Results of a glasshouse cuttings trial of *Eucalyptus loxophleba* subsp. *lissophloia*

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Introduction

Eucalyptus loxophleba subsp. *lissophloia* L.A.S.Johnson & K.D.Hill is a dryland mallee endemic to the Wheatbelt of Western Australia. This species was one of several identified in the early 1990s as a strong contender in the then Western Australian Department of Conservation and Land Managements (CALM) search for tree crops to combat dryland salinity. Control of the problem via reforestation was desirable but tree crops needed to be economically competitive with wheat and sheep in order to drive industry development (Bartle and Shea 2002). The species rapid growth rates combined with a high yield of eucalyptus oil containing around 70% of 1,8-cineole offered significant potential for development of a mallee industry producing high quality eucalyptus oil. In order to enhance industry prospects, CALM initiated a mallee breeding program with a view to developing seed from trees selected for high concentrations of 1,8-cineole in the leaves. The first progeny trials of *E. loxphleba* subsp. *lissophloia* were established in 1995. Currently the breeding program includes two populations, the first is comprised of progeny from some 100 parents selected in the field for high leaf cineole content. The second, established in 2011 by the then Department of Environment and Conservation in collaboration with the Future Farms Industry CRC, encompassed a further 135 families from across the natural distribution of *E. loxphleba* subsp. *lissophloia*. The latter population is deployed in four trials, one at each of Lake Bryde and Brookton in Western Australia, Monarto in South Australia and Condobolin New South Wales. Selection in this population will be focussed on improving biomass productivity to accommodate an emerging demand for renewable energy sources. Along with *Eucalyptus polybractea*, *E. loxphleba* subsp. *lissophloia* is now likely to form a large component of any future mallee industry in the wheat-sheep zone of southern Australia

The recent shift in the focus of the mallee industry away from eucalyptus oil production to that of biomass for energy production has necessitated a reversal in selection emphasis and the development of a new breeding strategy. Key to development of an efficient breeding strategy is the need to understand the breeding biology of the species concerned and its potential for vegetative multiplication. Trees for plantation forestry are produced in various ways including the production of seed from seed stands, seedling seed orchards (SSO), or clonal seed orchards (CSO), or via clonal propagation where the primary deployment propagule is a clone in the form of a rooted cutting or plantlet developed via tissue culture or other method (White et al 2009). The

deployment technique chosen in part relates to the ease with which a species may be clonally propagated and the relative cost of each pathway. Knowledge of how a species responds to various methods of cloning is therefore an important factor when planning a breeding strategy. To date, no study has been completed to determine if cloning of *E. loxophleba lissophloia* is possible, and with the exception of *E. polybractea* (Goodger 2008) (Peck unpublished data) and *E. impensa* (Bunn 2005) little research has been conducted on other mallee species.

Studies on other eucalypts have demonstrated a decrease in root development and growth of cuttings as the age of the parent plant increases (Giri *et al.* 2004; Paton *et al.* 1970). To reduce the effects of ortogenetic age, juvenile shoots can be produced by felling certain species of trees (Wendling 2014). This produces coppice which has been used as the source material for eucalyptus clonal forestry (Goodger *et al.* 2008; King 1980; Wendling 2014).

Application of indole-3-butyric acid (IBA) is known to improve the rooting success of cuttings in a range of species including conifers (Hunt *et al.* 2011), eucalypts (Fett-Neto *et al.* 2001; Trindade and Pais 1997; Fogaca and Neto 2005; Trueman *et al.* 2013; Gomes and Canhoto 2003; Trueman and Adkin 2013) and food production trees including apples (De Klerk GJ *et al.* 1995) and nuts (Contessa *et al.* 2011). IBA has also been used to increase the rooting capacity of recalcitrant eucalypt species (Fogaca and Neto 2005) (Serrano *et al.* 1996). Examples include improved rooting success of cuttings from recalcitrant *E. polybractea* families (Peck unpublished data) and in *in vitro* methods with *E. globulus* (Bennett *et al.* 1994; Ruaud *et al.* 1999; Trindade and Pais 1997).

This study examined the capacity of *E. loxophleba lissophloia* to be vegetatively cloned using cuttings in the glasshouse.

