2013

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Publication details

Post print of: Shepherd, M, Rose, TJ & Raymond, CA 2013, 'Rejuvenation of mature native tea tree (Melaleuca alternifolia (Maiden & Betche) Cheel) for vegetative propagation', *Propagation of Ornamental Plants,* vol. 13, no. 3, pp. 103-111.

REJUVENIATION OF MATURE NATIVE TEA TREE (*MELALEUCA ALTERNIFOLIA* (MAIDEN & BETCHE) CHEEL) FOR VEGETATIVE PROPAGATION

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ABSTRACT

Many situations arise in horticulture where it is desirable to vegetatively propagate mature specimens of woody species. Circumventing maturation effects often necessitates rejuvenation following decapitation or wounding but severe treatments may unacceptably modify plant form or endanger survival of individuals of high intrinsic value. This study quantified the maturation effect on strike rates for rooted cuttings and compared mild rejuvenation techniques for *Melaleuca alternifolia*. Paired samples of juvenile and mature foliage were obtained from most trees (37 out of 40) from one of four native stands. Juvenile foliage was sourced from epicormic shoots that were artificially induced or stimulated by natural stresses. Most trees could be cultured, with 33 out of 40 genotypes captured by vegetative propagation. Roots were first detected on cuttings around 41 days post-setting and rates continued to increase to at least 69 days post-setting. Cuttings derived from epicormic shoots rooted at significantly higher rates under a range of propagation conditions (rooting percentage for epicormic shoots over three experiments; 26.1 ± 3.4 %, range 12-42 %; for mature shoots 11.2 ± 1.9 %, range 6-20 %), and those originating closer to the plant base rooted more frequently. Relative to the large tissue-type effect, differences in stock-plant age and site conditions were small and non-significant, as were the differences between stands from the same region. The highest strike rates were obtained by inducing epicormic shoots late in winter, harvesting shoots in mid-summer, and setting cuttings (with a 3 g l^{-1} IBA treatment) under a misting system with >97% humidity and bottom heating of 20° C.

Key words: epicormic shoots, maturation, Myrtaceae, rooted cuttings

Running title: REJUVENIATION OF TEA TREE

INTRODUCTION

There are often situations in horticulture where it is desirable to vegetatively propagate woody plants that have reached reproductive maturity or where the stock-plants cannot be managed to produce optimal cutting material. This arises, for example, when it is necessary to assess floral attributes or other features i.e. bark, foliage, or form, of the mature plant, prior to mass propagation, or to facilitate the infusion of genes into domesticated populations via sexual reproduction. Vegetative propagation from mature trees is often difficult due to maturation effects, however, that may both reduce adventitious rooting rates on cuttings, as well as give rise to propagules that by virtue of their physiological age, exhibit undesirable growth and form i.e. cyclophysis (Greenwood and Hutchison 1993, Olesen 1978). Typically, maturation-related rooting problems in trees can be overcome through rejuvenation by wounding (Libby and Ahuja 1993). However, optimal induction treatments may involve decapitation or severe wounding (Eldridge et al. 1994, Amissah and Bassuk 2009), which may not be feasible in some situations, for example, where the risk of the loss due to extreme treatments for individuals of high value for conservation or uniqueness is too high, or severe alteration of the tree form is unacceptable for aesthetic reasons.

Melaleuca alternifolia (Maiden & Betche) Cheel is a small tree, native to the subtropics of eastern Australia. Like many in the genus, it has a history of cultivation for its ornamental

58 and amenity value (ANPSA 2012). Over the past few decades, *M. alternifolia* (or Tea Tree)
59 has been planted for the production of essential oil in Australia and overseas (Baker 1999).

60

61 Like many *Melaleuca* spp., *M. alternifolia* can be propagated from current season's growth
62 tip cuttings or seeds (ANPSA 2012, ANBG 2013). Previous reports of propagation through
63 rooted cuttings for this species have been based largely on the use of young, cultivated
64 stock-plants (Doran et al. 1997, List et al. 1996), that possess virtues which tend to minimise
65 the impact of maturation or other stock-plant factors upon clonal propagation rates in
66 woody species (Libby and Ahuja 1993, Mankessi et al. 2011). Even in situations where stock-
67 plants of *M. alternifolia* can be managed, attrition rates of 40% of genotypes occur (Doran
68 et al. 1997), indicating Tea tree is not unusual for a woody plant in showing a restriction in
69 genotypes that can be captured by vegetative propagation. Furthermore, as evident in the
70 more intensively studied and closely related Myrtaceae genus, *Eucalyptus* (e.g. (Mankessi et
71 al. 2010, Mankessi et al. 2011), pronounced maturation effects are expected in cuttings
72 taken from mature unmanaged stock-plants.

73

74 The aims of the present study were, firstly, to determine the impact maturation has upon
75 the range of genotypes that may be vegetatively propagated in *M. alternifolia*, as well as the
76 impact on rooting rate. Secondly, we compared the rooting rates of cuttings from shoots
77 following several mild rejuvenation approaches (basal wounding and branch severing), with
78 opportunistically collected naturally-stimulated epicormic shoots, and mature shoots. We
79 define propagation conditions that will be valuable in future propagation of *M. alternifolia*

or other *Melaleuca* species, when there is a requirement to culture mature, unmanaged stock-plants.

