 0.055]	
E88/03 Daspoort (113):		**Helvetia bulks[4]:**				
3	2.05	**1.7**	1.159	1.267	0.12	**-**
8		**1.9**	0.917		0.16	
					[0.13 ± 0.045]	
E88/03 Helvetia (94):						
3	0.29	**1.7**	1.159	1.267	0.15	**91.7 (63.5)**
8		**1.9**	0.917		0.16	
					[0.01 ± 0.026]	
E88/05 Babanango (112):		**Jaglust bulk[5]:**				
6	2.52	**1.4**	1.299	1.267	0.12	**71.7 (49.7)**
10		**1.3**	1.422		0.13	
					[0.18 ± 0.040]	
E88/06 Woodstock (76):		**Jaglust bulk[5]:**				
6	1.55	**1.4**	1.299	1.267	0.17	**71.7 (49.7)**
10		**1.3**	1.422		0.17	
					[0.14 ± 0.032]	
E88/07 Goedehoop (113)	3.07	-[6]	-	-	-	**-**
					[0.22 ± 0.055]	
E88/07 Arthur's Seat (113)	3.17	-[6]	-	-	-	-
					[0.27 ± 0.060]	

SE = Standard error of h^2

[1] Treatment numbers as described in Swain et al. (2013a),

[2] Selection Intensity,

[3] As described in Swain et al. (2013a),

[4] As no seed had been produced from this BSO by the time progeny trials were established, parameters from the related Helvetia BSO were used,

[5] Jaglust is a BSO representing the material in the Babanango and Woodstock trials,

[6] No seed had been produced from any of these trials or related BSOs by the time the progeny trials were established

Appendix 3 Equations used for predicting genetic gain by Shelbourne (1992), adapted by Verryn et al. (2000) for use in the algorithm *G-Assist (vs. 4.0)*, and adapted for the purposes of this *Eucalyptus nitens* study

a) Breeding population scenarios

i) B1.2 - Breeding population of open-pollinated families:

$\Delta G = 0.5(\Delta G_f) + 0.5(\Delta G_m)$, where

$$\Delta G_f = \left[\left(SI_1 \times r_g \times cr \times \frac{\sigma^2{}_A}{\sigma_{fm}} + SI_2 \times r_g \times \frac{(t-1)}{t} \times (1-cr) \times \frac{\sigma^2{}_A}{\sigma_{wf}} \right) \right]$$ and

$$\Delta G_m = \left[SI_1 \times r_g \times cr \times \frac{\sigma^2{}_A}{\sigma_{fm}} + SI_3 \times r_g \times \frac{(t-1)}{t} \times (1-cr) \times \frac{\sigma^2{}_A}{\sigma_{wf}} \right]$$ and:

ΔG = predicted genetic gain,

ΔG_f = predicted genetic gain from female selection,

ΔG_m = predicted genetic gain from male selection,

SI_1 = selection intensity between/among female or male families, respectively[1], i.e., number of effective flowering families remaining in seed orchard after roguing of poor families, assuming 75% flowering of families (Swain et al. 2013b, Chapter 6),

SI_2 = selection intensity within female families[1],

SI_3 = selection intensity within male families[1], taking thinning into account,

r_g = juvenile-mature genetic correlation,

cr = coefficient of relationship,

σ^2_A = additive genetic variance,

σ_{fm} = standard deviation between/among families,

t = number of trees per family,

σ_{wf} = standard deviation within families.

[1] Selection intensities were from the two standardised selection intensity tables of Becker (1984).

ii) B1.3 - Breeding population of open-pollinated families ex-archive:

$\Delta G = 0.5(\Delta G_f) + 0.5(\Delta G_m)$, where

$$\Delta G_f = \Delta G_m = \left[SI_1 \times r_g \times cr \times \frac{\sigma^2_A}{\sigma_{fm}} + SI_2 \times r_g \times (1 - cr) \times \frac{\sigma^2_A}{\sigma_{wf}} \right]$$ and:

ΔG = predicted genetic gain,

ΔG_f = predicted genetic gain from female selection,

ΔG_m = predicted genetic gain from male selection,

SI_1 = selection intensity between/among female and male families, respectively[1], i.e., number of effective flowering families selected for cloning after roguing of poor families,

SI_2 = selection intensity within female and male families, respectively[1].

The remainder of the variables are defined as for B1.2 above.

[1] Selection intensities were from the two standardised selection intensity tables of Becker (1984).

b) Production population scenarios

i) P2.1 – Half-pedigreed open-pollinated seedling orchard / forward selection (FS) from current F2 trials, and

ii) P2.9 – Seedling orchard from FS and thinning and roguing / FS from current F2 BSOs:

$$\Delta G = 0.5\left(\Delta G_{fp}\right) + 0.5\left(\Delta G_{mp}\right), \text{ where}$$

$$\Delta G_{fp} = \left[\left(SI_{1p} \times r_g \times cr \times \frac{\sigma^2{}_A}{\sigma_{fm}} + SI_{2p} \times r_g \times \left(1 - cr\right) \times \frac{\sigma^2{}_A}{\sigma_{wf}} \right)\right] \text{ and}$$

$$\Delta G_{mp} = \left[SI_{1p} \times r_g \times cr \times \frac{\sigma^2{}_A}{\sigma_{fm}} + SI_{3p} \times r_g \times \left(1 - cr\right) \times \frac{\sigma^2{}_A}{\sigma_{wf}} \right] \text{ and:}$$

