SHORT COMMUNICATION

The green-leaved variant of *Eucalyptus largiflorens*: a story involving hybridization and observant local people

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Abstract: *Eucalyptus largiflorens* (Black Box) is the most common tree in the Chowilla anabranch system on the Murray River floodplain. It typically has dull, glaucous, grey-green leaves. Occasional trees with smaller, glossy green leaves (Green Box) occur scattered amongst the Black Box. In areas with increasing salinity, they usually appear much healthier than adjacent, normal Black Box trees. Green Box plants are intermediate between normal *Eucalyptus largiflorens* plants and *Eucalyptus gracilis* plants in many morphological and allozyme characters, strongly suggesting that they are hybrids between those species. Green Box plants tolerate salinity better and use water more conservatively than normal Black Box plants, traits that they have probably inherited from *Eucalyptus gracilis*.

In 1994, the Botanic Gardens of Adelaide used tissue culture and micropropagation to produce nearly 9,000 cloned Green Box plants which were planted out on Riverland floodplains. Since the 1990s, the high cost of producing clonal plants has meant that no further such plantings have occurred. Because Green Box plants can be a considerable distance from the nearest plants of one putative parent (*Eucalyptus gracilis*), more detailed studies could contribute to the existing work on such phantom hybrids.

***Cunninghamia* (2010) 11(4): 413–416**

Introduction

Although a green-leaved variant of *Eucalyptus largiflorens* is mentioned in 'Trees for Saline Landscapes' (Marcar & Crawford 2004), it has never been dealt with in any mainstream taxonomic literature. However, the story of the discovery of this variant and the subsequent research on its evolution, physiology and use in saline landscapes is instructive in a number of ways and deserves to be more widely known. In this note, we bring together the sparse, widely-scattered literature to produce a summary of the topic. For brevity, we follow Nicholls (2009) in referring to the variant as Green Box. Plant nomenclature follows Chippendale (1988).

The Green Box Story

On the floodplain of the Murray River in the Chowilla anabranch system in southwestern New South Wales and eastern South Australia, the most common tree is *Eucalyptus largiflorens* (Black Box) (Zubrinich *et al*. 2000); it normally occurs upslope on less-flooded sites than *Eucalyptus camaldulensis* (River Red Gum) (Mensforth *et al*. 1994). Since European settlement, many of the floodplain eucalypts are dying because of rising saline groundwater and reduced flooding frequency (Zubrinich *et al.* 2000).

Typical trees of *Eucalyptus largiflorens* have dull, glaucous, grey-green leaves. While travelling through a stand of them on Chowilla Station in the mid 1930s, Dudley Foweraker, a nurseryman from Renmark, noticed a box-barked tree with smaller, glossy green leaves (Nicholls 2009). Later, in the 1970s, Janyce Frahn of Paringa noticed a similar tree on the nearby Pike River floodplain (Nicholls 2009). More of these Green Box trees turned up in a 1982 Society for Growing Australian Plants survey which was part of the Pike River Management Plan. Then, in 1989, Denis Frahn, a farmer and fruit grower from Paringa, showed one of the trees to the late Jack Seekamp, an agricultural scientist and fruit grower from Renmark (Nicholls 2009). Because Green Box trees usually appear much healthier than adjacent Black Box ones, Seekamp was quick to start studying them in the field and to alert others to their possible significance (Nicholls

2009). As a result, in 1992, one of us (TMZ) started her Ph.D. candidature at the Flinders University of South Australia under the supervision of Professor S.D. Tyerman and Dr. Molly Whalen to investigate the origin of Green Box and to determine its ability to cope with shallow, saline groundwater compared to the common form of Black Box (Zubrinich 1996).

This work established that Green Box trees have now been found on floodplains from at least as far east as Moulamein and Deniliquin in southwestern New South Wales and west to Sedan near Blanchetown in South Australia. They have also been recorded in Victoria along the Murray River as far upstream as Boundary Bend (Seekamp 1990–1994). They are much less common than normal Black Box trees, occurring as occasional, scattered trees in what appears to be normal Black Box habitat (Zubrinich 1996).

