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Tropical red gums — a source of 1,8-cineole-rich *Eucalyptus* oil

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Application. Screening of tropical provenances of *E. camaldulensis* and *E. tereticornis* showed that some of the most widely-planted and fast-growing sources have the potential to provide 1,8-cineole-rich *Eucalyptus* oil as an additional plantation product. Individual trees amongst some provenances produce much more oil of higher quality than the average trees of the population. There is potential to significantly improve the economics of oil production from the tropical red gums by selection and breeding for these traits.

Abstract. Tropical provenances of *Eucalyptus camaldulensis* Dehnh. and *E. tereticornis* Smith were studied, in their natural habitat in Australia and in a 3.75-year-old progeny trial in Zimbabwe, for their potential to produce medicinal-grade essential oils. Substantial inter- and intra-specific variation in the contents of five prominent monoterpenes, 1,8-cineole, α-pinene, β-pinene, limonene and p-cymene was found. Plantations of *E. camaldulensis* established in the wet/dry tropics using seed from Petford in northern Queensland offer immediate potential for oil production. Other widely-planted Queensland provenances with oil potential are Gilbert River Bridge *E. camaldulensis* and, after some genetic improvement, *E. tereticornis* from Morehead and Kennedy Rivers. Select individual trees at Petford provide oil of enhanced quality, at about double the yield of 'average' trees.

Introduction

Eucalyptus oil

Eucalypt leaves contain oil-bearing glands that if disturbed give off aromatic odours sometimes characteristic of individual species or even of local populations within species. These essential oils are complex mixtures of volatile organic compounds of possible value in industry although their

natural roles are not known. *Eucalyptus* oil is obtained commercially in a relatively simple process involving steam distillation of the leaves.

To be of interest for commercial development, a species should normally contain abundant oil (at least 1.5% oil on fresh foliage, equal to approximately 3% on dry weight), in which one or two important chemicals predominate. Oil-producing species are commonly grouped into three broad categories depending on the type of oil they produce. These types are the medicinal, industrial and perfumery/flavouring oils of which only medicinal oils will be further discussed here. Medicinal oils contain not less than 70% of 1,8-cineole (1,3,3-trimethyl-2-oxabicyclo [2.2.2.] octane, $C_{10}H_{18}O$), the principal therapeutic agent, and should be practically free of the possibly toxic α- and β-phellandrenes (Anon. 1988). Crude, rectified or blended cineole-rich oils and pure 1,8-cineole are used in preparations for the relief of cold and influenza symptoms (e.g. inhalants, chest rubs, lozenges). Other uses include liniments, gargles, dentrifices, soaps, disinfectants and as a solvent for spot and stain removal (Penfold and Willis 1961; Abbott 1977). A major future use of cineole could be as a component of petrol-ethanol fuel blends (Ammon et al. 1986).

The yield of oil from the leaves and its chemical constitution varies greatly, not only between and within species but also according to the type of leaf harvested and its physiological age, how the leaf is handled prior to distillation and on environmental conditions (Penfold and Willis 1961). Of the more than 600 species of *Eucalyptus* probably less than 20 have ever been exploited commercially for oil production (Lassak 1988). Only a handful of species, marginal in the quality of their oils, are suitably adapted for growth in the lowland tropics (e.g. *E. exserta, E. tereticornis*). This is a problem for tropical countries wishing to grow well-adapted, fast-growing species to produce *Eucalyptus* oil.

Importance of the tropical red gums

Eucalyptus camaldulensis and *E. tereticornis*, two closely-related species of the red gum group of eucalypts, are extremely important forest crop plants. As exotics both species are used for a wide range of purposes including shade and shelter, agroforestry and industrial wood production. The primary wood products are posts, poles, firewood, charcoal and pulp. Field trials throughout the wet/dry tropics invariably highlight the superior growth characteristics of red gum provenances from a region of northern Queensland between 14° and 18 °S latitude and 144° and 145 °E longitude (Fig. 1). This region contains many well-known provenances (Table 1) including Petford *E. camaldulensis* that is conspicuous in consistently having faster growth rate than other provenances in numerous trials.

Consequently Petford has become one of the most important seed sources for plantations throughout the tropics (Midgley et al. 1990).

Where the distributions of *E. camaldulensis* and *E. tereticornis* make contact, as in eastern Victoria and southern, central and northern Queensland, there are zones of apparent introgression (Turnbull 1973, 1975; Turnbull and Griffin 1986). There is speculation that stands near Petford are of an intermediate type, grading from pure *E. tereticornis* at the headwaters of watercourses feeding into the Walsh River system, to pure *E. camaldulensis* on the Walsh River west of Chillagoe (Fig. 1). Similar stands of an intermediate type occur on Cape York Peninsula north of Lakeland. This region includes the well-known and widely-utilised Laura, Kennedy River and Morehead River provenances. Traditionally botanists have named these populations *E. tereticornis* because of operculum length and shape.