ΔG = predicted genetic gain,

ΔG_{fp} = predicted genetic gain from female selection,

ΔG_{mp} = predicted genetic gain from male selection,

SI_{1p} = selection intensity between/among families of the breeding population for female and male production parents, respectively[1], i.e., number of effective flowering families remaining in seed orchard after roguing of poor families, assuming 75% flowering of families (Swain et al. 2013b, Chapter 6),

SI_{2p} = selection intensity within families of the breeding population for female production parents[1],

SI_{3p} = selection intensity within families of breeding population for male production parents[1], taking thinning into account,

The remainder of the variables are defined as for B1.2 above.

[1] Selection intensities were from the two standardised selection intensity tables of Becker (1984).

iii) P2.4 – Clonal orchard (CSO) from FS / FS using CSOs

$\Delta G = 0.5(\Delta G_{fp}) + 0.5(\Delta G_{mp})$, where

$$\Delta G_{fp} = \Delta G_{mp} = \left[SI_{1p} \times r_g \times cr \times \frac{\sigma^2{}_A}{\sigma_{fm}} + SI_{2p} \times r_g \times (1 - cr) \times \frac{\sigma^2{}_A}{\sigma_{wf}} \right]$$ and:

ΔG = predicted genetic gain,

ΔG_{fp} = predicted genetic gain from female selection,

ΔG_{mp} = predicted genetic gain from male selection,

SI_{1p} = selection intensity between/among families of the breeding population for female and male production parents, respectively[1], i.e., number of effective flowering families selected for cloning after roguing of poor families,

SI_{2p} = selection intensity within families of the breeding population for female and male production parents, respectively[1].

The remainder of the variables are defined as for B1.2 above.

[1] Selection intensities were from the two standardised selection intensity tables of Becker (1984).

iv) P2.6 – Clonal orchard from FS and roguing / FS using CSO and roguing (backward selection (BS)):

$\Delta G = 0.5(\Delta G_{fp}) + 0.5(\Delta G_{mp})$, where

$$\Delta G_{fp} = \Delta G_{mp} = \left[SI_{1p} \times r_g \times cr \times \frac{\sigma^2{}_A}{\sigma_{fm}} + SI_{2p} \times r_g \times (1 - cr) \times \frac{\sigma^2{}_A}{\sigma_{wf}} + 2 \times SI_{3p} \times r_g \times cr_p \times \frac{\sigma^2{}_{Ap}}{\sigma_{fp}} \right]$$

and:

ΔG = predicted genetic gain,

ΔG_{fp} = predicted genetic gain from female selection,

ΔG_{mp} = predicted genetic gain from male selection,

SI_{1p} = selection intensity between/among families of the breeding population for female and male production parents, respectively[1], i.e., number of effective flowering families selected for cloning after roguing of poor families,

SI_{2p} = selection intensity within families of the breeding population for female and male production parents, respectively[1],

SI_{3p} = backward selection intensity for roguing of the CSO using progeny test information[1],

cr_p = coefficient of relationship in production population,

σ^2_{Ap} = additive genetic variance of progeny test,

σ_{fp} = standard deviation of family means in progeny test used for backward selection.

The remainder of the variables are defined as for B1.2 above.

[1] Selection intensities were from the two standardised selection intensity tables of Becker (1984).

c) Parental reconstruction

This comprises two parts, summarised in the formula below:

i) B1.4 – Breeding population of full-sib families:

As above for B1.2, but using full-sib information from genotyping study

ii) Bi-clonal production orchard:

$\Delta G = 0.5(\Delta G_{fp}) + 0.5(\Delta G_{mp})$, where

$$\Delta G_{fp} = \Delta G_{mp} = \left[SI_{1p} \times cr \times \frac{\sigma^2{}_A}{\sigma_{fm}} + SI_{2p} \times r_g \times (1 - cr) \times \frac{\sigma^2{}_A}{\sigma_{wf}} + 2 \times SI_{3p} \times cr_p \times \frac{\sigma^2{}_{AD}}{\sigma_{fp}} \right]$$ and

ΔG = predicted genetic gain from bi-clonal orchard,

ΔG_{fp} = predicted genetic gain from female selection,

ΔG_{mp} = predicted genetic gain from male selection,

SI_{1p} = selection intensity between/among families of the breeding population for top 10 female and male production parents, respectively, i.e., number of families selected for cloning after roguing of poor families, as bi-clonal production parents[1],

SI_{2p} = selection intensity within families of the breeding population for female and male bi-clonal production parents, respectively[1],

SI_{3p} = backward selection intensity for roguing bi-clonal pairs of orchards, using progeny test information[1],

cr = cr_p = coefficient of relationship = 0.5, given molecular studies,

σ^2_A = additive genetic variance,

σ^2_{AD} = broad sense genetic variance,

σ_{fm} = standard deviation between/among families,

σ_{wf} = standard deviation within families,

σ_{fp} = standard deviation of clonal family means in a progeny test of the bi-clonal orchards

[1] Selection intensities were from the two standardised selection intensity tables of Becker (1984).

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