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Designing food and habitat trees for urban koalas: tree height, foliage palatability and clonal propagation of *Eucalyptus kabiana*


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Graphical abstract

Highlights

- Koala populations are vulnerable because of habitat fragmentation and inbreeding.
• Urban planners and landowners often do not like to plant tall Eucalyptus trees.
• We have developed shorter koala-food Eucalyptus trees for planting in urban areas.
• Eucalyptus kabiana is short, palatable to koalas, and easy to propagate.
• E. kabiana trees are now planted in wildlife corridors, parks, schools and gardens.

ABSTRACT
Koalas are iconic Australian tree-dwelling marsupials that are classified as vulnerable because of threatening processes that include urban development, habitat fragmentation and inbreeding. Koalas eat the leaves of specific eucalypt trees but urban planners and landowners often prefer to plant smaller trees that pose less risk from falling limbs. We have conducted a long-term project to develop shorter koala-food trees for planting in parklands, schools, streets and gardens. We identified a little-known and geographically-confined species, Eucalyptus kabiana, that had potential for urban plantings. We assessed the height of E. kabiana trees in cultivation, determined whether their foliage was palatable to koalas, and compared the amenability to vegetative propagation of E. kabiana with that of an extensively-propagated related species, E. tereticornis. Cultivated E. kabiana trees were short, reaching around 3–5 m height after 6 years. Their foliage was highly palatable to koalas, and their cuttings proved to be amenable to propagation. Average rooting percentages for E. kabiana cuttings were 31–46%, similar to values obtained with E. tereticornis cuttings. Over 600 E. kabiana trees have thus far been distributed for planting in wildlife corridors, parklands, schools and gardens. The planting of more koala-food trees will help to alleviate the risks of inbreeding faced by koala populations in fragmented urban landscapes. School plantings also provide opportunities for students to learn about and interact with organisms such as koalas that inhabit the Eucalyptus trees.
Keywords: Adventitious roots, Cuttings, *Eucalyptus tereticornis*, *Phascolarctos cinereus*, Street trees, Urban fauna.

1. Introduction

Urban trees provide a wide range of economic, social and environmental benefits such as boosting property values, reducing energy costs, lowering crime, absorbing ultraviolet radiation, reducing heatstroke, sequestering carbon, removing air pollutants and decreasing stormwater runoff (Berland and Hopton 2104; Livesley *et al*. 2014; Na *et al*. 2014; Mullaney *et al*. 2015a–c; Graham *et al*. 2016; Gratani *et al*. 2016; McPherson *et al*. 2016; Thom *et al*. 2016; Donovan 2017; Nowak *et al*. 2017; Tan *et al*. 2017). Trees also provide habitat and movement corridors for fauna (Munshi-South 2012; de Oliveira *et al*. 2014; Zhang and Jim 2014; Huang *et al*. 2015; Matsuba *et al*. 2016; Shimazaki *et al*. 2016) and they offer opportunities for residents to interact with other organisms (Pearce *et al*. 2015; Askerland and Almers 2016). However, tree clearing for urban development can reduce fauna populations, especially for herbivores that rely on tree species for both food and habitat. One example of a declining species in urban environments is the iconic Australian arboreal marsupial, the koala (*Phascolarctos cinereus* Goldfuss), which is now listed as Vulnerable across most of its range in eastern Australia because of threatening processes that include urban development, habitat fragmentation, fire, drought, disease and inbreeding (Lee *et al*. 2010; Seabrook *et al*. 2014; McAlpine *et al*. 2015; Adams-Hosking *et al*. 2016).

Koalas feed almost exclusively on the leaves of some *Eucalyptus* and *Corymbia* species (Moore *et al*. 2010; Wu *et al*. 2012; Melzer *et al*. 2014). Large tracts of prime koala habitat have been cleared for urban development along the outskirts of cities and towns in eastern Australia. Most of the eucalypt species eaten by koalas in this region are tall trees, reaching...
between 12 m and 40 m height in cultivation (Nicolle 2016b). Many local governments and urban landowners prefer to plant small trees that pose less risk of branches falling from a great height or becoming entangled with overhead power lines during storms (Kendal et al. 2012; Kirkpatrick et al. 2012; Nicolle 2016a; Plant and Sipe 2016; Roy et al. 2017). Local governments have, therefore, recognised a need to develop shorter koala-food trees for planting in urban parklands, schools and gardens. Koalas prefer to roost in tall forest trees, particularly on hot days (Moore et al. 2010; Crowther et al. 2013; Marsh et al. 2013), although they frequently inhabit small forest remnants, rehabilitated sites, juvenile forest trees, isolated farm trees, and trees in urban streets and gardens (White 1999; Sullivan et al. 2004; Cristescu et al. 2013; de Oliveira et al. 2014; Melzer et al. 2014).

