Self-incompatibility in *Eucalyptus globulus* 

and *E. nitens*

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B.Sc. (Hons)

Submitted in fulfilment of requirements for the degree of

Doctor of Philosophy

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University of Adelaide

November, 2002
# Table of Contents

Abstract ................................................................................................................................. 1
Declarations ............................................................................................................................ iii
Acknowledgements ............................................................................................................. iv

List of Figures ...................................................................................................................... v
List of Tables ....................................................................................................................... v
List of Plates ......................................................................................................................... vii

Chapter 1
General Introduction ............................................................................................................. 1
1.1 Introduction ..................................................................................................................... 1
1.2 Project aims ..................................................................................................................... 3

Chapter 2
Literature Review .................................................................................................................. 5
2.1 Taxonomy ....................................................................................................................... 5
2.2 Plantations ..................................................................................................................... 5
2.3 Genetic improvement .................................................................................................... 6
2.4 Reproductive biology/ecology ...................................................................................... 10
2.4.1 Floral morphology .................................................................................................... 10
2.4.2 Mating system ......................................................................................................... 13
2.4.3 Self-incompatibility ................................................................................................. 14
2.4.4 Mechanisms of self-incompatibility ....................................................................... 16
2.4.5 Eucalypt pollinators and their effect on mating systems ....................................... 17
2.5 Inbreeding depression ................................................................................................. 18
2.6 Pollen competition ....................................................................................................... 20
2.7 Implications for forestry industry ................................................................................. 22
2.8 Conclusions .................................................................................................................. 23

Chapter 3
Characterisation of the self-incompatibility mechanism in *Eucalyptus nitens* ... 25
3.1 Introduction .................................................................................................................... 25
3.2 Materials and methods ................................................................................................. 27
3.2.1 Plants ....................................................................................................................... 27
3.2.2 Pollinations ............................................................................................................. 27
3.2.3 Pollen ....................................................................................................................... 27
3.2.4 Field harvests and microscopy .............................................................................. 28
3.2.5 Seed set .................................................................................................................... 30
3.2.6 Statistical analysis ................................................................................................. 30
3.2.6.1 Seed set ............................................................................................................. 30
3.2.6.2 Pollen tubes and ovule penetration ........................................ 30
3.2.6.3 Ovule fertilisation ............................................................ 31
3.2.6.4 Ovule dimensions .............................................................. 31
3.3 Results ................................................................................. 31
3.3.1 Seed set and self-incompatibility ........................................... 31
3.3.2 Pollen tubes in styles .......................................................... 36
3.3.3 Ovary arrangement and ovule penetration ............................. 36
3.3.4 Ovule structure ................................................................. 36
3.3.5 Ovule fertilisation .............................................................. 40
3.3.6 Ovule dimensions .............................................................. 43
3.4 Discussion ............................................................................ 48

Chapter 4
Pollen tube growth and seed set in Eucalyptus globulus ..................... 52
4.1 Introduction .......................................................................... 52
4.2 Materials and methods ......................................................... 53
4.2.1 Trees .............................................................................. 53
4.2.2 Pollination ....................................................................... 54
4.2.3 Field harvests and microscopy .......................................... 54
4.2.4 Seed set .......................................................................... 55
4.2.5 Statistical analysis ............................................................. 55
4.2.5.1 Pollen tube growth in styles .......................................... 55
4.2.5.2 Ovary arrangement ....................................................... 56
4.2.5.3 Ovule penetration by pollen tubes ............................... 56
4.2.5.3 Seed set ...................................................................... 57
4.3 Results ................................................................................ 57
4.3.1 Seed set .......................................................................... 57
4.3.2 Pollen tube growth in styles ............................................. 57
4.3.3 Ovary arrangement ........................................................... 61
4.3.4 Ovule penetration by pollen tubes ................................... 61
4.4 Discussion ............................................................................ 65

Chapter 5
Early ovule development in Eucalyptus globulus .............................. 71
5.1 Introduction .......................................................................... 71
5.2 Materials and methods ......................................................... 72
5.2.1 Plants and pollinations ..................................................... 72
5.2.2 Field harvests and microscopy .......................................... 72
5.2.3 Statistical analysis ............................................................. 73
5.3 Results ................................................................................ 74
5.3.1 Ovule structure ............................................................... 74
5.3.2 Ovule fertilisation ............................................................ 78
5.3.3 Ovule dimensions ............................................................ 82
5.4 Discussion ............................................................................ 86
Chapter 6
Pollen competition does not affect the success of self-pollination in *Eucalyptus globulus* ................................................. 93