MATERIALS AND METHODS

Materials

Four native stands, two inland and two coastal, were selected from an earlier survey of 10 sites, to broadly represent geographic extremes in the natural range of Tea Tree. The two coastal lowland sites were Dilkoon (29°29’25”S 152°59’15”E) and Leeville (28°59’20”S 153°00’36”E), whereas the two inland sites were, Cannon Creek (28°34’48”S 151°50’58”E) and Ballandean (28°47’10”S 151°49’06”E). Ten mature trees from each stand (more than 50 m apart to avoid relatives; tree age unknown but based on size likely to be > 10 years of age), were selected for the present study, and in addition to the collection of material for cuttings as described below, a botanical specimen including reproductive material was collected from each tree, along with data on tree height, main stem diameter, ecology and soil type.

Experimental design, propagation conditions and timing of wounding and cutting harvests

Nominally, the study utilised 20 cuttings from each tree (10 from epicormic and 10 from mature shoots) (i.e. a total of 800 cuttings). Ten cuttings of each tissue-type (epicormic or mature-shoot derived) were set as two plots of five cuttings as a column in 8 x 5 cell propagation trays. Plots were arranged into two replicates, a plot of mature and epicormic

from each tree paired together, and arrayed in order of tree index. Trays within a replicate were arranged as a block within the propagation facility, with tray position within each block randomised. Trays were shuffled periodically within the growing space.

Due to logistical constraints in the availability of the growth facilities and the conduct of field work, the study was carried out as a series of three experiments, each experiment utilised material from different sites and propagation systems.

Experiment 1

Experiment 1 utilised material from 10 trees from the coastal lowland site of Dilkoon. Wound induction was carried on 14 May 2012 late in autumn and cutting material was harvested early in spring (14 Sept 2012). Cuttings were set in 8 x 5 cell propagation trays (BCC Hiko V93 Seedling Tray; BCC AB**,** Landskrona, Sweden), and grown in a light and humidity regulated Versatile Environment Chamber MLR-360H (Sanyo Oceania P/L, North Sydney) set at 25□□C with a 16 hr light cycle (photosynthetic photon flux density 6160 lux) and humidity at 90%. Cuttings were watered by hand until saturation of the rooting substratum at an approximately 2-day interval. The rooting substratum used was mixture of perlite, vermiculite and sphagnum moss (1:1:1 ratio) with pH adjusted to 7.0 with dolomite.

Experiment 2

Experiment 2 utilised material from 20 trees from the two upland inland sites, Cannon Creek and Ballandean. Wounding treatments were applied in mid winter (25 July 2013) and cutting material was collected late spring (7 Nov 2012). Cuttings were set in a rooting substratum consisting of perlite and sphagnum moss (4:1 ratio) in Hiko trays as described below, and grown in a custom propagation chamber at the NSW Department of Primary Industries, Centre for Tropical Horticulture Alstonville under ambient temperatures (daytime max. 27-32□□C and night time min. 16-22□□C; 20% of ambient light), where humidity of > 95% was maintained by misting controlled by a balance arm switch (Sage Horticulture, Cheltham, Victoria).

Experiment 3

Experiment 3 utilised material from 10 trees from a second coastal lowlands site, Leeville. Wounding treatments were applied late winter (29 Aug 2013) and cutting material was harvested midsummer (16 Jan 2013). Cuttings were set in Hiko trays in rooting substratum consisting of perlite, vermiculite and sphagnum moss (1:1:1 ratio) and supplemented with fertilisers (Osmocote Exact 12-14 month at 5 kg m^{-3}; Everris Australia P/L, Bella Vista NSW); Micromax 0.5 kg m^{-3} (Everris Australia P/L, Bella Vista NSW) and Hydroflo II (granular wetting agent) 1kg m^{-3}) (Everris Australia P/L, Bella Vista NSW). Cuttings were placed in a commercial heated propagation chamber (Sage Horticulture; 1.8 x 0.76 m propagation bed) installed in glasshouses at SCU. Bottom heating was applied at 20□□ C but otherwise cuttings were subjected to ambient temperatures in the glasshouse (day-time max. 30-35□□C; night-time min. 16-22□□C) and 20% of ambient light. Humidity of > 95% was

maintained within the chamber by misting controlled by a balance arm switch (Sage Horticulture, Cheltham, Victoria).

Induction of epicormic shoots

Each tree was subjected to wounding in an attempt to induce epicormic regrowth of a standard age within each experiment. Where possible a large branch, usually attached to the main stem within 1 m from ground height and from the northern side of the tree, was severed with a hand saw.

Additionally, in Experiment 1, the trees were subject to debarking of a window (approximately 100 x 100 mm) on the main stem close to ground level, in order to test the efficacy of the two wounding approaches. In Experiment 3 (Leeville site), the branch removal was varied so that the branch was cut through about half its thickness, then fractured but left attached to the main stem. This approach was trialled in an attempt to mimic damage induced by flooding where a proliferation of epicormic regrowth was noted to be induced along partially severed horizontal branches.