Materials and methods

Coppice was collected from two open-pollinated *E. loxophleba lissophloia* seed orchard in Merredin, Western Australia planted in 1995 and 1996. Trees were cut down with a chainsaw on the 10th of June 2014 as part of a routing thinning operation. Coppice shoots were collected from 25 randomly selected stumps on the 19th of November 2014. Apical tips of coppice shoots were too small in diameter for use as cuttings and were trimmed on site to reduce transpiration and required storage space. The coppice shoots were then wrapped in wet paper, placed in eskies with icepacks to keep them cool and transported to Perth. Where possible, cuttings taken from a coppice shoot retained two leaf nodes. Otherwise cuttings contained one or three leaf nodes. Leaves on the cutting were

cut to about one third of their original size to reduce leaf area. No leaves were retained on the bottom half of a cutting. The bark on each side of the base of a cutting was lightly scraped for a distance of about 5mm to expose the cambium and induce a wound response. Where required, the base of the cutting was dipped into a rooting hormone prior to being set in the soil medium to a depth of approximately 1 cm. All cuttings were struck the same day they were collected. The apical portion of the coppice shoots was discarded.

The trial was set up in a split-plot design consisting of three replicates and 25 ortets. A main plot plots consisted of a single 40 cell tray with eight rows of five cells, containing thirty cuttings arranged in three groups or subplots of ten cuttings. Subplots were separated from each other by an intervening row of five empty cells. Ortets were randomly assigned to main plots. One of three treatments where then randomly assigned to subplots. Treatments included the control (C) with no IBA and one of two commercially available rooting compounds for either hardwood (H) containing 8g l^{-1} IBA or softwood (S) containing 3g l^{-1}IBA. All cuttings were submerged into Thyram (1.5g l^{-1}, 1 minute minimum soak) then, if not part of the control, dipped 1 cm into the relevant IBA solution. Cuttings where then placed into propagation tubes containing a soil medium comprising of 3 parts coco peat to 2 parts perlite (P400 medium) which had been drenched with Plantmate fungicide (0.5 g l^{-1}). The tubes were immediately placed under mist irrigation in an air-conditioned glasshouse. The irrigation provided mist for 13 seconds every 13 minutes during the day and five seconds every hour at night. On three occasions during the trial, following a week of watering, the accumulated volume of water from a vial placed in each tray was recorded. Glasshouse temperatures ranged between 20 and 30 degrees centigrade during the course of the trial.

After three weeks, obviously dead cuttings were removed from the trial to reduce mould infection. Mortality of the remaining 1301 cuttings occured at about 7 weeks after they were set. Mortality was probably caused by a faulty air conditioner thermostat resulting in desiccation. All cuttings were subsequently harvested and assessed for presence or absence of callus tissue or roots at the base. The existence of roots was accepted as evidence of rooting success. The cuttings ranged in diameter between 1.4 mm to 5.8 mm, the average diameter was 3.1 mm. The average length of a cutting was 78.8 mm and ranged between 39 and 140 mm.

Statistical analysis

The proportion of cuttings with roots in a plot were arcsine square root transformed to meet assumptions of normality and homescedascisity. Plot data was analysed using the mixed procedure in SAS® 9.3 according to the model $y_{ijk} = \mu + r_i + o_j + t_k + o.t_{jk} + o.r_{ij} + e_{ijk}$ where μ = the trial mean, r_i =

the random replicate effect, o_j= fixed ortet effect, t_k = the fixed hormone treatment effect, $o.t_{jk}$ = the fixed ortet by treatment interaction, $o.r_{ij}$ = the random ortet by replicate interaction and e_{ijk} = the random error. The mixed procedure automatically performs F-tests using the correct error terms for all effects in the model (SAS technical report 1992).

The effect of watering was examined using watering effect as a covariate (not shown).

Results

All data reported is on the untransformed scale unless otherwise stated. Analysis of variance indicated highly significant differences between ortets ($P<0.001$) and between treatments ($P<0.001$) for rooting success. The interaction between ortet and hormone treatment was also significant ($P< 0.05$). Localised variation in watering was found not to be significant and was eliminated from the analysis.

Across the trial, overall rooting success was 15.1%. Mean treatment yields for rooting success were 21.8%, 14.1% and 9.4% for treatments H, S and C respectively.

