Reproductive and Molecular Biology of

_Eucalyptus marginata_ Donn ex Smith

Margaret Anne Wheeler Bachelor of Science (Hon.)
University of New England

This thesis is presented for the degree of Doctor of Philosophy
of Murdoch University (2003)
I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary institution.

Margaret Wheeler
Abstract

This thesis examined aspects of the reproductive and molecular biology of *Eucalyptus marginata* (jarrah). The aims were to develop protocols for controlled pollination, that could be used in clonal orchard trees to breed jarrah seedlings that have a known genetic resistance to *Phytophthora cinnamomi* (dieback), for use in rehabilitation after mining and logging. An intimate knowledge of the breeding biology of jarrah was necessary to achieve this aim. The project also aimed to increase knowledge of the genetic diversity and structure of jarrah, in order to make informed decisions regarding the collection of material to be used for clonal propagation. Previous research has had little success in producing viable seed from any controlled pollinations, but clonal material resistant to *P. cinnamomi* has been produced using tissue culture. The question posed in this thesis was ‘Can we improve breeding and propagation techniques of jarrah?’

Techniques were developed for testing of in vitro pollen viability and pollen storage, pollination and fertilisation success after controlled pollinations, including determination of stigma receptivity and development of bud isolation techniques using alfoil. The variation in female fertility between genotypes was examined. The use of paclobutrazol was explored as a method of increasing the level of viable seed production in clonal orchard trees. The use of fertiliser as well as the growth retardant was also explored to see if it increased the level of seed production even more. Genetic diversity, genetic differentiation and phylogeny within *Eucalyptus*
marginata were examined using nuclear and chloroplast DNA analysis with Restricted Fragment Length Polymorphisms.

While it was first thought that the fertilisation rate was quite low, it was confirmed that the fertilisation rate is similar to other eucalypt species. The zygote abortion rate was quite high in one clone, but one wild tree had a similar seed production rate to other eucalypt species. The zygote and endosperm appeared to be different in the clone and the wild tree observed. The level of seed production was examined in clones and wild trees and it was found that the level was often quite low, particularly in the clones (0 – 13% in clones, 0 – 18% in wild trees) in comparison with other Eucalyptus species, and varied between genotypes. The use of a growth retardant such as paclobutrazol may increase the production of viable seed, if it is applied during autumn. The results were inconclusive for the fertiliser/paclobutrazol experiment, since the paclobutrazol was applied during spring which was the worst time of year for increasing seed production. There were differences between genotypes in reaction to both the paclobutrazol and the fertiliser/paclobutrazol. Genetic diversity was moderate in comparison with other Eucalyptus species, and there was a low level of genetic differentiation between populations in the nuclear genome. No differentiation was observed between the morphologically recognised subspecies in the nuclear genome, but differentiation between the populations on the Swan Coastal Plain and populations on the Darling Plateau was seen in the chloroplast genome, indicating that there was historical separation of these two areas.
The conclusions arising from this work are that while controlled pollinations are possible in *Eucalyptus marginata* the clones that were used in these experiments have often behaved differently to the wild trees in the time of anthesis and levels of viable seed production, and in one clone (5J119) the zygote and endosperm nuclei appeared to be very different to the zygote and endosperm nuclei of a wild tree. Further investigation is necessary to see if these differences are related to the low level of seed production observed in the clonal populations. Paclobutrazol may be worth exploring further as a means of increasing seed production. Material to be used for rehabilitation and seed orchards can be collected from a wide area in the main distribution of the species, although trees on the Swan Coastal Plain are distinct from the trees in the main forest area in the chloroplast genome.
# Table of Contents