Harvesting of cutting material

Cutting material was collected around 3-4 months after wounding. If wound-induced epicormic regrowth was unavailable, epicormic shoots that occurred spontaneously were collected opportunistically as the closest alternative. Vigorously growing epicormic shoots (approximately 2mm diameter) from the base of the plant and from a northerly aspect were collected where possible. In addition, for each tree, mature mid-crown foliage (branch originating from 1-4m height of stem) from a northerly aspect was also obtained. The total stem length between the position at which shoots were collected (both epicormic and mature) and the ground was estimated to the nearest 200 mm and recorded. Harvested shoots of 200-500 mm length were cut and stored moist and cool in plastic bags inside a cooler for transport until cuttings were set.

Setting of cuttings

Standard cuttings, prepared by using an oblique cut to remove an 80-100 mm section of the stem tip, followed by removal of foliage from the lower half of the cutting, were used in all three experiments. IBA (3 g l^{-1}) was applied by dipping the cutting base into a commercial preparation (Clonex Purple, Yates) for around 10 seconds, the rate being based on earlier reports that rates between 0.5 and 4 g l^{-1} IBA have been suitable for cuttings of *Melaleuca alternifolia* (Whish 1994). Cuttings were set into saturated rooting substratum to a depth of around half their length, by creating a hole with a dibble stick, inserting the cutting, then gently pressing to firm the rooting substratum around the base of the cutting. Foliage of

cuttings was maintained saturated with a hand sprayer till placed in the propagation chambers.

Root and shoot assessment

Assessment of rooting was facilitated by the use of clear plastic inserts (crackpot liners) that allowed visual inspection for root development and detection of roots that reached the side of the container or emerged from the drain hole. Monitoring of rooting was carried out three times per week during Experiment 1 to allow early detection of root development, then approximately weekly during Experiments 2 and 3. The presence of newly developed shoots was also recorded at Day 52 in Experiment 3 to allow testing for correspondence in root and shoot development.

Statistical analysis

Pooled and individual experiment analysis – tissue-type nested within stock-plant

A nested design was first used to examine the sources of variation within each experiment and provide the most sensitive test of the tissue-type effect (TT) i.e. contrasting epicormic and mature shoot derived cuttings. The model allowed testing of replicate (R), stock-plant

(SP) and tissue-type within stock-plant terms (TT(SP)). The F tests performed used the Mean Square ratios R/SP, SP/TT(SP) TT(SP)/Error.

This analysis was repeated for each of the multiple assessment time points in Experiments 1 and 3, and, in addition to testing each experiment individually, a pooled analysis of data from all three experiments was also conducted. For the pooled analysis rooting rates assessed in the window of 64-67 days post-setting were chosen. Assessment dates of 64-69 days post-setting were chosen for this pooled analysis because the first evidence of roots was noted around 20 days earlier, at 30-41 days post-setting in each experiment, and little additional rooting occurred beyond this time (See results).

A second model was also used on Experiment 2 data to test for site and tissue-type effects, however, in this analysis tissue-type could not be nested because the same stock-plants do not occur at both sites. The F tests were constructed using the Mean Squares as follows; S/TT and TT/Error.

Experiment and tissue-type subcategory effects

A second set of analyses was conducted for experimental level differences on the total pooled data set. In this case three subcategories of tissue-type (TTSC) were identified, namely mature, induced epicormic, and spontaneous epicormic sources. A model with an Experiment term and TTSC factor was utilised and all factors were tested on the Error term.

For all statistical analyses, factors were treated as fixed unless otherwise noted, and ANOVA and estimated margin means were generated using the General Linear Model (GLM) Univariate module of SPSS 20. For all analyses, rooting rate was expressed as the proportion of rooted cuttings in each plot of five cuttings at the time of the assessment.

The relationship between the stem height at which epicormic shoots were sourced and tree mean rooting proportion for the 37 trees where epicormic shoots were obtained was quantified by a Pearson's correlation coefficient, estimated in the Correlation module of SPSS.

The degree of relationship between root and shoot production was assessed in Experiment 3 using the Descriptives / Crosstabs Module of SPSS and selecting the Contingency coefficient test for nominal variables. New shoot growth was recorded as a presence-absence variable during the Day 52 post-setting assessment of rooting.

RESULTS

Response to wounding treatments

Experiment 1 – Dilkoon – Stem basal wound versus branch severing.

Of the 10 trees at the Dilkoon site, no regrowth occurred from basal wounds and only four trees (06-08 & 10) sprouted epicormic shoots from branch stumps, four months after basal wounding and branch removal. Spontaneous epicormic shoots were present on five out of the six remaining trees (No epicormic shoots on Tree 09).

Experiment 2 - Cannon Creek and Ballandean - Branch removal during winter

Induction of epicormic regrowth by severing a low branch during winter (25th July 2012) was more successful when applied at the Cannon Creek site, as all 10 treated trees had produced epicormic shoots at the branch stump approximately four months later by 7th Nov 2012. The stump resprouts were typically not adequately developed enough to sample for cutting material (<200 mm in length and unlignified), hence induced resprouts were only collected from two trees (05 and 08). Spontaneous epicormic shoots were collected from the eight remaining trees.