Detailed studies of adult and seedling morphology showed that Green Box plants are intermediate in many cotyledon, leaf, bark, flower and fruit characters between *Eucalyptus largiflorens* and *Eucalyptus gracilis* (White Mallee) and so are considered to be hybrids between those two species (Zubrinich *et al.* 2000). A large-scale allozyme analysis also provided substantial evidence that Green Box plants are hybrids between those species (Zubrinich 1996). A later Ph.D. project using AFLP DNA markers also supports that view (Koerber 2004).

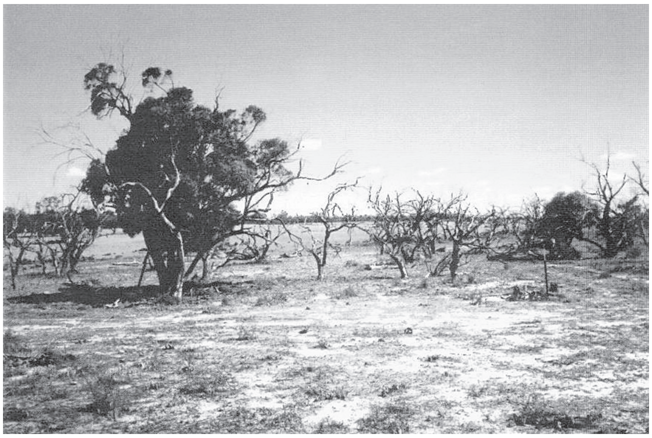

Fig. 1. Murray floodplain at Tareena Station, New South Wales. Live Green Box tree with dead *Eucalyptus largiflorens* trees nearby. Photo by J.V. Seekamp, 29 January 1999.

Fig. 2. Murray floodplain at Coombool Inlet, South Australia. Live Green Box tree with dead *Eucalyptus largiflorens* trees nearby. Photo by J.V. Seekamp, 3 October 1994.

An important character is that while *Eucalyptus largiflorens* has all stamens fertile, *Eucalyptus gracilis* has an outer ring of staminodes (filaments lacking anthers). Green Box resembles *Eucalyptus gracilis* in usually having an outer ring of staminodes, but with fewer staminodes in total than *Eucalyptus gracilis* (Zubrinich 1996).

Eucalyptus gracilis is a mallee (i.e. a tall shrub with many stems arising from an underground lignotuber) or tree found on non-flooded, well-drained, calcareous soils adjacent to the Chowilla floodplain and elsewhere (Zubrinich *et al.* 2000). *Eucalyptus largiflorens* and *Eucalyptus gracilis* are both from subgenus *Symphyomyrtus*, so that hybridization between them is not unexpected (Potts & Wiltshire 1997). However, they are members of different sections within the subgenus and are apparently only remotely related (Pryor & Johnson 1971). Hybridization between a tree species and a mallee species, as in this case, is known in many other cases (D. Nicolle pers. comm.).

Eucalyptus largiflorens can flower opportunistically more than once a year, depending on flooding and rainfall. *Eucalyptus gracilis* has a shorter flowering period with a different peak, but some synchronous flowering occurs, with a lot of overlap (Zubrinich 1996). Because *Eucalyptus gracilis* has larger flowers with longer styles than *Eucalyptus largiflorens* (while Green Box is intermediate in those characters), it may be unlikely that the pollen tube of *Eucalyptus largiflorens* can grow the full length of the style of *Eucalyptus gracilis*. If so, hybridization may only occur when *Eucalyptus largiflorens* is the maternal parent (Zubrinich 1996) as in the very similar case described by Gore *et al*. (1990). The Green Box trees are more variable than their putative parents in a number of adult leaf and fruit characters (Zubrinich 1996).

Detailed ecophysiological studies showed that Green Box tolerates saline conditions better than normal Black Box plants. Its more conservative water use, and ability to operate at lower water potential, may underlie its superior performance on the floodplain under present conditions (Zubrinich *et al*. 2000).

