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Article

Testing New Provenances of *Eucalyptus polybractea*: A Eucalypt Oil Mallee Adapted to Semi-Arid Environments

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Abstract: Novel genetic accessions of *Eucalyptus polybractea* from a previously untested, hotter and drier part of the species' natural range were tested in a common garden trial at a semi-arid site in NSW, Australia. *Eucalyptus polybractea* is a mallee eucalypt cultivated for essential oils (1,8-cineole), bioenergy and carbon sequestration on dryland sites in southern Australia (sites receiving about 450 mm mean annual rainfall, MAR). A trial of six previously untested provenances from the relatively hot, dry part of the species' natural range in South Australia (SA) (250–450 mm MAR) was established alongside seven provenances from New South Wales (NSW) and Victoria within a commercial plantation in NSW. The trial was assessed at age 3.7 years for growth and oil characteristics. While survival was excellent, most of the SA sources were slower growing and of sub-standard oil concentration and quality relative to those from Victoria and NSW. However, a single SA provenance, with the highest oil concentration and 1,8-cineole percentage of all provenances tested, may have potential as a source of selected germplasm. Infusion of SA material into the breeding populations of *E. polybractea*, which are currently based on NSW and Victorian selections only, may provide more resilience in the face of hotter and drier temperatures expected under projected climate change scenarios, and/or allow the introduction of the species to hotter and drier climates in Australia or other parts of the world with semi-arid climates. However, high-intensity selection of infusions will be required to maintain the growth and oil characteristics in the existing breeding population.

Keywords: *Eucalyptus* oil; biomass; climate change; 1,8-cineole; blue mallee

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1. Introduction

Eucalyptus polybractea R.T. Baker (blue-leaved or blue mallee) is a short (5–10 m), multi-stemmed mallee eucalypt. Mallee eucalypts are typically adapted to harsh, drought- and fire-prone environments and are able to regrow their aboveground shoots repeatedly following their loss to fires, animal browsing or harvesting. Two disjunct regions of provenance (ROP), West Wyalong, New South Wales (NSW) and Bendigo, Victoria (Vic), have been harvested as a source of 1,8-cineole-rich *Eucalyptus* oil since the early 1900s (Shiel, 1985). However, it has more recently been confirmed that a third ROP exists in South Australia (SA) [1,2]. The SA ROP comprises discrete upland provenances from the Flinders Ranges to the Gammon Ranges (Figure 1). SA provenances of *E. polybractea* have not been harvested commercially for their foliar oils, nor, to our knowledge, have they previously been cultivated for any purpose.