Surprisingly, the same technique for induction of resprouting, applied at the same time, was less successful at the Ballandean site, as only four out of 10 treated trees produced sprouts. Two trees (01 and 02) were sampled for wound-induced epicormic, whereas seven out of eight of the remaining trees were sampled for spontaneous epicormic (Tree 07 had no epicormic shoots). The main difference between the two sites was that the trees from Cannon Creek retained a more natural upright form, whereas most of the canopies of trees at Ballandean were newly regenerated from stems damaged by floods in January 2011. As the Ballandean trees were already undergoing extensive canopy replacement, they may not have responded to our additional wounding challenge (around 18 months later).

Experiment 3 – Leeville – Partial severance of a branch in late winter

Induction of epicormic regrowth, and subsequent rooting of cuttings (see below) was most successful in the Experiment 3, where eight out of 10 trees responded to partial severing of a branch in late winter (29th Aug 12). The cutting material collected from regrowth 4.5 months later (16 Jan 2013) was considered more suitable for rooting than that in the earlier experiments because shoots showed some lignification and were around 500-1000 mm in length. Induced epicormic regrowth was utilised for seven out of 10 trees, epicormic shoots were sampled opportunistically from two trees (05 and 06), and no epicormic shoots were available for the remaining tree (07) at this site.

Testing for tissue-type and stock-plant effects within experiments or pooled across experiments

Tissue-type within stock-plant

Considering the pooled analysis of all three experiments, a wider (71 % 27/38) range of genotypes rooted from epicormic shoots than from cuttings derived from mature shoots (52.5 % 21/40), when assessed 65-69 days post-setting. Additionally, cuttings from epicormic shoots rooted at significantly higher rates (26.0 ± 3.5 %) than those derived from mature shoots (11.2 ± 2.0 %) (ANOVA p-value = 0.0, Tables 1 and 2).

At the individual experiment level, a significant tissue-type within stock-plant effect indicated epicormic-derived cuttings rooted more frequently than those from mature foliage at all assessment days for Experiments 2 and 3 but not for Experiment 1 (Table 1). The replicate effect was not significant in any of the ANOVA at the individual experiment level or in the pooled analysis (Tables 1 and 2).

Experiment 1, differed from the other two experiments in that its mean rooting percentage for epicormic-derived cuttings (25.6 ± 7.6 %) was not significantly different to that for mature shoots (20.0 ± 5.2 %) at Day 69 (p-value = 0.26, Table 1). However, at an earlier assessment time point (Day 41), the difference between rooting rates of cuttings from different tissue types approached significance (p-value = 0.062, Table 1), which suggested mature-shoot derived cuttings may root more slowly than cuttings derived from epicormic-shoots. Plotting rooting rates over four assessment time points indicated that the rate of

311 rooting was more or less linear for cuttings from both tissue-types and increased at a similar
312 rate over the time-span assessed (41-69 days post-setting) (Fig. 1). Furthermore, an
313 assessment of cuttings 81 days post-setting in Experiment 3 also tended to support the
314 observation that rooting was largely completed by day 69, as no mature-shoot derived
315 cuttings were found to have rooted beyond Day 69 (data not shown).
316
317 *Stock-plant*
318
319 Differences among stock-plants (i.e. due to genotype, plant age or health), were not
320 significant for the pooled data set or at the individual experiment level except at the three
321 later assessment dates in Experiment 1 (Table 1). Some stock-plants from each site did not
322 root at all, whereas other genotypes reached the maximum rooting percentage (100%) for
323 cuttings from at least one tissue-type in the case of Dilkoon and Leeville sources, and a
324 maximum of 60 and 80% for Cannon Creek and Ballandean sources, respectively.
325
326 *Site*
327
328 A test for a site effect based on the mean for both tissue types was not significant (p-value =
329 0. 239; Table 1) for the two sites in Experiment 2, the only comparison possible in this study
330 (Fig 2.).
331

Variance components supported the greater importance of tissue-type relative to stock-plant

Analysis of tissue-type within stock-plant and stock-plant effects as random variables on the pooled data set allowed estimation of variance components and indicated that the variance explained by tissue-type within stock-plant (Estimate ± SE; 0.029 ±0.01) was around four fold larger than that due to the stock-plant (Estimate ± SE; 0.007 ±0.008). The variance due to tissue-type within stock-plant was of a similar order of magnitude to that of the residual term (Estimate ± SE; 0.030 ± 0.005), which in this analysis included unaccounted for variation due to factors such as site, or other experimental level differences, including differences in the propagation systems or the season in which shoots were harvested.

Differences among experiments on tissue-type subcategories

An ANOVA using Experiment as a factor indicated there were significant differences among the overall means for each experiment (ANOVA not shown; df=2; p-value for F test on Experiment = 0.008). The mean for Experiment 2 (16 ± 3 %) was lower than the mean for Experiment 3 (34 ± 5 %) but it was not different to Experiment 1 (23 ± 4 %), and Experiment 1 was significantly lower than Experiment 3 (Fig. 3).