Differences of adjusted treatment means (Table 1) indicate that all three treatments were significantly different from each other.

Table 1 – The difference of least square means of the three hormone treatments

Effect	Treatment Code	Treatment Code	Estimate*	Standard Error	DF	t Value	Pr > |t|
Treatment Code	C	H	-0.193	0.0307	100	-6.29	<.0001
Treatment Code	C	S	-0.0864	0.0307	100	-2.82	0.0058
Treatment Code	H	S	0.1066	0.0307	100	3.47	0.0008

* Estimate is square root arcsine transformation of the proportion of root success

Examination of the two way table (table 2) shows that whilst the capacity of cuttings to root was highly influenced by the ortet, the effect of the interaction between ortet and treatment can be clearly seen.

Sixteen of the 25 ortets achieved greater rooting success with the highest concentration of IBA, six ortets performed best with the moderate IBA concentration and two ortets exhibited highest rooting with no IBA (Table 2). One recalcitrant ortet did not exhibit any rooting.

Table 2 –Two way table showing rooting success expressed as proportions with different IBA treatments, H, S and C. The table was ranked from highest proportion of rooting success to lowest.

Ortet	H	S	C	Ortet mean
13	0.53	0.18	0.58	0.43
4	0.56	0.38	0.17	0.37
12	0.43	0.39	0.15	0.32
2	0.35	0.19	0.28	0.27
15	0.42	0.16	0.18	0.26
20	0.37	0.28	0.08	0.25
1	0.44	0.20	0.07	0.24
5	0.25	0.33	0.06	0.21
8	0.32	0.08	0.19	0.20
9	0.33	0.18	0.04	0.18
14	0.22	0.22	0.07	0.17
10	0.15	0.05	0.18	0.13
21	0.08	0.19	0.00	0.09
17	0.20	0.05	0.02	0.09
27	0.04	0.15	0.06	0.08
6	0.15	0.00	0.09	0.08
26	0.14	0.03	0.06	0.08
3	0.05	0.15	0.00	0.07
7	0.04	0.08	0.07	0.06
11	0.05	0.13	0.00	0.06
16	0.12	0.03	0.00	0.05
29	0.12	0.00	0.00	0.04
18	0.03	0.07	0.00	0.03
25	0.05	0.00	0.00	0.02
28	0.00	0.00	0.00	0.00
Treatment mean	0.09	0.22	0.14	0.15

Discussion

These data demonstrate that *E. loxophleba lissophloia* is not an easy-to-clone species (15.1% successfully rooted overall). To be viable for clonal forestry, commercial nurseries require a minimum of 70% rooting success (Trueman, 2006; Hunt *et al.*, 2011). Although rooting success was increased with higher concentrations of IBA (up to 21.8% overall with 8 g l^{-1} IBA) clonal propagation of *E. loxophleba subsp lissophloia* at best might be used for situations requiring smaller numbers of clones, for example the establishment of clonal seed orchards of elite clones. In accord with observations in our trial, Goodgar *et al.* (2008) demonstrated that the amount of variation in root development was clone-specific in *E. polybractea.* Similarly, studies of *In vitro* propagation of E.

globulus have shown that rooting success is heritable (Ruand *et al.* 1999) and that rooting and shooting is highly clone dependant (Trindade and Pais 1996; Bennett *et al.* 1994). The implication for establishment of *E. loxophleba* subsp *lissophloia* clonal seed orchards is that selection of clones may be limited to those clones which have good rooting ability.

Similar results were found within the DPAW *E. polybractea* breeding program (Peck unpublished data), where recalcitrant families where eliminated from the clonal orchard program, despite attempts over four successive years to clone them.

Conclusion

Since the experiment was terminated only seven weeks after the cuttings were struck, these results should be treated with caution . Nevertheless, the available evidence suggests that like *E. polybractea, E. loxophleba* subsp. *lissophloia* is relatively difficult to propagate using cuttings. It is likely that the use of cuttings would at best, be restricted to the establishment of clonal seed orchards using only those clones capable of setting roots.

The experiment needs to be repeated with a different set of ortets and a more reliable glasshouse environment in order to confirm the trends.

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