

**Final Report: Hybridisation & rapid evolution in eucalypts of the Murray-Darling Basin**

*Eucalyptus largiflorens* is a floodplain tree, common on the Chowilla Floodplain of the Murray River, South Australia. It typically has glaucous leaves however occasionally unusual trees with smaller, glossy green leaves (Green Box) are found among *E. largiflorens*. In areas where salinity and waterlogging are increased these individuals usually appear healthier than adjacent *E. largiflorens*. Previous research established that Green Box individuals are hybrids between *E. largiflorens* and *Eucalyptus gracilis* (Zubrinich, 1996; Koerber, 2004). *E. gracilis* is a mallee eucalypt, found distant from the floodplain, the closest individuals are found approximately three kilometres from stands of *E. largiflorens*. It is thought that Green Box inherited its salt tolerance and conservative water use from *E. gracilis*. This study aimed to investigate some of the reproductive parameters of Green Box compared with its parental taxa as little was known of its reproductive fitness and therefore its ability to persist or expand in the altered Chowilla Floodplain.

The pollination of the parental species and hybrid was investigated through an analysis of the nectar rewards offered by each, and by observations of floral visitors. *E. gracilis* was found to offer much higher volumes of concentrated nectar than *E. largiflorens*. Green Box offered higher nectar rewards than either parental species. The different reward schedules offered by the parental species indicate that they may be differentially attractive to pollinator groups. Birds, honeyeaters and lorikeets, and insects, honeybees and flies, were observed at the parental species and the hybrids. It was hypothesised in this thesis that birds may be responsible for long distance pollination between the parental taxa, crucial in hybridisation events, and insects may be responsible for intrapopulational gene movement and therefore involved in backcrossing between the parental taxon, *E. largiflorens*, and the hybrid, Green Box. If Green Box becomes more common in the landscape a shift in pollinator activity or pollinator assemblages may occur together with a higher incidence of gene fixation at various loci.

The reproductive output of the hybrid was compared to the parental species through an assessment of fruit and seed set. The number of Green Box fruit set per inflorescence was equivalent to its parental species but its seed per fruit was lower. A small number of open pollinated seeds from each taxon was germinated and germination of hybrid seed was as successful as the parental taxa, confirming the findings of Zubrinich (1996). Earlier work in the 1990's found that seed of Green Box individuals was highly variable and often displayed slow growth rates. Further investigation into the success of Green Box seedlings is warranted.

Maternity of the hybrid was assumed to be unidirectional owing to the presumed shorter style length in *E. largiflorens* compared to *E. gracilis* (Zubrinich, 1996). Style measurements conducted in this study demonstrated that *E. largiflorens* had a longer style but chloroplast SSR analysis confirmed that it is consistently the maternal taxon of Green Box.

As leaf colour is one of the major identifying characters of Green Box, it was analysed using reflectance spectrometry. Leaf colour of hybrids was found to be intermediate between the parental taxa and more variable than either. Leaves of *E. largiflorens* individuals from the Chowilla Floodplain were more variable in colour than individuals from distant (pure) populations of this species. Potentially, this may be due to interbreeding between Green Box and *E. largiflorens* on the floodplain, or it might reflect different environmental stresses. Major reflectance differences between the parental taxa were found in the UV range (~240 – 390 nm) which may result from differences in leaf waxes, as the glaucous leaves of *E. largiflorens* have a denser covering of wax than *E. gracilis*. Further investigation into the leaf waxes of the hybrid in relation to the parents may reveal patterns of leaf wax inheritance of these hybrids.

This project has covered much groundwork that will assist in further research into these apparently well adapted hybrids. Further work should include perfecting controlled pollination techniques which were attempted in this study but were not successful as the small size of flowers led to damage of the hypanthium during emasculation and caused flowers to abort. Once hand pollination techniques are perfected more information about the breeding systems, levels of self-incompatibility of the parental species, whether *E. gracilis* is able to act as the maternal parent and ideally an artificial breeding programme could be established. Furthermore, aspects of pollen longevity and stigma receptivity need to be established using the time from operculum shed, to anther dehiscence and stigma receptivity as developmental timeframes

In summary, this study found that Green Box is capable of reproducing and may be a conduit for transferring *E. gracilis* traits to the *E. largiflorens* gene pool. The research has been presented at the McCormick Centre, Renmark (26<sup>th</sup> October 2011), and La Trobe University, Bundoora, (26<sup>th</sup> March 2012).

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## References

- Koerber GR. 2004.** *Morphological, physiological and AFLP molecular marker assessment of a putative hybrid origin for 'green variant plants' of Eucalyptus largiflorens F. Muell. and its apparent 'salt tolerance' in order to select plants for revegetation studies.* Flinders University of South Australia. PhD thesis.
- Zubrinich TM. 1996.** *An investigation of the ecophysiological, morphological and genetic characteristics of Eucalyptus largiflorens F. Muell and Eucalyptus gracilis F. Muell. in relation to soil salinity and groundwater conditions throughout the Chowilla Anabranch.* Flinders University of South Australia. PhD thesis.



# "Out & About"

Community Environmental Education



## **Flux and Box**

**7pm Wednesday  
26 October 2011**

### **McCormick Centre for the Environment**

**Ral Ral Ave, RENMARK**

**Exciting research projects at Calperum Station**

#### **Calperum Flux Tower**

**Timothy Lubcke**, University of Adelaide will discuss how as part of an International initiative this project will allow better understanding of climate, water & carbon transfer in this mallee ecosystem.

#### **Green Box**

**Louise Romanin**, La Trobe University, Melbourne will present her further study of the Green Box, a naturally occurring hybrid of the Eucalyptus largiflorens (Black Box) & E. gracilis (Yorrell Mallee).

***Come & enjoy an interesting evening***

**Supper provided**

RSVP or for more information contact Teresa  
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