Within each tissue-type sub-categorisation (mature, epicormic induced or epicormic spontaneous), the factorial effect was also highly significant (ANOVA not shown; p-values =0). The better performance of Experiment 3 relative to the other two experiments could

largely be attributed to better rooting on epicormic shoots-derived cuttings (Fig. 2 and 3). Experiment 1 differed from the other two experiments in that the rooting rate for cuttings derived from the induced epicormic shoots was on average lower (12.5 ± 8.1 %, No of trees = 4) than cuttings from spontaneous epicormic shoots (36.0 ± 7.2 %, No. of trees = 5), or mature shoots (20.0 ± 5.1 %, No. of trees = 10), but not significantly so (One way ANOVA F-value = 1.8, p-value = 0.175; Fig. 3).

Stem height of epicormic shoots

The height above ground level at which a tree is decapitated to produce coppice has been found to be critical for rooting rates of cuttings (e.g. (Haines et al. 1993). In our study, total stem length (i.e. the sum of the length of the main stem plus the branch length) was used rather than vertical height above ground level because this was thought to moderate among tree forms (multi-stem “mallee” forms versus small trees with short single main stems and damaged forms where trees had been prostrated by flood water). For the 37 trees sampled for induced or spontaneous epicormic, the shoots were sourced at stem lengths ranging from around 0.2m to 4m. There was a significant (p-value= 0.022) negative correlation (r = -0.375) between stem length and rooting rate.

Are new shoots on a cutting a reliable indicator of rooting?

In Experiment 3, the production of new shoots was recorded as well as roots to test whether new shoots were an indicator of rooting. Although there was a significant positive correlation (Contingency coefficient = 0.252 p-value = 0), shooting was only a weak indicator of rooting, with many cuttings rooting but not shooting, and other cuttings shooting but not rooting by Day 52.

DISCUSSION

Impact of maturation on rooting rates in M. alternifolia

This study has shown that it's possible to capture a wide range of genetic material from *M. alternifolia* directly from natural stands via vegetative propagation. It was clear, that under most circumstances, there would be an advantage in targeting juvenile tissue from epicormic shoots when sourcing cuttings, either opportunistically or by inducing epicormic regrowth by wounding. The use of juvenile foliage both maximised the range (increasing the proportion of genotypes brought into cultivation from 53% to 71%), and the rate of rooting (ranging from 26-42% to 8-20% in three experiments) for epicormic and mature tissue-types, respectively.

Maturation effects profoundly influence the morphology and physiology of ramets and are subject to both genetic and epigenetic control (Olesen 1978, Greenwood and Hutchison 1993, Eldridge et al. 1994, Fraga et al. 2002, Shepherd et al. 2009). As the plant ages there is a loss of totipotency in the tissue that must undergo dedifferentiation to give rise to adventitious roots and thus a reduction of rooting from stem cuttings. Within the Myrtaceae family, maturation effects have been reported for well-studied groups like the eucalypts (Genera *Eucalyptus, Corymbia and Angophora*) (Hartney 1980, Eldridge et al. 1994, Mankessi et al. 2010). For example, Mankessi et al. 2010 found cuttings from juvenile shoots rooted at 38.7% which was significantly higher than the 28.7% for those from mature shoots for *E. grandis* X *E. urophylla* hybrids across settings in both the dry and wet season.

The usual response to circumvent maturation-related rooting problems is to rejuvenate through induction of epicormic shoots (Libby and Ahuja 1993). Epicormic shoots are often a more successful source for cuttings because they are initially juvenile and in eucalypts, for example, may be induced by decapitation or wounding (Eldridge et al. 1994, Jacobs 1955). In eucalypts, epicormic regrowth arises from dormant bud strands (meristematic tissue) buried beneath the bark and are found at the base of every leaf (Burrows 2002, Burrows et al. 2008) and may give rise to a foliage phase with strikingly different leaf form and physiological attributes (Wiltshire et al. 1998).

Like eucalypts, the epicormic foliage of *M. alternifolia* differed in morphology and chemistry from mature foliage and was generally readily recognised. The newly-induced epicormic shoots on *M. alternifolia* tended to have a larger leaf form like the broader and longer foliage found on young seedlings (~ 3 mths of age before lateral branching begins), but this progressed to narrower, adult-like foliage so that by harvest time 4 mths later, it resembled the leaf form of the mature canopy. Epicormic shoots also differ in foliar oil composition and yield compared to adult foliage from the same tree (data not shown), similar to the differences found between the juvenile foliage of seedlings and adult foliage (Southwell and Stiff 1989, Russell and Southwell 2002, Russell and Southwell 2003). Sourcing shoots from juvenile sources has significant advantages for vegetative propagation from *M. alternifolia*.

Mild induction of epicormic regrowth

Relative to branch severance or even partial branch severance, a basal wound was not as effective at simulating regrowth. It is likely that relatively minor wounds (removal of a bark window of about 10 cm^2) were insufficient to provide the necessary hormonal signals to stimulate regeneration. It is worth noting, however, that the relatively mild treatment of removing one branch can be effective if applied at the appropriate time of year. Partial branch severance followed by fracturing of the limb (but leaving it attached) was used in Experiment 3 and was also highly successful. Here we attempted to mimic the natural stimulus of flood damage, where extensive epicormic regrowth occurred along the lengths of branches that had been fractured and prostrated. It is likely a more drastic treatment (ie. decapitation) would be more successful in stimulating more extensive regrowth and provide a greater abundance of shoots, but this approach is also more risky, as trees may not recover (Eldridge et al. 1994), and may not be desirable in situations of high conservation value or for aesthetic reasons.