We have conducted a long-term project to develop shorter eucalypt trees for planting in urban areas. In this study, we identified a poorly known and geographically confined species, Eucalyptus kabiana L.A.S.Johnson & K.D.Hill, that has potential as a short tree for urban plantings. Eucalyptus kabiana (Mount Beerwah mallee) is only found in two populations on rocky outcrops of the Glasshouse Mountains National Park, Queensland, Australia (Brooker and Kleinig 1994). It is listed as Vulnerable under the Commonwealth of Australia Environment Protection and Biodiversity Conservation Act (1999). The palatability of E. kabiana foliage to koalas was previously unknown although E. kabiana is closely related to two prime koala-food species, E. tereticornis Sm. and E. camaldulensis Dehnh. We assessed the height of E. kabiana trees in cultivation, determined whether the foliage of cultivated E. kabiana trees was palatable to koalas, and compared the amenability to clonal propagation of E. kabiana with that of an extensively-propagated related species, E. tereticornis.

2. Materials and methods
2.1. Seed collection

We collected seeds from *E. kabiana* trees in the Mount Beerwah section (26°53’S, 152°52’E) of Glasshouse Mountains National Park, Queensland, Australia, in April 2009. Seeds were collected under Permit No. WITK05599808 (Environmental Protection Agency, Queensland). We obtained seeds of *E. tereticornis* (seedlot 20357) from the Australian Tree Seed Centre, Canberra, Australia.

2.2. Production and height of field plants

We sowed *E. kabiana* seeds to produce nursery stock plants, from which cuttings could be harvested and the rooted cuttings used for evaluating tree height in cultivation and the palatability of *E. kabiana* foliage to koalas. These seeds were sown directly into 70-mL tubes filled with potting medium consisting of a 75/25 (v/v) mixture of shredded pine bark and perlite, with 3 kg of 8-9 month slow release Osmocote™ fertiliser (Scotts International, Heerlen, The Netherlands), 3 kg lime (Unimin, Lilydale, VIC), 1 kg gypsum (Queensland Organics, Narangba, QLD), 1 kg Micromax® micronutrients (Scotts Australia, Baulkham Hills, NSW) and 1 kg Hydroflow™ wetting agent (Scotts Australia, Baulkham Hills, NSW) incorporated per m³ (Trueman *et al.* 2013a–c, 2014). Seeds were covered with a thin layer of vermiculite and the tubes were placed in 120-L plastic translucent tubs in an Adaptis A1000 plant growth chamber (Conviron, Winnipeg, Canada). Germination conditions were 250 μmol m² s⁻¹ irradiance inside the tub at 25°C for 16 h daily and in darkness at 18°C for 8 h daily. Tubs were filled with water to 3-cm depth to ensure that the propagation medium remained moist.
We transplanted 13 seedlings successfully after 1 month into 1.6-L pots containing the same potting medium and transferred them to a white translucent polyethylene chamber with mist irrigation at the University of the Sunshine Coast (26°43’S, 153°03’E). These seedlings were maintained as nursery stock plants, by regular pruning at 30-cm height and 20-cm canopy diameter, for the harvest of cuttings (Trueman et al. 2013a–c). Harvested cuttings, each containing two nodes and with approximately two-thirds of the length of each leaf removed, were dipped 0.5 cm into powder containing 3 g kg\(^{-1}\) indole-3-butyric acid (IBA) (Kilkenny et al. 2012; Trueman and Adkins 2013). They were then placed 1 cm deep into a 70-mL tube containing a 75/25 (v/v) mixture of perlite and shredded pine bark with 3 kg of 8-9 month slow release Osmocote\textsuperscript{TM} fertiliser and 1 kg gypsum incorporated per m\(^3\) (Trueman et al. 2013a–c). The cuttings were returned to mist irrigation in the polyethylene chamber, and then transferred to full sunlight after roots had penetrated the base of the propagation tube.

