"Eucalyptus is not only the Universal Australian, it is the Ideal Australian - versatile, tough, sardonic, contrary, self-mocking, with a deceptive complexity amid the appearance of massive homogeneity"

(Stephen J. Pyne *The Burning Bush* 1992, pp. 22)

This natural F1 hybrid between *Eucalyptus stoatei* and *E. tetraptera* was grown from seed collected in the Jerdacuttup area, south east of Ravensthorpe in Western Australia by Dean Nicolle. The photograph was taken at Dean's Currency Creek Arboretum, in South Australia.

Printed on carbon neutral paper

Exotic gene flow from plantation to native eucalypts

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B.Sc. (Hons)

Submitted in fulfilment of the requirements for the degree of Doctorate of Philosophy

School of Biological Sciences, University of Tasmania

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Declarations

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Signature: Date:

The *Candidate* Matthew James Larcombe

We the undersigned agree with the above declarations, and submission of the final corrected version of this thesis:

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Statement of co-authorship/contribution

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Below are the details of which papers the co-authors contributed to, the contributions they made and where the papers can be found in the thesis:

Chapter 3 is published:

Larcombe MJ, Vaillancourt RE, Jones RC, Potts BM (2014) Assessing a Bayesian approach for detecting exotic hybrids between plantation and native eucalypts. *International Journal of Forestry Research* **2014**: DOI:10.1155/2014/650202.

Candidate was the primary author, undertook all analysis, field and lab work. The Candidate as well as authors 1, 2 and 3 contributed to developing the idea, and approach. Authors 1, 2 and 3 assisted with refining the text.

Chapter 4 is published:

Larcombe MJ, Barbour RC, Vaillancourt RE, Potts BM (2014) Assessing the risk of exotic gene flow from *Eucalyptus globulus* plantations to native *E. ovata* forests. *Forest Ecology and Management* **312**,193-202.

Candidate was the primary author, undertook most field, glasshouse, lab work and analysis. Author 4 established the paired hybrid trial in 2006, author 1 assisted with analysis. The candidate as well as author 1, and 2 contributed to developing the main idea, and approach. The paired hybrid trial was the idea of Author 4. Author's 1 and 2 assisted with refining the text.

Chapter 5 is published**:**

Larcombe MJ, Silva JS, Vaillancourt RE, Potts BM (2013) Assessing the invasive potential of *Eucalyptus globulus* in Australia: quantification of wildling establishment from plantations. *Biological Invasions* **15**, 2763-2781.

Candidate was the primary author and was involved in all field work. Field work undertaken in Victoria was done in partnership with author 5. The candidate undertook most analysis with assistance from authors 1 and 5. The candidate as well as author 1, and 5 developed the main ideas, and approach. Author's 1, and 2 and 5 assisted with refining the text.

The following people made contributions to the unpublished Chapter 2:

The candidate was the lead author, undertook some analysis, all field work, glasshouse work and project specific lab work (with the exception of the generating the DArT data see below).

Barbara R. Holland, School of Maths and Physics, University of Tasmania. Barbara gave statistical advice and assisted with analysis, particularly the computer code (R code) used to undertake the modelling.

Rebecca C. Jones, School of Plant Science, National Centre for Future Forest Industries, University of Tasmania, Hobart, Australia – provided the species-level DArT dataset and commented on the text.

Dorothy A. Steane, School of Plant Science, National Centre for Future Forest Industries, University of Tasmania, Hobart, Australia – provided the section-level DArT dataset and commented on the text.

Dean Nicolle, Currency Creek Arboretum, South Australia – provided access to the Arboretum where most of the crossing was undertaken. He also contributed his unpublished classification of *Eucalyptus*, and commented on the text.

We the undersigned agree with the above stated "proportion of work undertaken" for the published chapters 3, 4 and 5, and the unpublished Chapter 2 of this thesis:

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Abstract

The movement of species around the world by humans has created situations where "exotic" gene flow can arise between species that would not naturally co-occur. *Eucalyptus globulus* has been planted widely throughout temperate Australia over the past 15 years, with around 538,000 ha of plantations now growing, mainly outside the species native range. Concerns have been raised that these plantations could genetically contaminate natural eucalypt populations. This thesis aimed to assess the risk, and management, of pollen- and seed-mediated gene flow from *E. globulus* plantations.

The thesis initially addresses the risk of introgression through pollen-mediated gene flow from *E. globulus* plantations and hybridisation with co-occurring native species. Prior to this study there were no known complete barriers to hybridisation between *E. globulus* (as the pollen parent) and other species in subgenus *Symphyomyrtus*. This meant that as many as 484 species could have been considered at risk of hybridisation if they occurred within the pollen dispersal zone of *E. globulus* plantations. A controlled crossing program (where *E. globulus* pollen was applied to the stigma of 100 other eucalypts species) was undertaken to identify phylogenetically controlled barriers to hybridisation in subgenus *Symphyomyrtus*. This crossing suggests the presence of a complete barrier to hybridisation between *E. globulus* and more divergent groups within *Symphyomyrtus*, probably reducing the number of at-risk species by over 70% (to 138). Hybridisation success declined with increasing genetic distance, meaning the most at risk species were those within the same taxonomic section as *E. globulus*, *Maidenaria* (68 species). The results also provided new insights into the evolution of reproductive barriers in forest trees.

Because hybrid identification is vital for management of exotic gene flow and can be difficult in eucalypts, a Bayesian modelling approach to detect hybrids in at-risk species was tested. Range-wide samples from five at risk species, as well as samples from *E. globulus* (total n = 606 individuals) were genotyped at 10 microsatellite loci. The ability of Bayesian clustering to identify hybrids using this database was tested

using simulations. The technique was highly effective at identifying F_1 hybrids, which are currently the primary concern in the Australian *E. globulus* estate.