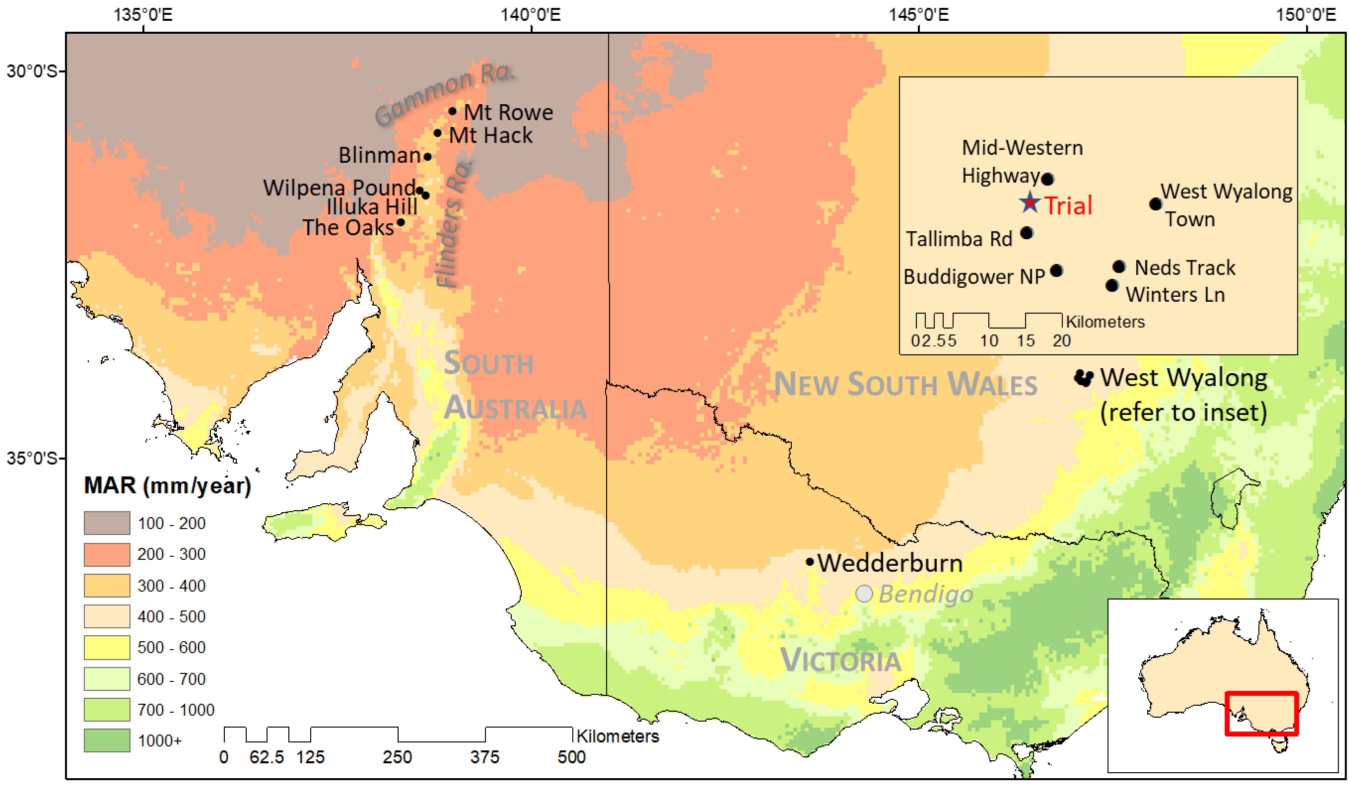

Figure 1. Locations of the trial near West Wyalong, NSW; provenances included in the trial from NSW, Vic and SA, and mean annual rainfall (MAR) distribution.

Eucalyptus polybractea is commercially significant as the major domestic source of *Eucalyptus* oil in Australia. It is increasingly sourced from plantations rather than harvested from natural woodlands. It produces exceptionally high-quality, medicinal-grade oil used in health care products, disinfectants and solvents [3,4]. It is this grade of oil that commands the greatest share of the current international market. *Eucalyptus* oils are classified and priced according to the percentage of 1,8-cineole (typically ranging from 70%, to near-pure) and must legally satisfy the requirements of various pharmacopoeial monographs [5,6]. *E. polybractea* oil is among the few that meet all international and national standards direct from the still. The 1,8-cineole levels (%cineole) in this species generally range between 75% and 90% of total oil while oil concentration in fresh foliage from coppiced trees is typically between 4% and 6 % (w/w% dry weight) with a range of 2% to 10% [7].

In the 1970s, two of Australia's largest *E. polybractea* oil-producing companies, GR Davis Pty Ltd. at West Wyalong, NSW, and Felton, Grimwade and Bosisto's Pty Ltd., 40 km west of Bendigo, Vic, commenced transition from harvesting wild stands to plantations. Early plantations were established from wild-collected seed with only rudimentary levels of selection for oil yield and %cineole. By the 1990s, this had changed [8], with the realisation that the drivers of oil quality and yield per hectare, namely %cineole, concentration of oils in the leaves, and leaf biomass, were independent traits of low (growth traits) to high (oil traits) heritability and all amenable to concurrent improvement by selection and breeding [9,10]. The companies in NSW and Vic commenced tree improvement based on seed and clones from stands near their centres of operation in the 1990s: though formal provenance and family testing was not carried out, the local region of provenance is thought to perform best at both locations. The Government of Western Australia also commenced planting and domestication of *E. polybractea* in the 1990s, motivated by the need for economic incentives to encourage revegetation for dryland salinity control. Returns from integrated production of biomass bioenergy, activated charcoal and *Eucalyptus* oil provide an impetus for planting on privately owned farmland [10–12]. An additional use for *E. polybractea* in southern Australia is carbon sequestration, where it is planted and retained in perpetuity [13].