We planted field-ready cuttings, ~30-cm height, on the adjoining campuses of the University of the Sunshine Coast (USC) and Chancellor State College (CSC) at Sippy Downs (26°42’S, 153°04’E) in April 2010. These sites were campus lawns that were mowed regularly (Fig. 1a). Cuttings were also planted on a site to be developed as public parkland at Kybong (26°19’S, 152°42’E) in September 2010. This site was a former pasture that was slashed only when required to prevent the build-up of excessive fuel load for wildfires. Soil type and tree spacing at CSC and Kybong have been described previously (Trueman et al. 2017). The soil type at USC was the same as at CSC but the trees at USC were planted along the perimeters of native forest where it adjoined lawns, rather than in rows within the lawns. Irrigation was not provided at any site. Mean annual rainfall is approximately 1500 mm at USC and CSC and 1150 mm at Kybong (Bureau of Meteorology, Australian Government...
2017). The numbers of trees per site were 23 (USC), 20 (CSC) and 19 (Kybong). Tree
heights were measured after 6 years (Fig. 1b).

2.3. Palatability of foliage

We harvested *E. kabiana* branches from 20 trees at USC in May 2016. The branches, approximately 1 m in length, were transported to the Moggill Koala Hospital, Moggill, Queensland (27°34’S, 152°52’E) and installed in enclosures that housed individual koalas (Fig. 1c). The koalas had been well-fed overnight on the foliage of other eucalypt species. Fresh *E. kabiana* foliage was placed in the enclosures of six koalas on the first day and left overnight. Foliage of both *E. kabiana* and the prime koala-food species, *E. tereticornis*, was placed in the enclosures of eight koalas on the second day and left overnight. Koalas were observed each day to assess whether they consumed *E. kabiana* foliage (Fig. 1d), and branches were inspected on the following morning to determine how much foliage had been consumed (Fig. 1e).

2.4. Amenability to clonal propagation

We sowed remaining *E. kabiana* seeds to produce nursery stock plants for evaluating the clonal propagation capacity of *E. kabiana* and to produce trees for community release. We also sowed seeds of *E. tereticornis* to compare *E. kabiana* with a closely related species that was known to be amenable to clonal propagation (Marques *et al.* 1999; Chinnaraj and Malimuthu 2011; Shanmugapriya *et al.* 2015). Seeds were sown into germination trays containing the same germination medium used for ‘Production and height of field plants’ (above). The trays were placed under mist irrigation in a glasshouse at USC. Twenty-two *E.
kabiana seedlings and 25 E. tereticornis seedlings were transplanted into 1.6-L pots after 2 weeks and moved to another glasshouse where irrigation was provided by overhead sprinklers. The seedlings were managed as stock plants for 6 months by regular pruning to maintain a height of 15 cm and a canopy diameter of 20 cm (Wendling et al. 2015a). All available cuttings of each stock plant were then harvested at five 4-week intervals from late spring to early autumn (November to March), and the total fresh mass and number of cuttings were recorded for each plant on each occasion; i.e. at 4, 8, 12, 16 and 20 weeks after commencement. Cumulative fresh mass and cumulative number of harvested cuttings were then calculated by adding the fresh mass and cutting number for each occasion to the corresponding data from all previous occasions.

We randomly allocated the cuttings from each stock plant on the first, third and fifth harvests to the following four treatments: 0, 1, 3 or 8 g kg⁻¹ IBA (Trueman and Richardson 2011; Kilkenny et al. 2012; Trueman and Adkins 2013; Wendling et al. 2015b). The cuttings were prepared and placed into the same propagation medium as for ‘Production and height of field plants’ (above) and then placed into a translucent white polyethylene chamber with mist irrigation. We transferred all cuttings after 9 weeks to a glasshouse with overhead sprinkler irrigation. The proportion of cuttings that formed roots for each IBA treatment within each stock plant was assessed after another 6 weeks; i.e. at 15 weeks after harvest. The production capacity of rooted cuttings per stock plant on each occasion was calculated by multiplying the total number of cuttings harvested per stock plant by the proportion of cuttings that formed roots in each treatment. We estimated the proportions of cuttings that would have formed roots on the second and fourth occasions (i.e. 8 and 16 weeks after commencement) by averaging the proportions that formed roots on the first and third occasions and on the third and fifth occasions, respectively, for each stock plant. The cumulative production capacity of
rooted cuttings per stock plant was then calculated by adding the production capacity of rooted cuttings for each occasion to the corresponding data from all previous occasions.