Some of the larger Green Box trees obviously pre-date European settlement, but it is clear that their numbers have increased through time, especially in the season following the 1956 floods (Seekamp 1999). Details are lacking about the extent to which established Green Box trees are producing seed and contributing to the spread of Green Box. By 1999, there were floodplain areas (e.g. near Tareena) carrying healthy Green Box trees but where nearly all of the *Eucalyptus largiflorens* trees were dead (Seekamp 1999, Fig. 1, Fig. 2).

Under pre-European conditions, the *Eucalyptus largiflorens* habitats on the floodplain were generally more favourable for plant growth than the adjacent non-flooded *Eucalyptus gracilis* habitats because of the extra water they received from floods. Thus, it is not surprising that Zubrinich (1996), in her seedling experiments, recorded faster, genetically-determined growth rates in *Eucalyptus largiflorens* than in *Eucalyptus gracilis*, probably caused by selection for conservative water use in the latter (Parsons 1968). The Green Box seedling growth rates were slower than normal Black Box, a feature presumably inherited from *Eucalyptus gracilis* (Zubrinich 1996).

In areas more distant from the Murray River, there are salt-affected sites which carry eucalypts in non-floodplain areas near the margins of salt lakes and saltpans. The predominant eucalypt on such sites is *Eucalyptus gracilis* (unpublished data; see also Rowan & Downes 1963 p. 54 and Rowan 1971 p. 21). Thus it is likely to be the most salinity-tolerant eucalypt native to this part of Australia. This notion is supported by Barrett *et al*. (2005), who found that it tolerated high leaf salt concentrations, with summer sodium concentrations as high as or higher than those reported for any other eucalypt. Of the species of subgenus *Symphyomyrtus* adjacent to the floodplain (*Eucalyptus gracilis, oleosa, porosa* and *socialis;* Zubrinich 1996), morphology and flowering time suggest *Eucalyptus gracilis* as the species involved in the present story. In addition, its high salinity tolerance may make it the most likely local eucalypt to confer extra salt tolerance on *Eucalyptus largiflorens* by hybridizing with it.

Turning to the efforts to produce Green Box progeny for revegetation of sites losing their Black Box trees to rising salinity, as early as March 1991 the South Australian Woods and Forests Department plant nursery at Murray Bridge was raising seedlings using seed from Chowilla Green Box trees, as were students at Glossop High School in 1992 and 1993 (Seekamp 1990–1994). Such seedlings segregate into a wide range of plant types, many of which do not become green and have low survival and very slow growth (Seekamp 1999). Possibly for this reason, in 1993 the Botanic Gardens of Adelaide began to study the possibility of cloning Green Box by micropropagation. Eventually, clones were established from epicormic shoots from five trees. A grant of $15,000 from the Murraylands Conservation Trust enabled mass propagation from these to finally produce 8,819 acclimatised and hardened-off young plants by October 1994 (Botanic Gardens of Adelaide 1993, 1994, 1995).

These tissue-cultured plants were then planted out by community groups and school children 'onto the floodplains throughout the Riverland' (Nicholls 2009) including Chowilla Game Reserve and Banrock Station (Michael Harper pers. comm.) where they are said to be 'magnificently green and healthy amongst the dying Black Box' (Nicholls 2009). When planted above fresh water tables in Renmark parking lots, nature strips and gardens, the Green Box clonal plants 'grew rapidly into attractive and shapely trees' (Seekamp 1999). At least some of this batch of clonal plants were planted out as early as 1994 (Seekamp 1999).

In 1994 the Botanic Gardens of Adelaide sent cultures from four Green Box trees to Biotech Plants Pty Ltd at Somersby, New South Wales, who had a contract from the Murraylands Conservation Trust (MCT) to supply 20,000 cloned plants (Botanic Gardens of Adelaide 1995). It seems certain that no plants were produced (Michael Harper pers. comm.) This is hard to confirm because the company is now defunct; the MCT is also defunct and its records have disappeared (Michael Harper pers. comm.) It is thought that a final batch of clonal plants was produced by a Hobart company in the late 1990s, but again records are lacking (Michael Harper pers. comm.).