Adjusting the breeding programs of key crops to provide adaptation to future climate-change-impacted environments is now a global priority [14,15]. Testing of crop wild relatives from warmer and drier places is seen as an informative strategy, applicable to analogous climates that are likely to become hotter and drier due to climate change, as these populations will already have been under natural selection and be well adapted [16]. Deploying provenances of long-lived forest trees from hotter and drier climates to those that are currently more mesic, but likely to undergo significant climate change within the next generation and beyond, has also been suggested [17]. The importance of common-garden testing of a wider range of forest tree species in this context has also been argued for [18].

Climate change is projected to lead to a hotter and drier climate in southern Australia [19] where *E. polybractea* is grown, presenting challenges to plantation establishment and productivity [20]. The historical and present climates (Australian Bureau of Meteorology (BoM) climate records. Available online: http://www.bom.gov.au/climate/ accessed 17 June 2022) of the NSW and Victorian ROPs are similar—Mean Annual Rainfall (MAR) is around 475 mm while Mean Maximum Temperature (MT_{max}) is 21.2 and 24.1 °C at Bendigo and West Wyalong, respectively (see Figure 1 for MAR and Table 1 for additional detail). The climate in the South Australian ROP is significantly drier (310–395 mm MAR) and exposed to hotter maximum temperatures (25.3 to 26.5 °C MT_{max}), though due to their higher elevation, Mean Annual Temperature experienced by these provenances is similar or slightly lower than those of the NSW ROP. The SA provenances may therefore be better adapted to future climates in the present industrial plantation regions and could potentially be used to expand the plantation range to hotter and drier environments.

Table 1. Geographic, climatic and CSIRO Australian Tree Seed Centre (ATSC) family composition data for provenances included in the *E. polybractea* trial at West Wyalong established in 2016. Climate data were accessed from the Bureau of Meteorology (BoM) (Australia) http://www.bom.gov.au/climate/. accessed on 17 June 2022.

Region-Of-Provenance and Provenance	ATSC Seedlot Number	No. of Families in Provenance	Latitude (Decimal Degrees)	Longitude (Decimal Degrees)	Altitude (m above Sea Level)	MAR (mm/Year)	MAT (°C)
South Australia-Modified BoM Koeppen climate is *Grassland, warm (persistently dry)*							
W of Blinman, Flinders Ranges	21,336	2	−31.1033	138.6674	630	330	16
ENE Mt Rowe, Vulkathuna-Gammon Ranges	21,337	13	−30.5200	138.9828	750	350	15
Mt Hack, Warraweena Station	21,338	15	−30.7917	138.7947	700	320	15
Illuka Hill, Flinders Ranges	21,340	5	−31.6006	138.6317	610	360	14
The Oaks, SW of Hawker	21,342	9	−31.9464	138.3231	500	310	16
Wilpena Pound, Flinders Ranges	21,343	10	−31.5431	138.5667	586	395	16

Table 1. *Cont.*

Region-Of-Provenance and Provenance	ATSC Seedlot Number	No. of Families in Provenance	Latitude (Decimal Degrees)	Longitude (Decimal Degrees)	Altitude (m above Sea Level)	MAR (mm/Year)	MAT (°C)
Victoria-Modified BoM Koeppen climate is *Temperate, no dry season (hot summer)*							
Wedderburn	19,361	7	−36.3333	143.6000	180	510	14
New South Wales-Modified BoM Koeppen climate is *Temperate, no dry season (hot summer)*							
Mid-Western Hwy	19,920, 21,060	6	−33.8928	147.0722	310	470	16
West Wyalong township	19,649, 21,059, 21,060	8	−33.9237	147.2047	260	460	16
Tallimba Rd	19,649, 19,920, 21,059, 21,060, 21,365	9	−33.9589	147.0453	290	470	16
Neds Track	19,920, 20,680, 21,060	10	−34.0011	147.1594	280	460	16
Winters Lane	19,649, 21,060, 21,365	7	−34.0244	147.1508	265	460	16
Buddigower NP	19,649, 21,060	8	−34.0061	147.0822	270	450	16
TOTAL		109					

The objective of this study was to test the SA provenances relative to those of NSW, in a common-garden trial situated within a commercial plantation on a semi-arid site at West Wyalong in southern Australia. Traits assessed included survival, growth performance, oil yield and oil quality. The implications of the results are discussed in the context of germplasm exploration and genetic selection for semiarid environments and environments that are likely to become hotter and drier in the face of climate change.