Genetic differences among provenances and site conditioning factors may be relatively small relative to propagation system effects

It was also clear from this study that there could be strong interactions between the performance of cuttings from different tissue-types and experiment level effects (i. e. propagation system, timing of wounding and the harvest of cutting). While it wasn't possible to separate these factors in our analysis, testing for a site effect in Experiment 2,

446 nonetheless suggested that the site effects may be relatively small, at least for sites located
447 within the same bioregion. Other experiment level factors such as the propagation system,
448 and the timing of epicormic induction and shoot harvest, therefore, may be more important
449 in determining rooting rates.

450

451 ***Optimising rooting rates***

452

453 By optimising the propagation system, the timing of treatments, and harvesting of shoots, it
454 should be possible to at least reach rooting rates of around 48% for *M. alternifolia* trees
455 from a native stand (equal to the average rooting rate for wound-induced epicormic shoots
456 across the stock-plants in Experiment 3). Differences among stock-plants (including those
457 due to genotype, plant age and condition) were usually not significant, and the effect of
458 tissue-type was comparatively larger (around 3.6 fold); hence, tissue-type appears to be the
459 single-most important factor influencing rooting success within each experiment. In general,
460 it seems that it is worth trying to induce epicormic growth at an optimal stage rather than
461 relying on serendipitous production, although opportunistic collection of appropriate
462 material may be a reserve option.

463

464 The highest overall level of rooting was obtained in Experiment 3 (Leeville), largely due to
465 the high rooting rates on cuttings from epicormic shoots. The large deviation in rooting
466 response between cuttings derived from wound-induced epicormic and mature-shoots was
467 unique in this experiment and suggests the timing of induction and harvesting of shoots

were the most appropriately investigated, with the shoots produced here providing a model to aim for in future work.

Our results from sourcing cutting material mid-summer, were consistent with general recommendations for epicormic shoot induction in eucalypts (Eldridge et al. 1994) where the aim is to cutback at the beginning of the active growth period - almost any time in the tropics but late spring in temperate climates, so that resprouts are available 2 – 3 mths after induction. The advantage of sourcing cuttings from more actively growing stock-plants is exemplified in a recent study of two subtropical eucalypts that showed higher rooting rates from stock-plants maintained at higher temperatures (Trueman et al. 2013). But this general guideline may not be universal, as was recently found for *E. grandis* x *E. urophylla* where whilst high rooting and survival of cuttings was found in dry season harvests, rates for juvenile and mature shoots were not different for the rainy season (Mankessi et al. 2010). These authors note that this has been observed before both in eucalypts and conifers and attribute it to the "influence of endogeneous rhythms on time-related fluctuations in adventitious rooting capacity". Because of difficulties in comparing responses across experiments in our study, we recommend further investigation of optimal timing for epicormic induction in *Melaleuca* sp., where propagation and genetic material are standardised during experimentation.

In terms of the timing of harvest and selection of appropriate shoots for cuttings, general recommendations for eucalypts also appear appropriate. Regrowth should be of an

490 “appropriate shade of green, with some lignification but less than 1m long, shoots should
491 not be too succulent, and shoot tips should be avoided in most species” (Eldridge et al.
492 1994). The most effective cutting material we used had some lignification and was around
493 500 -1000 mm in length, induced by partial severing of branches in autumn, and harvested
494 4.5 mths later in mid-summer (Experiment 3). The epicormic regrowth was growing
495 vigorously, and the trees were not flowering at this stage (they flower late October- early
496 November). We found that the use of relatively “soft” and unlignified induced epicormic
497 shoots performed poorly, wilting quickly and decaying more than “harder” cuttings.

498

499 We also found that there was a negative correlation between rooting rates and the stem
500 length at which epicormic shoots were sourced in *M. alternifolia*. This effect has also been
501 found in a wide range of woody plants (Haines et al. 1993, Eldridge et al. 1994). Maturation
502 advances unevenly in a tree so that juvenility declines with height on the main stem or
503 towards the tips of lateral branches (Olesen 1978). Our study showed that targeting
504 branches that joined the main stem within 0.5m of ground level for cuttings, tended to
505 improve rooting rates, which was consistent with the differential maturation effects
506 observed in other trees.

507

508 Targeting or inducing suitable epicormic resprouts provided an advantage by allowing the
509 capture of more genotypes from mature native forest stands of *M. alternifolia*. Further
510 optimisation of propagation via cuttings may benefit by studying the influence of hormone
511 application, as micropropagation studies indicate that exogenous IBA applications are sub-

optimal for stimulation of rooting (de Oliveira et al. 2010), and hormone type can influence the quality of the root system on a Tea Tree cutting (Whish 1994).

ACKNOWLEDGEMENTS

The authors thank R Dyason, M &L Simpson, D & M Hood and J Wicken for allowing access to their properties for collections, T Olesen for cooperation with use of NSW DPI propagation facility at Alstonville, G Ablett, A Wiseman, M Abasolo, R Wood and J McDowell for assistance with collections, setting or assessments of the experiments, and T Olesen, D Cliffe, T Shapter and G Baker for valuable discussions on the propagation of Tea Tree.