In 1999, Seekamp wrote 'the current recipe for producing Green Box rootlings by tissue culture has a low survival rate through the process and commercial production would only be possible at a prohibitive price per plant.' This is supported by workers currently involved in revegetation work; the high cost of producing limited numbers of plants (e.g. 1–2,000) is the only reason that no clonal plants have been planted in the Riverland since the 1990s (Michael Harper pers. comm.). Seekamp believed that more research would solve this cost problem (Seekamp 1999). In any case, any future revegetation work using large numbers of Green Box needs to consider both their long-term regeneration prospects and their possible long-term genetic effects on *Eucalyptus largiflorens*, as well as the advisability of using large numbers of plants cloned from a few parent trees compared to using seedlings.

Seekamp had pointed out that there were both 'rounded and vertical-growing types' of Green Box and had hoped to use cloned plants from rounded mother trees for shade and as widely-spaced plantings on degraded floodplain and those from vertical-growing mother trees in plantations for firewood, trellis posts and sawn timber (Seekamp 1999).

While writing this paper our enquiries failed to identify anyone currently selling Green Box seed or raising Green Box seedlings.

Apart from a mention in passing in Barrett *et al*. (2005) and the brief summary in Nicholls (2009), the only published reference to Green Box we can find since Zubrinich *et al*. (2000) is a record of it from Penfolds Lagoon 6 km downstream from Morgan (Marsland & Nicol 2009).

Concluding Discussion

The Green Box story raises a number of general issues. Firstly, it helps to exemplify the important role that observant local farmers and naturalists have played in research on Australia's plants, a point that is clear from papers like that of Willis (1975) and from the body of work published since the late nineteenth century in journals like the South Australian Naturalist and the Victorian Naturalist.

Secondly, further study of the Green Box story may shed light on important evolutionary issues related to the subject of phantom hybrids, namely hybrids that are geographically isolated from one or both putative parents (Pryor & Johnson 1971). While Green Box plants co-exist on the floodplain with one putative parent, populations of the other one, *Eucalyptus gracilis*, are usually more than three km from the floodplain and frequently much further away (Zubrinich 1996).

Using the *Eucalyptus gracilis* distribution data in Australia's Virtual Herbarium, it seems that the record of Green Box from Deniliquin (Zubrinich 1996) could easily be at least 50 km from the nearest *Eucalyptus gracilis* population. Furthermore, Seekamp (1990–1994) records Green Box from 'along the Darling up as far as Bourke' which could be much further from *Eucalyptus gracilis* plants. This aspect of Green Box biology needs careful checking and intensive work for the light it might shine on important issues like eucalypt gene flow via pollen and seed (Potts & Wiltshire 1997).

Furthermore, the present unusual case of a parent species dying on a large scale from anthropogenic causes, leaving behind only its hybrid progeny deserves further work to help elucidate crucial current issues in the study of hybrid speciation, adaptive divergence and other evolutionary processes (see e.g. Donovan *et al*. 2010). In the present story, given the prominence and importance of hybrids that may stabilize and become species, it can be argued that Australian herbaria should encourage taxonomists to provide a level of formal recognition for natural hybrids.

Finally, it would be valuable for the people responsible for policy and funding related to the rehabilitation of degraded Black Box country in New South Wales, South Australia and Victoria to discuss what role there is for Green Box and what funding and research are needed as a consequence.

Acknowledgements

It is a pleasure to thank Trevor Edwards, Stephen Forbes and his staff at the Botanic Gardens of Adelaide, Denis Frahn, Mike Harper, Susan Hoebee and Dean Nicolle for a great deal of help. We especially thank Teresa ter Bogt of the McCormick Centre of the Australian Landscape Trust for very kindly selecting and sending us extracts from the seven volumes of Jack Seekamp material held there.

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Manuscript accepted 4 November 2010