2. Materials and Methods

2.1. Selection of Seedlots for Trial

Eucalyptus polybractea herbarium records for SA were downloaded from the Atlas of Living Australia (Available online: http://www.ala.org.au accessed on 17 May 2014) and used to target sampling areas. Seed and leaf samples were collected by the CSIRO Australian Tree Seed Centre (ATSC) from 54 trees representing six provenances, which were further grouped into three ROPs, namely New South Wales, Victoria and South Australia (Table 1, Figure 1). Shoot samples (leaves, fruit, flower capsules) from each mother tree were examined and verified as *E. polybractea* by staff at the Australian National Herbarium (CANB). Leaf tissue and extracted DNA samples from these trees were also retained for future reference. Seed of families of Vic and NSW origin were chosen from those available at ATSC. The 48 families from West Wyalong were assigned to six putative provenances, each a remnant of a much wider occurrence before clearing for agriculture started more than 100 years ago. A single provenance from Wedderburn, Vic, was also included as a performance checklot.

2.2. Trial Establishment and Design

Seedlings for the trial were sown in August 2016 at Narromine Transplants Pty Ltd., Narromine, NSW, Australia, in 90 cm^3 Hyco® tray cells using the open-pollinated seedlots specified in Table 1. The trial was planted in January 2017 amongst GR Davis Pty Ltd. commercial oil plantations of *E. polybractea* west of West Wyalong, NSW (Figure 1) when the plants were around 30 cm tall. The planting site had a slight southerly aspect. Soils are classified as sandy clay loam. The MAR for the region is about 470 mm, uniformly distributed. MT_{max} is 24.1 °C.

An Alpha trial design was utilised with families randomly allocated to five-tree row plots with six replicates of 109 treatments. Spacing was 1 m within rows and 3 m between rows. A single-row surround of *E. polybractea* was planted to minimise edge effects. The trees were watered-in at time of planting, a routine establishment practice in summer.

2.3. Growth Traits

At age 3.7 years after planting and 1.6 years after the first coppice harvest, all trees in replicates one to five were measured for height (HT), width of crown (CW) across the row and crown leaf density (scale: 1 sparse to 6 very dense) (CD). To estimate green, aboveground biomass (BM) 50 (23 NSW, 3 Vic and 24 SA), trees covering the range of height growth in the trial, with sample sizes proportional to their representation in the trial (see Table 1), were harvested and weighed in the field. A crown volume index (CVI) was calculated as CVI = HT $\times$ CW^2.

2.4. Foliar Oil Characteristics

Large provenance bulk samples were constructed by stripping 20 g of leaf from the mid-crown of all individual trees from four replicates, with replicates kept separate. Two 100 g samples for distillation and a 10 g sample for moisture determination were subsampled from the large, thoroughly mixed bulks, in the field. These samples were placed in paper bags and allowed to air-dry.

Oil extraction was undertaken by hydrodistillation in the laboratory of GR Davis Pty Ltd., NSW, using Clevenger-type stills. A two-hour distillation time for each sample was employed following optimisation trials to determine time to complete extraction. Oil concentrations (*w*/*w*% fresh weight) were derived from the known weight (100 g) of fresh leaves, the volume of oil extracted from that known weight of leaves multiplied by a specific gravity of 0.92 [21]. Moisture content of the 10 g samples of leaves was determined after oven drying (70 °C, 12 h), allowing calculation of oil concentration on a dry weight basis (*w*/*w*% dry weight).

Gas chromatography of distilled oils was carried out on a GCMS-QP2010SE (Shimadzu, Japan) with a DB-35 GC column. Compounds were identified by matching their GLC retention time to that of known compounds. The oils of *E. polybractea* are known to contain at least 55 terpenoid compounds [22]. This study assessed 16 priority compounds that determine commercial oil potential under the BP [5] standard and that are known to influence physical properties and fragrance.

2.5. Data Analysis

Determination of the best proxy estimator for BM was carried out by exploratory linear (FIT directive) and non-linear model fitting (FITCURVE directive) using regression and linear fitting functionality in Genstat 19 (VSN International, Hemel Hempstead, UK). The relationships between BM and both untransformed and log-transformed explanatory variates (HT, CD, CW, CVI) were considered. Preliminary analysis showed that the best fits were achieved by linear regression and exponential curve fits with ROP fitted as an explanatory factor. Relationships among traits were visualised using the R software [23] library GGPLOT2 [24].