Abstract i
Table of Contents iv
Acknowledgements ix
List of Abbreviations x
List of Publications arising from the project xi
Chapter 1. Introduction 1
Chapter 2. In-vitro Pollen Viability and Pollen Storage in *Eucalyptus marginata* 13
  2.1. Introduction 13
  2.2. Materials and Methods 16
    - 2.2.1. Trees used and pollen collection 16
    - 2.2.2. Developing germination media 17
    - 2.2.3. Pollen germination tests 17
    - 2.2.4. Testing pollen viability at varying times of day and weather conditions 18
    - 2.2.5. Storage test 19
    - 2.2.6. Viability testing 20
  2.3. Results 20
    - 2.3.1. Germination media 20
    - 2.3.2. Varying weather conditions and times of day 22
    - 2.3.3. Storage 23
    - 2.3.4. Viability testing methods 24
  2.4. Discussion 26
Chapter 3. Pollination success in *Eucalyptus marginata* 31
  3.1. Introduction 31
    - 3.1.1. Floral morphology and phenology 31
    - 3.1.2. Pollination ecology of *Eucalyptus* 32
    - 3.1.3. Pollen-stigma interaction 34
    - 3.1.4. Controlled pollination techniques for *Eucalyptus marginata* 35
  3.2 Materials and Methods 37
3.2.1. Tests of pollination techniques 37
3.2.2. Trees used 38
3.2.3. Isolation methods 42
3.2.4. Emasculation and pollination 42
3.2.5. Pollen processing 42
3.2.6. Stigmatic receptivity and pollination success 43
3.2.7. Statistical analysis 44

3.3. Results 44
3.3.1. Stigmatic receptivity 44
3.3.2. Emasculation and bud isolation 46
3.3.3. Pollen processing 47
3.3.4. Pollination trials 49

3.4. Discussion 55

Chapter 4. Variation in numbers of fertile seeds between genotypes, and natural bud, flower and fruit drop in *Eucalyptus marginata* trees 63

4.1. Introduction 63
4.2 Materials and methods 66
4.2.1. Trees used 66
4.2.2. Capsule drying and seed counts 67
4.2.3. Calculation of seeds/tree 68
4.2.4. Viability testing 69
4.2.5. Insect predators 71
4.2.6. Natural bud, flower and fruit drop 71
4.2.7. Statistical analysis 71

4.3. Results 71
4.3.1. Viability tests (season of 2000) 71
4.3.2. Predators 72
4.3.3. Fruit fertility 73
4.3.4. Bud, flower and fruit drops 81

4.4 Discussion 83
Chapter 5. Gamete development and self-incompatibility in *Eucalyptus marginata* 90

5.1 Introduction 90
5.1.1. Development of the megasporangium and the microsporangium 93

5.2. Materials and methods 95
5.2.1. Trees 95
5.2.2. Pollination 96
5.2.3. Collections and microscopy 96

5.3 Results 99
Paraffin embedded material 99
5.3.1. Gamete development 99
5.3.1.1. Microsporogenesis 100
5.3.1.2. Post-fertilisation 103
GMA embedded material 104
5.3.2. Position and number of ovules in ovaries 104
5.3.3. Pollen tubes in the lower style and ovary 109
5.3.4. Fertilisation and self-incompatibility in control pollinated material 110

5.4 Discussion 123

Chapter 6. The use of paclobutrazol to promote flowering and fruiting in orchard trees of *Eucalyptus marginata* (jarrah) 128

6.1. Introduction 128

6.2. Materials and methods 134
6.2.1. Trees 134
6.2.2. Application technique 135
6.2.3. Dose and time of application 136
6.2.4. Paclobutrazol application with the addition of fertilizer 136
6.2.5. Monitoring 136
6.2.6. Wild fire 137
6.2.7. Analysis 137