TABLES

Table 1. Factors affecting the rooting rates for each of three experiments and pooled data set.

Expt. No.	Provenance	No. of Trees	Assessment (Days post-setting)	First roots (Days post-setting)	Rooting Percentage [1]		ANOVA factors											
							Site[5]			Replicate			Stock-plant			Tissue-type(Stock-plant)		
					Epicormic	Mature	df[2]	MS[3]	Sig[4]	df	MS	Sig	df	MS	Sig	df	MS	Sig
					% (SE)	% (SE)												
1	Dilkoon	10	41	38-41	12.2 (4.9)[a]	4.0 (2.3)[a]	na	na	na	1	0.009	0.675	9	0.054	0.061	9	0.032	0.062
1	Dilkoon	"	48	"	17.8 (7.3)[a]	11.0 (4.4)[a]	na	na	na	1	0.127	0.394	9	0.159	0.009	9	0.029	0.539
1	Dilkoon	"	58	"	21.1 (6.9)[a]	16.0 (4.4)[a]	na	na	na	1	0.127	0.384	9	0.152	0.004	9	0.022	0.702
1	Dilkoon	"	69	"	25.6 (7.6)[a]	20.0 (5.2)[a]	na	na	na	1	0.127	0.445	9	0.044	0.018	9	0.044	0.260
2	Cannon Creek	10	64	<40	21.0 (5.1)[a]	9.0 (3.1)[b]	1	0.05	0.239	1	0.032	0.418	18	0.047	0.538	20	0.049	0.043
2	Ballandean	10	64	"	12.0 (4.7)[a]	8.0 (3.7)[b]	"	"	"	"	"	"	18	0.047	0.538	20	0.05	0.043
3	Leeville	10	52	"	42.2 (8.2)[a]	6.0 (2.1)[b]	na	na	na	1	0.105	0.359	9	0.112	0.821	9	0.212	0.000
3	Leeville	"	64	"	47.8 (8.1)[a]	8.0 (2.7)[b]	na	na	na	1	0.052	0.550	9	0.134	0.770	9	0.222	0.000
Pooled			64-69	NA	26.0 (3.5)[a]	11.2 (2.0)[b]				1	0.004	0.851	39	0.115	0.212	38	0.089	0.000

[1] Differences in rooting percentage means at p-value <0.5 indicated by different letters based on ANOVA F test.

[2] df = degrees of freedom

[3] MS = Mean Square

[4] Sig = Significance of F test.

[5] NB. Site factor could only be tested in Experiment 2 where material from two sites was subjected to the same propagation conditions. Tests for differences among tissue-types in Experiment 1 and 3 are shown for multiple assessment times, 4 in the case of Dilkoon and 2, in the case of the Leeville material..

532 Table 2. ANOVA for the effect of tissue-type, stock-plant and replicate upon rooting

533 percentages based on the pooled data from three experiments.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	13.204[a]	79	0.167	5.557	0.000
Replicate	0.004	1	0.004	0.036	0.851
Stock-plant	4.503	39	0.115	1.298	0.212
Tissue-type(Stock-plant)	3.380	38	0.089	2.957	0.000
Error	2.316	77	0.030		
Total	15.520	156			

a. R Squared = .851 (Adjusted R Squared = .698)

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FIGURE LEGENDS

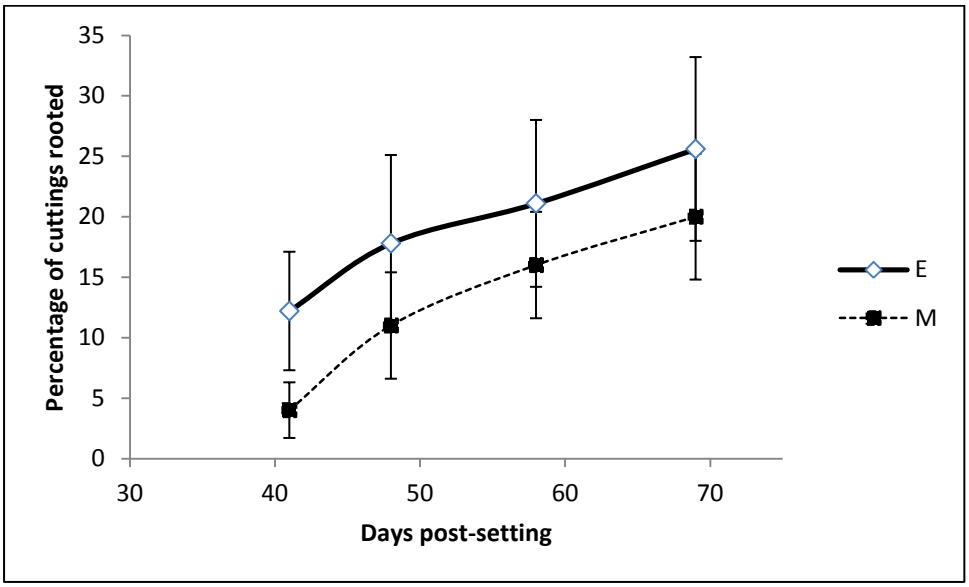

Fig. 1. Progression of rooting during days 41-69 post-setting for cuttings derived from epicormic (E) or mature (M) shoots for 10 *Melaleuca alternifolia* trees from Dilkoon.