Linear Mixed Models of individual tree data for each trait were analysed as follows:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}\mathbf{u} + \mathbf{e} \quad (1)$$

where $\mathbf{y}$ is the vector of observations on the trait; $\mathbf{b}$ and $\mathbf{u}$ are vectors of fixed- and random-effect estimates, respectively; $\mathbf{X}$ and $\mathbf{Z}$ are incidence matrices for fixed and random model terms, respectively, and $\mathbf{e}$ is a vector of random residual effects. For the growth traits, vector $\mathbf{b}$ contained sub-vector estimates for fixed effects of replicate and subpopulation (provenance) effects, and $\mathbf{u}$ contained sub-vectors for the random effects of incomplete blocks (field rows and columns) and plots. Survival was analysed using a Generalised Linear Mixed Model as described in Equation (1), but assuming a binomial data distribution and a logit link function. Oil trait data (provenance bulks from four replicates) were analysed using a simplified form of Equation (1) incorporating fixed effects only, with vector **b** containing replicate and genetic effects. Significance of fixed effects was gauged using Wald *F* tests and approximate significance of differences between pairs of provenances gauged by comparing predicted means and their average standard errors of difference, with means more than twice the SED apart considered to be significantly different. All analyses were implemented in Genstat.

3. Results

3.1. Survival and Growth

Survival was high for all provenances and averaged 94% overall with no significant difference among provenances ($p = 0.28$). Linear regression between BM and each of HT, CW and CVI traits (untransformed data) resulted in significant relationships ($p < 0.001$) with 66.7%, 77.4% and 91.4% of variance accounted for, respectively. Inclusion of the region of provenance (ROP) factor was significant for CVI and CW ($p < 0.001$ and $p = 0.03$, respectively) although dropping these terms only reduced explained variance by around 2% in both cases. Analysis of log-transformed data gave slightly better relationships between BM and both HT (86% variance explained) and CW (78.7% variance explained). Similarly, an exponential curve fit involving the CW and BM data was superior to the straight-linear fit (85.5% versus 77.4% variance explained, respectively) between these variates. Fits for the best linear and curvilinear models for each trait are shown in Figure 2.