2.5. Statistical analyses

We analysed tree height data by 1-way ANOVA (comparing three planting sites) after confirming that the data had a normal distribution and homogeneous variance (IBM SPSS Statistics v. 24). Propagation data were analysed by 1-way ANOVA (comparing four IBA treatments within each species) or t test (comparing two species) because extensive treatment × species interactions were detected by 2-way ANOVA. Post-hoc Tukey’s honestly significant difference (HSD) tests were performed when differences among planting sites or IBA treatments were detected by ANOVA. Differences or interactions were regarded as significant at $P < 0.05$. Means are presented with standard errors.

3. Results and discussion

*Eucalyptus kabiana* were short trees, only reaching about 3–5 m height after 6 years in cultivation (Table 1). They were shortest where they were planted in infrequently-slash parkland on the lower-rainfall site (Kybong) and tallest where they were planted in regularly-mowed garden lawns on one of the higher-rainfall sites (CSC; Fig. 1b). The *E. kabiana* trees at Kybong were much shorter than trees of the koala-food species, *E. tereticornis*, *E. robusta* Sm., *E. resinifera* Sm., *E. propinqua* H.Deane & Maiden, *E. moluccana* Roxb. and *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson, planted at the same site, which were around 8–14 m tall after 5 years (Trueman *et al.* 2014). *E. kabiana* trees also appeared shorter than tall ecotypes of *C. intermedia* (R.T.Baker) K.D.Hill & L.A.S.Johnson, which were around 8–
12 m tall at CSC and Kybong after 8 years in cultivation (Trueman et al. 2017). Their growth rate was similar to short ecotypes of *C. intermedia*, which were 5–7 m tall at CSC and Kybong after 8 years (Trueman et al. 2017). However, the foliage of *C. intermedia* trees is known to be consumed by koalas (Tucker et al. 2007; Callaghan et al. 2011) whereas the palatability of *E. kabiana* foliage to koalas was previously unknown.

We observed all eight koalas consuming *E. kabiana* foliage (Fig. 1d). Many of the *E. kabiana* branches placed in the koala enclosures had been heavily stripped of foliage by the following morning (Fig. 1e). Furthermore, six of the eight koalas ate *E. kabiana* foliage before they ate *E. tereticornis* foliage when branches of both species were placed in the enclosures on the second day. These six koalas had also eaten more *E. kabiana* foliage than *E. tereticornis* foliage by the third morning. The palatability of *E. kabiana* foliage was not surprising because this species is related to two prime koala-food species, *E. tereticornis* and *E. camaldulensis* (Wu et al. 2012; Cristescu et al. 2013; de Oliveira et al. 2014; Melzer et al. 2014; Nicolle 2016b), that are in the same *Eucalyptus* taxonomic section, *Exsertaria* (Brooker 2000). In fact, *E. kabiana* is very closely related to *E. tereticornis*, belonging to the same taxonomic series, *Erythroxylon* (Brooker 2000). *E. tereticornis* and *E. camaldulensis* are much better known trees than *E. kabiana*, having wide natural distributions in Australia and being established internationally in forestry plantations (Brooker and Kleinig 1994; Teulières et al. 2007; Nicolle 2016b). However, *E. tereticornis* and *E. camaldulensis* are unpopular with urban landowners because they reach heights up to 35 m and are perceived to be prone to “sudden limb failure”, in which apparently-healthy branches are shed from the tree (Nicelle 2016b). *E. kabiana*, in contrast, is a short species but it shares the foliage-palatability characteristics of its much-taller related species. The effects on body mass, behaviour and physiology of continuous feeding on *E. kabiana* foliage by koalas await investigation. However, *E. kabiana* will typically be planted in the vicinity of other eucalypt
trees and so koalas feeding on this short species are unlikely to have a monospecific-foliage diet.