6.1 Introduction ...................................................................... 93
6.2 Materials and methods .................................................. 94
6.2.1 Plants ........................................................................... 94
6.2.3 Pollinations .................................................................. 95
6.2.4 Pollen treatments ....................................................... 95
6.2.5 Testing pollen mixtures .............................................. 95
6.2.6 Seed set ...................................................................... 96
6.2.7 Isozyme analysis ....................................................... 99
6.2.8 Statistical analysis .................................................... 104
6.2.8.1 Seed set ............................................................... 104
6.2.8.2 Seed paternity ..................................................... 104
6.3 Results ........................................................................... 105
6.4 Discussion .................................................................... 109

Chapter 7
General Discussion ................................................................ 114

Bibliography ........................................................................ 119

Appendix 1: Publications ..................................................... 134
Appendix 2: Industry Communication ................................. 135
Abstract

*Eucalyptus globulus* and *E. nitens* are two species within the subgenus *Symphyomyrtus*, widely grown in plantations predominantly for pulpwood production. This study investigated aspects of the breeding system to determine the mechanism of self-incompatibility operating in each species, and whether cross-pollen can outcompete self-pollen following mixed pollinations in *E. globulus*.

The self-incompatibility mechanism in each species was investigated by conducting controlled self- and cross-pollinations on five mature trees. Pollen tube growth in the style and pollen tube penetration of ovules was visualised using fluorescence microscopy, and embryology was examined by bright-field microscopy on sectioned material. Some pollinated flowers were left on the trees until seed maturity, then seed counts were used to establish the self-incompatibility level of each tree.

Self-incompatibility levels of *E. nitens* trees ranged from 25.8 to 93.6%. There was no significant difference between self- and cross-pollen treatments in the number of pollen tubes present in styles or in the percentage of ovules penetrated by pollen tubes. Fertilisation had taken place by 2 weeks after pollination with nearly every ovule showing evidence of fertilisation. Cross-pollination resulted in a greater proportion of healthy, developing ovules, at both 2 and 4 weeks after pollination, compared with self-pollination. The proportion of degenerating ovules increased from 2 to 4 weeks after pollination. Self-incompatibility in *E. nitens* appears to be a post-zygotic mechanism. Differences in ovule size from 6 weeks after pollination onwards show potential for identifying self-incompatible trees.

*Eucalyptus globulus* trees had self-incompatibility levels ranging from 76 to 106%. There was no significant difference in the number of pollen tubes in the style between self- and cross-pollen treatments although variation was present between
trees. The number of pollen tubes present was similar to the number of ovules present within flowers. At 2 weeks after pollination there was no significant difference between pollen treatments in the number of ovules penetrated by a pollen tube. By 4 weeks after pollination, there was slightly greater penetration by cross-pollen tubes. Fertilisation had taken place by 4 weeks after pollination with zygotes and free nuclear endosperm visible. There was a greater proportion of healthy, fertilised ovules in the cross- compared with the self-pollination treatment. Approximately half the ovules examined from both pollen treatments were not fertilised or were degenerating. By 6 weeks after pollination a few zygotes were starting to divide. The mechanism of self-incompatibility appears to have both late- and post-zygotic components. Fertilised ovules were significantly larger than non-fertilised or degenerating ovules and this difference was detectable by eye at 6 and 8 weeks after pollination. As in E. nitens, these differences in ovule size show potential for the identification of self-incompatible trees.

To investigate whether pollen competition occurs favouring cross- over self-pollination in Eucalyptus globulus, controlled pollinations using self-pollen, cross-pollen and a mixture of self- and cross-pollen were conducted on three partially self-incompatible trees. The paternity of individual seeds resulting from mixed pollination was determined by isozyme analysis. No evidence for pollen competition was found. Instead, seed paternity reflected the level of self-incompatibility of each tree as determined by separate self- and cross-pollinations. Furthermore, the number of seeds set per capsule following mixed pollination was significantly less than following cross-pollination in the two least self-compatible trees. These results suggest that both self- and cross-pollen tubes reach ovules following mixed pollination, and that a late-acting self-incompatibility mechanism operates to abort a certain proportion of self-penetrated ovules.

This research has added to our knowledge on reproductive biology strategies within Eucalyptus and has produced useful information for eucalypt breeders.