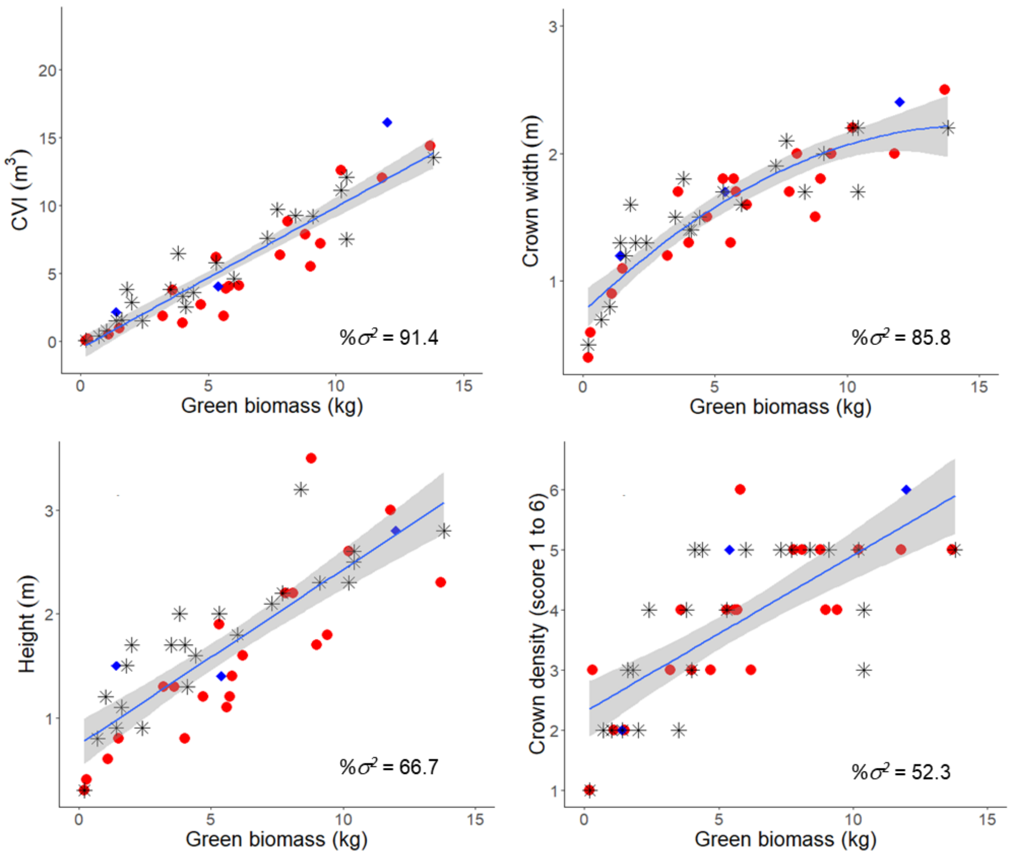

Figure 2. Fitted relationships between green biomass (BM) and explanatory variables including crown volume index (CVI), height (HT), crown width (CW) and crown density score (CW). Regions of provenance are represented by red circles (SA); asterisks (NSW) and blue diamonds (Victoria). The 95% confidence intervals for the fitted blue lines are indicated by the surrounding grey regions.

The relationship between BM and the subjectively scored CD trait was also significant ($p < 0.001$), explaining 52.3% of the variance.

Significant differences ($p < 0.001$) were found among ROP and provenances in all growth and oil quality variates. Provenances from NSW and Vic were generally not significantly different from each other but accumulated more biomass (estimated by CVI) than those from SA (Table 1). Buddigower and Blinman provenances performed particularly poorly (although the latter was only represented by seed sampled from two mother trees), while Wedderburn performed best.

3.2. Oil Characteristics

Significant differences ($p < 0.001$) were found among provenances for both oil yield and %cineole. Generally, there was little within-provenance variation of oil yield and %cineole (distributions are shown in Figure 3; %CVp in Table 2) and oil yield was consistent among the NSW provenances (around 5%–6% w/w dry weight). The Victorian checklot (Wedderburn) was also within this range. Oil yields were generally much lower for the SA provenances (around 3%–4%), with the major exception of The Oaks. The latter provenance had an oil concentration 31% higher than the eastern provenances and 50% higher than other SA provenances. The Oaks also had the highest %cineole of any oil, while SA provenances Blinman, Mt Hack and Wilpena contained less than 70% cineole (below the threshold for medicinal oil). Significant differences among ROP and provenances were also detected for some minor oil constituents (see Supplementary Material Table S1 for a full table of oil analysis results).