_Eucalyptus kabiana_ also shared the amenability to clonal propagation of _E. tereticornis_ (Fig. 2). Stock plants of _E. kabiana_ and _E. tereticornis_ did not differ significantly in the number of cuttings, 91 ± 9 and 89 ± 7, or the biomass of cuttings, 28.5 ± 3.0 g and 28.2 ± 1.2 g, respectively, that were produced in a 14-week period (Fig. 2a,b). The percentage of cuttings that formed roots also did not differ significantly between the two species, although _E. tereticornis_ cuttings proved to be sensitive to the highest IBA dose (8 g kg⁻¹) in a way that _E. kabiana_ cuttings did not (Fig. 2c,d). Rooted cutting production did not differ significantly between _E. kabiana_ and _E. tereticornis_, with stock plants having the capacity to produce up to 48 ± 8 and 45 ± 4 rooted cuttings, respectively, following the 14-week harvest period (Fig. 2e,f). However, treatment of _E. tereticornis_ cuttings with 8 g kg⁻¹ IBA reduced the production capacity to 22 ± 3 rooted cuttings (Fig. 2f). This IBA dose also reduces rooted cutting production in the eucalypts, _Corymbia citriodora_ and _C. torelliana_ (F.Muell.) K.D.Hill & L.A.S.Johnson × _C. citriodora_ (Trueman and Richardson 2008; Wendling _et al._ 2015b). The average rooting percentages for _E. kabiana_ cuttings (31 ± 5% to 46 ± 5% across the IBA treatments) were reasonably high for eucalypt species, which are often considered difficult-to-propagate from cuttings. Mass vegetative propagation has been used successfully for some _Eucalyptus_ species, such as _E. grandis_ W.Hill, _E. tereticornis_ and _E. camaldulensis_ (Naidu and Jones 2009; Chinnaraj and Malimuthu 2011; Makouanzi _et al._ 2014; Bryant and Trueman 2015; Shanmugapriya _et al._ 2015), that often grow naturally in flood-prone environments. This may be because adventitious root formation by shoots has evolved as an adaptive response to the anoxic conditions experienced by submerged roots during waterlogging (Steffens and Rasmussen 2016). Vegetative propagation has proven difficult for many other eucalypt species, especially from dryland environments (Brondani _et al._ 2010; Dickinson _et al._ 2010).
al. 2013; Trueman et al. 2013a,b). The natural habitat of *E. kabiana* on highly-exposed rocky outcrops of the Glasshouse Mountains National Park did not suggest that the species would be amenable to propagation from cuttings. Fortunately, *E. kabiana* shared the amenability to propagation of its closely-related lowland species.

More than 600 *E. kabiana* trees that were propagated from cuttings (Fig. 1f) have been distributed to Moreton Bay Regional Council and the Pine Rivers Koala Care Association for planting in wildlife corridors, parklands, and urban and peri-urban gardens. Foliage has also been provided for koala fodder to the Wildlife HQ zoo (Nambour, Queensland, Australia). The number of *E. kabiana* trees released to the community will soon overtake the number of trees occurring naturally in the two wild populations (~1000), most of which grow in the population on Mount Beerwah. The project, therefore, represents a case study of a threatened plant species whose conservation has been assisted by propagation and planting *ex situ* (Pohio et al. 2005; Dwan and Trueman 2014; Roberts and Trueman 2016). The Moreton Bay region, in which the *E. kabiana* trees have been planted, includes large tracts of prime koala habitat that have been fragmented by urban development (Dique et al. 2003a; Dexter et al. 2016). Wildlife corridors have been maintained and constructed in this region to allow the movement of koalas between forest remnants (Loose and Haase 2004; Dexter et al. 2016). Koalas can disperse many kilometres and establish home ranges larger than 50 hectares in urban and peri-urban areas (Dique et al. 2003b; de Oliveira et al. 2014; Goldingay and Dobner 2014; Matthews et al. 2016). Therefore, the planting of koala-food trees in wildlife corridors and parklands helps to alleviate the risks of inbreeding faced by koala populations in these fragmented landscapes. Koalas are also at risk of car strikes and dog attacks (de Oliveira et al. 2014; McAlpine et al. 2015; Adams-Hosking et al. 2016). The planting of small eucalypt trees will provide additional food and shelter for koalas that transit between...
larger tracts of forest but it remains to be determined whether these wildlife corridor and parkland trees reduce or increase the risks to koalas from cars and dogs in urban areas.