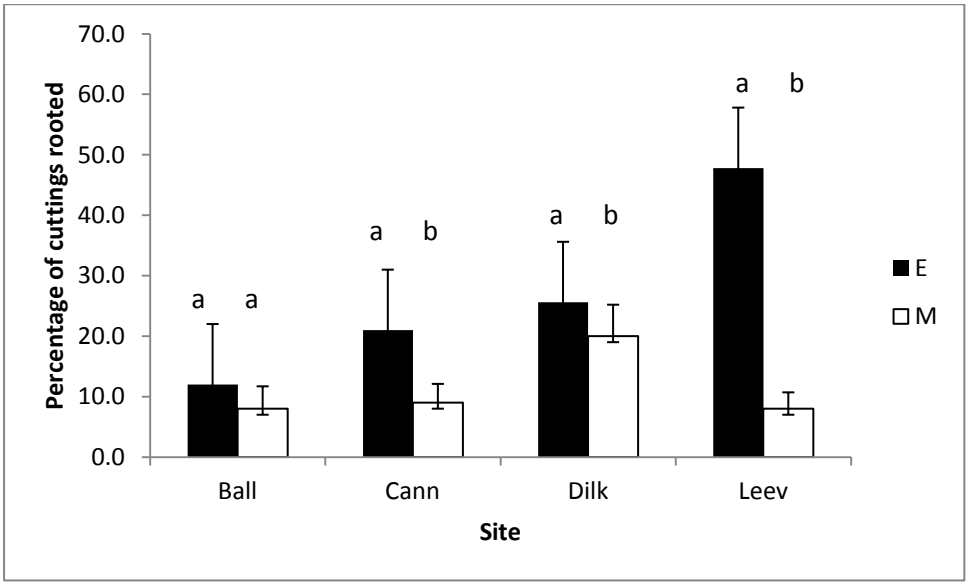

Fig. 2. Mean rooting rates for cuttings derived from epicormic (E) or mature (M) shoots for *Melaleuca alternifolia* at each of 4 sites. Error bars represent the standard error of the mean. Different letters denote significant differences at the 95% level, between tissue-types, within an experiment.

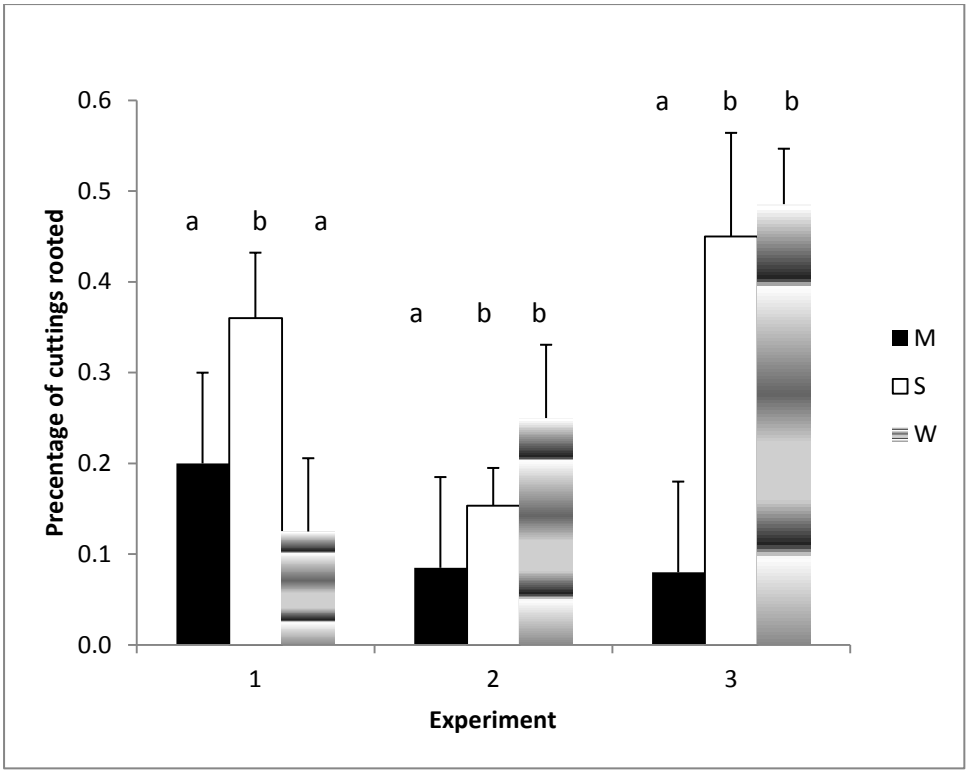

Fig. 3. Mean rooting proportions for *Melaleuca alternifolia* cuttings for three tissue-type subcategories: (M) mature shoots, (S) spontaneous epicormic shoots, or (W) wound-induced epicormic shoots in each of three experiments. ANOVA-based experimental means are shown above the clusters with error bars that represent the standard error of the mean. Significant differences in experiment means at 95% level for a Least Significant Difference test (LSD) are indicated by different letter following each mean. Differences among tissue-type subcategories *within* each experiment are denoted by different letters under each experimental mean.

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