The crossing study showed that species in section *Maidenaria* should be the focus of management attention. The frequent proximity of *E. ovata* (*Maidenaria*) to plantations and its known cross-compatibility with *E. globulus* makes it a prime candidate for exotic gene flow. However, by conducting a case study in *E. ovata* forests around plantations, the actual risk posed was found to be low. Hybridisation was assessed in 24,322 open pollinated progeny from 142 trees in 25 native forest remnants. Although patch size and tree position affected hybridisation risk (small patches and edge trees were at highest risk), the rate of hybridisation declined very rapidly inside *E. ovata* patches, and hybrid establishment along native forest-plantation boundaries was low. Furthermore, hybrids showed a 78% reduction in survival compared to pure *E. ovata* after six years, making it unlikely that hybrids will reach reproductive maturity to enable backcrossing and subsequent introgression. However this study showed that pure *E. globulus* seedlings (wildings) were establishing in far higher numbers than hybrids at the edge of plantations, raising the concern that they could pose a threat to native forests.

As well as having ecological impacts as locally exotic species, wildlings could cause introgression via hybridisation if they reach reproductive maturity. To assess the risk that wildings pose to native forests in Australia, surveys to quantify current levels of establishment were undertaken along 290 km of *E. globulus* plantation edges. Wildling establishment was low with the vast majority occurring within the plantation disturbance zone. It also appears that current management practices, including short rotations and firebreak maintenance, are reducing the risk of wildling spread.

In conclusion this thesis has found that there are significant barriers to hybridisation between *E. globulus* and native eucalypts that will limit the opportunity for exotic gene flow. If these barriers are overcome, avenues for management exist. While wildling establishment appears to currently be limited, the Australian plantation estate is young and on-going monitoring is warranted.

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List of publications and presentations arising from this thesis

Refereed journal articles:

Larcombe MJ, Vaillancourt RE, Jones RC, Potts BM (2014) Assessing a Bayesian approach for detecting exotic hybrids between plantation and native eucalypts. *International Journal of Forestry Research* **2014** DOI:10.1155/2014/650202.

Larcombe MJ, Barbour RC, Vaillancourt RE, Potts BM (2014) Assessing the risk of exotic gene flow from *Eucalyptus globulus* plantations to native *E. ovata* forests. *Forest Ecology and Management* 312 (0):193-202.

Larcombe MJ, Silva JS, Vaillancourt RE, Potts BM (2013) Assessing the invasive potential of Eucalyptus globulus in Australia: quantification of wildling establishment from plantations. *Biological Invasions* **15**, 2763-2781.

Refereed Conference Proceedings:

Larcombe MJ, Barbour R, Vaillancourt, RE and Potts, BM (2012) Gene flow from Australia's eucalypt plantations. Australian Forest Growers National Conference: Diverse sub-tropical forestry, 14th – 17th October 2012. Gympie, Queensland. pp 82-91.

Presentations:

Invited Presentations:

Larcombe MJ, Barbour R, Vaillancourt, RE and Potts, BM (2012) Gene flow from Australia's eucalypt plantations. Invited presentation at the Australian Forest Growers National Conference: Diverse sub-tropical forestry, 14th – 17th October 2012. Gympie, Queensland.

Other presentations:

Larcombe MJ, Steane D, Jones RC, Nicolle D, Holland B, Vaillancourt RE, Potts BM. Phylogenetic patterns of reproductive isolation in *Eucalyptus*. Presentation to Phylomania 2013, University of Tasmania, School of Maths and Physics, 6-8 November 2013.

Larcombe MJ, Silva JS, Vaillancourt RE, Potts BM (2013) Quantification of wildling establishment from Australian *Eucalyptus globulus* plantations. Presentation at the VII Southern Connection Congress: Southern lands and southern oceans - life on the edge? 21st – 25th January 2013. Dunedin, New Zealand.

Larcombe M. Potts, BM and Vaillancourt, RE (2012) Recent progress in measuring gene flow from *Eucalyptus globulus* plantations. Presentation at the workshop on 'Gene flow from planted eucalypts workshop'. CRC Annual Science Meeting, 5th March 2012. Mooloolaba, Queensland

Potts BM, Barbour R, Larcombe M, Vaillancourt RE (2012) Assessing and managing the risk of gene flow from eucalypt plantations in Australia: an overview. Presentation at the workshop on 'Gene flow from planted eucalypts workshop'. CRC Annual Science Meeting, 5th March 2012. Mooloolaba, Queensland.

Posters:

Larcombe MJ, Vaillancourt, RE and Potts, BM (2012) The landscape context of pollen-mediated gene flow from *Eucalyptus globulus* plantations. Poster at the CRC Annual Science Meeting, 6th – 8th March 2012. Mooloolaba, Queensland.

Larcombe MJ, Vaillancourt, RE and Potts, BM (2010) Managing gene flow from plantation to native eucalypts. Poster at the CRC Annual Science Meeting, 18th – 20th May 2010. Fremantle, Western Australia

Format of thesis chapters

The experimental chapters of this thesis (chapters 2 – 5) have been written in paper style and chapters 3 – 5 are published. Being paper style means that some repetition of concepts and ideas was unavoidable, particularly in the introduction sections. The published papers have been reformatted (including figure and table numbering), and the acknowledgments and references have been consolidated into single sections in the thesis. Chapter 1, the general introduction, includes text that was published as a peer reviewed conference proceedings and the full paper is provided in the General Appendix (with all publications arising from the thesis) in the back of the thesis.

Table of Contents