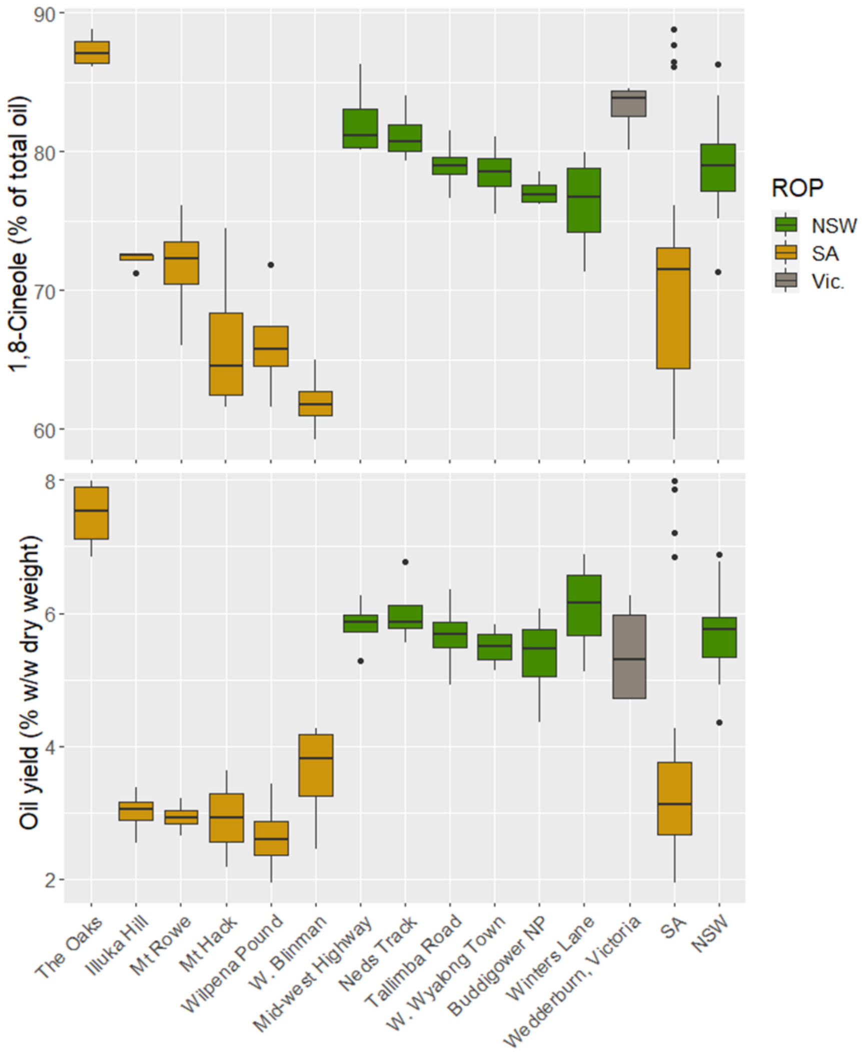

Figure 3. Box plots showing: *boxes* the mean andinterquartile range; *whiskers* the extent of the 0th and 100th percentiles, and; *dots* outlying values for oil yield and %cineole traits by provenance and ROP.

Table 2. Provenance means (best linear unbiased estimates), average standard errors of difference (SED) and coefficients of phenotypic variation (%CV_p) for CVI, oil yield and %cineole.

Provenance	CVI (m^3)		Oil Yield *w*/*w* (g/100 g Dry Leaf)		%Cineole	
	Mean	%CV_p	Mean	%CV_p	Mean	%CV_p
South Australia						
Illuka Hill	4.8	71.8	3.0	11.5	72.2	0.9
Mt Hack	4.5	86.6	2.9	21.4	66.3	8.8
Mt Rowe	4.4	101.1	2.9	7.9	71.7	5.9
The Oaks	4.7	73.3	7.5	7.3	87.3	1.4
W Blinman	3.4	105.1	3.6	23.0	61.9	3.8
Wilpena Pound	5.1	80.9	2.6	23.1	66.2	6.4
Victoria						
Wedderburn	6.7	72.3	5.4	14.8	83.1	2.5
New South Wales						
Buddigower NP	3.8	88.6	5.3	13.6	77.1	1.4
Mid-Western Highway	5.9	64.7	5.8	6.9	82.2	3.5
Neds Lane	5.2	64.3	6.0	8.8	81.2	2.5
Winters Lane	6.0	71.0	6.1	12.6	76.2	5.0
Talimba Rd	5.3	67.9	5.7	10.4	79.0	2.5
West Wyalong Town	5.6	61.6	5.5	5.5	78.4	3.0
Average SED *	*0.47*		*0.42*		*1.9*	

* Where trait means differ by more than 2 × average SED, they can be considered significantly different.

4. Discussion

Previously untested provenances of *E. polybractea* from relatively dry provenances in SA demonstrated mixed potential in an industrial plantation trial at West Wyalong, NSW. While the survival of SA material was excellent, matching that of NSW and Vic provenances, the growth performance was generally inferior to those locally native to West Wyalong and to the Wedderburn, Victoria provenance. Oil yield of SA provenances was generally low, around 3%, but one, The Oaks, had the highest yield (7.5%) on a dry leaf-weight basis of any provenance. Moreover, this provenance had the highest %cineole composition. The average oil concentration of the eastern state provenances was 5.7%, which is consistent with published averages for the species [7]. The %cineole composition of some SA provenances was slightly below the threshold for medicinal-grade oils, but still well above many other eucalypt species. The implication is that selection for oil traits from SA material should be targeted to the better provenances, though high-intensity selection for vigour would also be required. Testing of more families from The Oaks provenance and further exploration of still-untested provenances in South Australia is certainly warranted.