6.3 Results 141
6.3.1. Tree height, leaf length and tree girth 141
6.3.1.1. Tree height 141
Time and dose trial
Fertiliser trial
6.3.1.2. Leaf length
Time and dose trial
Fertiliser trial
6.3.1.3. Tree girth
Time and dose trial and fertilizer trial
6.3.2. Bud, capsule and viable seed numbers
Time and dose trial
Fertiliser trial
6.3.3. Effects of fire
6.3.4. Clone 700F50
6.4. Discussion
Chapter 7. Phylogenetic study of the nuclear DNA of *Eucalyptus marginata*
7.1. Introduction
7.2. Materials and methods
7.2.1 Plant material
7.2.2 Data analysis
7.3. Results
7.4. Discussion
Chapter 8. Chloroplast DNA variation in *Eucalyptus marginata* using RFLPs
8.1. Introduction
8.2. Materials and methods
8.2.1. Plant material
8.2.2. Data analysis
8.3. Results
8.3.1. Polymorphism in cpDNA
8.3.2. Haplotype relationships
8.3.3. Nucleotide diversity
8.4. Discussion
Chapter 9. General Conclusions
Acknowledgments

I thank my supervisors Jen McComb, Margaret Byrne, Ian Colquhoun and Mike Stukely for their continuous efforts and support throughout the duration of this project.

Mike Calver, Margaret Byrne, Bernie Dell, Michael Borowitzka, Lesley Brain and Julia Hobson have generously supported me with advice on statistics, writing and other advice.

John Kuo from the University of Western Australia also supported me with technical advice, making possible the embedding of specimens in GMA resin for the work in Chapter 5. Jamie O'Shea, also from the University of Western Australia allowed me access to the Harry Waring Mammal Reserve at Wattleup, where wild trees for Chapters 2, 4 and 5 were used. Melinda Moir and Brian Heterick from Curtin University are thanked for identification of invertebrates.

Alcoa World Alumina Australia have given financial support, both through the APA(I) scholarship and extra funding for the research in Chapter 8. David Willyams (Alcoa) has given me access to facilities and John Koch has allowed me access to unpublished data. The work in Chapter 7 was made possible by the financial support of a Murdoch University Research Grant.

Mike Jones and Dave Berriman have allowed me access and given assistance to the microscope facilities in the SABC centre at Murdoch University.

I thank Bronwyn MacDonald and Gordon Thompson for their excellent technical support, and Jolante Damas, Monika Wheeler, Ben Dawson, Meredith Fairbanks and Libby Burgess for laboratory and field assistance. Max Dawson also generously gave me access to facilities at Murdoch University.

Finally, I would like to thank David Wheeler and Joff Bennell for supporting me and managing to live with me during this period.
List of Abbreviations

Jarrah  \( Eucalyptus \ marginata \)

Dieback  \( Phytophthora \ cinnamomi \)

nDNA  deoxyribonucleic acid from the nuclear genome

cpDNA  deoxyribonucleic acid from the chloroplast genome

RFLP  Restricted Fragment Length Polymorphism

ssp.  Subspecies

BK medium  pollen germination medium based on Brewbaker and Kwack (1963)

BA medium  pollen germination medium based on maltose and 150ppm boric acid

ANOVA  Analysis of variance statistical analysis

CALM  Conservation and Land Management

GMA  glycol methacrylate

PAS/TBO  periodic acid-Schiff’s reagent/toluidine blue O

FAA  Formalin:Acetic Acid:70%Ethanol (1:1:18)

GA3  Giberellic acid 3

Ca  Calcium

N  Nitrogen

P  Phosphorous

K  Potassium

DBHOB  Diameter at breast height

\( A \)  number of alleles per locus

\( P \)  mean number of polymorphic loci

\( H_o \)  observed heterozygosity

\( H_e \)  expected heterozygosity

\( H_T \)  total heterozygosity

\( G_{ST} \)  percentage of genetic diversity between populations

\( F_{IS} \)  Wright’s inbreeding co-efficient

\( \theta \)  differentiation between populations

D  genetic divergence

\( f \)  degree of inbreeding within populations

F  overall inbreeding co-efficient
List of Publications arising from the project

Refereed Publications:


Conference Publications:


Posters:


Oral Presentations:


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