*Eucalyptus kabiana* trees have also been planted on school campuses, which sustain healthy and mobile koala populations. The availability of smaller eucalypt trees such as *E. kabiana* allows the planting of koala-food species closer to school buildings, playgrounds and sports fields where the planting of much larger koala-food trees such as *E. tereticornis* would be discouraged. School plantings provide opportunities for students to learn about their local environment and to interact with organisms such as koalas and other fauna that inhabit the *Eucalyptus* trees (Pearce *et al*. 2015; Askerland and Almers 2016). The amount of tree cover around school buildings has also been linked positively with students’ academic performance (Matsuoka 2010; Kweon *et al*. 2017). Another benefit of the project has been the release of *E. kabiana* trees to koala carers, who often nurse sick or injured koalas in their urban backyards. These sites are often unsuitable for large *Eucalyptus* trees but smaller trees such as *E. kabiana* can be planted to produce fodder for koalas during their recovery and to provide climbing and roosting sites for koalas when they are being conditioned for release back to the wild. These specific benefits of planting *E. kabiana* trees are in addition to the many other economic, social and environmental benefits that all urban trees provide to improve the liveability of our cities and towns (Mullaney *et al*. 2015a; McPherson *et al*. 2016).

4. **Conclusions**

Trees of the threatened species, *Eucalyptus kabiana*, remained small in cultivation, reaching only about 3–5 m tall after 6 years. They shared the foliage palatability to koalas and the amenability to vegetative propagation of their much-taller closely-related species, *E. tereticornis*. Over 600 *E. kabiana* plants produced from cuttings have been distributed for
planting in wildlife corridors, parklands, schools and gardens. The project demonstrates the practical value of urban trees in providing valuable food, habitat and movement corridors for fauna and in offering opportunities for residents to learn about, and interact with, other organisms.

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Figure captions

Fig. 1. (a) *Eucalyptus kabiana* trees planted in two rows at Chancellor State College (CSC) alongside an existing windbreak of *Melaleuca quinquenervia* trees; (b) *E. kabiana* trees at CSC at 6 years after planting; (c) *E. kabiana* foliage placed in an enclosure with a koala (*Phascolarctos cinereus*); (d) *E. kabiana* foliage being consumed by a koala; (e) *E. kabiana* branches after a koala had consumed foliage during an 18-hour period; and (f) rooted cuttings of *E. kabiana* for planting in wildlife corridors, parklands, schools and gardens.

Fig. 2. (a) Cumulative number and (b) cumulative mass of cuttings produced per *Eucalyptus kabiana* and *E. tereticornis* stock plant; percentage of cuttings that formed roots after (c) *E. kabiana* or (d) *E. tereticornis* cuttings were treated with different doses of indole-3-butyric acid (IBA); and rooted-cutting production capacity of (e) *E. kabiana* or (f) *E. tereticornis* stock plants when cuttings were treated with different doses of IBA. The IBA doses are in g kg$^{-1}$ powder. ‘Avg. refers to the average rooting percentage across the three harvests. Means (± SE) with different letters within a time point and species are significantly different (ANOVA and Tukey’s HSD test, P<0.05, n=22–25). Means do not differ between species at any time point (t test, P>0.05).
Table 1. Height of *Eucalyptus kabiana* trees at 6 years after planting

<table>
<thead>
<tr>
<th>Planting site</th>
<th>Tree height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of the Sunshine Coast (USC)</td>
<td>4.17 ± 0.33b</td>
</tr>
<tr>
<td>Chancellor State College (CSC)</td>
<td>5.17 ± 0.26a</td>
</tr>
<tr>
<td>Kybong</td>
<td>3.13 ± 0.26c</td>
</tr>
</tbody>
</table>

Means (± SE) with different letters are significantly different (ANOVA and Tukey’s HSD test, P<0.05, n=19–23)