The relationship between green, aboveground biomass (BM) and measured growth traits (HT, CW) was strong, particularly when the measured traits were combined into a CVI in the first coppice rotation of this trial. This finding echoes those of other studies of mallee eucalypts that have made use of CVI, e.g., [25,26]. A difference between this study and others is that our CVI involved only a single measure of width, rather than two orthogonal crown measures: the decision to take only one measure was partly driven by the practical difficulty of identifying crown width in adjacent trees once crown closure has occurred. Taking a single measure is quicker, allowing the assessment of more trees per unit time, and it is notable that the single width (CW) measure explained 86% of the green biomass variance. The crown density score (CD), which was assessed qualitatively, was also a reasonably effective estimator of BM (54% variance explained), though markedly inferior to CVI (91%). Rapid biomass assessment techniques are potentially useful for mass screening of trees, allowing for higher selection intensity, leading to increased genetic gain. To this end, aerial assessment of crown width using unmanned aerial vehicle (UAV) technology, e.g., [27,28] might significantly increase the efficiency of screening of trials such as this, especially as the CW parameter, which can readily be obtained from an aerial view, is an effective estimator of biomass.

Climate change is expected to impact southern Australia including the current and target *E. polybractea* plantation zones in NSW, Victoria and Western Australia. There is very high confidence in continued, substantial increases in projected temperatures, and high confidence that cool season (winter and spring) rainfall will decrease, driven by further increases in greenhouse gas concentrations [19]. Testing and selection of *E. polybractea* from more xeric environments is therefore a good strategy to provide resilience to hotter and drier climates in the current planting zones. Another potential application for the SA provenances of *E. polybractea* is planting in drier sections of the Western Australia wheatbelt or further west in NSW. *Eucalyptus polybractea*, based mainly on NSW selections, is currently planted in the relatively high rainfall part of the WA wheatbelt with *E. lissophloia* being used on drier sites [26]. Testing the SA material on these sites would not only provide an indication of adaptation there, but also serve as an analogue of the climate futures that the plantations might experience in the current plantation zones in both Western Australia and the eastern States. The species would likely be suitable to other parts of the world with Mediterranean or dry temperate climates, although it has not been widely tested outside of Australia.

Although a considerable number of Australia's > 600 *Eucalyptus* species have been widely tested in Australia and overseas, much of the testing has been focused on fast-growing species and provenances endemic to, and suitable for planting in, high rainfall environments. Plantation forestry is likely to experience a shift towards more climatically and edaphically challenging sites [20], and be called upon for goods such as biomass energy [29], biofuels [30], chemical constituents and carbon sequestration, in addition to traditional timber and fibre products. Testing of previously unexplored genetic material including novel species from more xeric environments, and as demonstrated here, novel provenances of species that are already under domestication, will be an important element of industrial forest crop development.

5. Conclusions

This paper presents a first-time comparison of the growth and foliar oils of SA provenances of *E. polybractea* with seedlots from the main centres of production of the 1,8-cineole oil type near West Wyalong, NSW and Wedderburn, Vic in a trial at West Wyalong. The main finding was that although survival was generally excellent, most of the SA sources were slow growing, had low foliar oil concentrations and some yielded oils below the standard for 1,8-cineole composition. The exception was The Oaks, SA provenance, that, although slower growing than most eastern provenances, had a high oil concentration containing a high proportion of 1,8-cineole. Further testing of families of this provenance and other currently untested provenances from South Australia is now warranted. The inclusion of selections from the hotter, drier part of the *E. polybractea* natural range is a recommended strategy for increasing the resilience of the breed. This may provide insurance against projected climate change and also allow the introduction of the species into hotter and drier climates for uses including *Eucalyptus* oil production, biomass cropping and carbon sequestration.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f13071109/s1, Table S1: *Eucalyptus polybractea* oil composition (expressed as % of total oil) in the *E. polybractea* trial established at West Wyalong, New South Wales (NSW) in 2016.

Author Contributions: All authors contributed to the study conception and design. Data Collection and preliminary processing was carried out by D.S. and J.D. Oil distillations were carried out by D.S. and J.D. Statistical analysis was performed by D.B. The first draft of the manuscript was written by D.B. and J.D. and all authors commented on this and subsequent versions of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors do not have competing interest to disclose.

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