The present study of the oils of the tropical red gums has its origins in a reforestation program using fast-growing red gums to replace degraded forest in the central Terai of Nepal (White 1986). White established several red gum provenance trials and observed that a number of provenances had characteristic oils. Petford provenance was especially distinctive because of its more pungent odour, characteristic of cineole-rich oils. Subsequent tests by the Nepal Department of Medicinal Plants confirmed that some provenances produced oils rich in cineole (S.B. Malla, pers. comm.).

In 1987 we began a series of investigations centred on natural stands of *E. camaldulensis* and *E. tereticornis* in northern Queensland. The aims were to determine

- the extent of inter- and intra-specific variation in chemical composition of the oils of both species in northern Australia,
- the potential of selected provenances to provide a source of cineole-rich oil, and
- if chemical analysis of the oils would aid in the botanical classification of 'intermediate' populations.

Experiments, objectives and methods

Experiment 1: Survey, northeastern Queensland

The objective of this experiment was to compare the oil composition of populations of *E. camaldulensis* and *E. tereticornis* of authentic botanical classification and Petford provenance to find:

— if there were qualitative differences useful for distinguishing the pure species from each other and from a population where introgression of the two species is suspected;
— if any of the oils met the standards for medicinal use.

Collection of plant material and extraction and analysis of volatile oils
The sites chosen were Einasleigh River, Walkamin and Emu Creek near Petford (Sites, 12, 40 and 5 in Fig. 1 and Table 1). In the field, approximately 500 g of fresh leaves and terminal branchlets were sampled from each of 5 widely-spaced mature trees selected at random and a bulk sample harvested from 5 other trees at each of the three sites. Care was taken in this and subsequent trials to take only leaves that were estimated to be at a similar age and stage of maturity in each tree. These were obtained from within the crown, below the immature leaves but above leaves showing signs of senescence. Samples were placed in calico bags and stored over ice for a few days prior to air despatch to Sydney where the leaves were steam-distilled with cohobation (Lassak 1979) for 8 hours to yield colourless to golden-yellow oils. Chemical analysis of the oils was by gas chromatography and mass spectrometry (see Table 2).

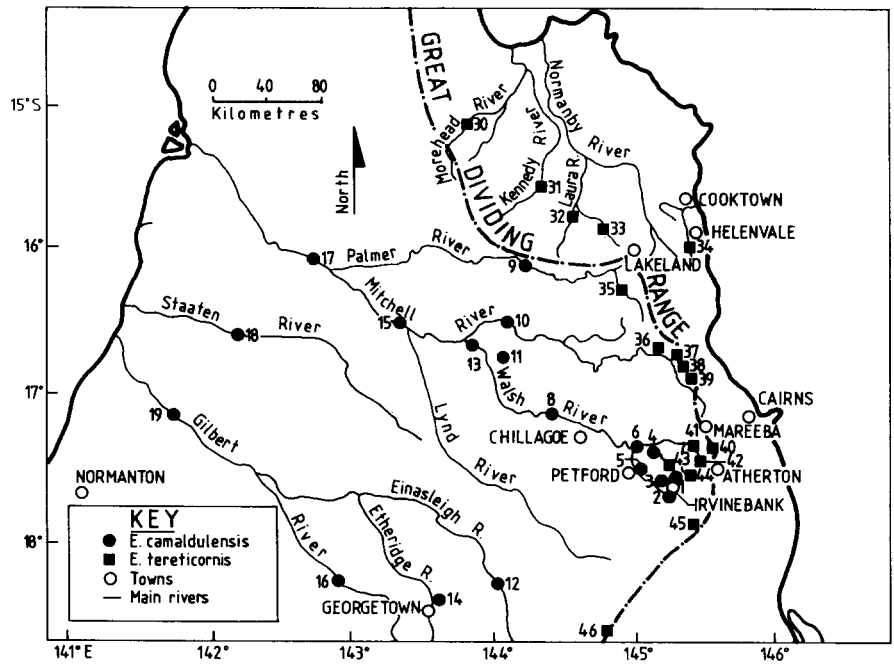
Experiment 2: Survey, northern Australia

The purpose of this experiment was to survey natural populations of *E. camaldulensis* and *E. tereticornis* in northern Australia at a broad scale to find if there were:

— geographic variations in yield (w/w fresh weight) of the major oil constituents, the monoterpenenes — 1,8-cineole, α-pinene, β-pinene and limonene, within and between species; and
— cineole-rich provenances in addition to Petford that might merit further investigation as cineole producers.

Collection of plant material and extraction and analysis of volatile oils
In January, April and June 1987 leaf samples were collected from 32 natural populations of *E. camaldulensis* and *E. tereticornis* including several provenances considered to be intermediate in character to core populations (see Fig. 1 and Table 1). At each collecting site, about twelve mature leaves were taken from the crowns of each of five widely-spaced trees. They were placed in plastic bags, kept separate by individual trees, and held on ice to prevent sweating until the field parties returned to the laboratory, often a period of three to four weeks. In the laboratory, 100

Map 1



Map 2

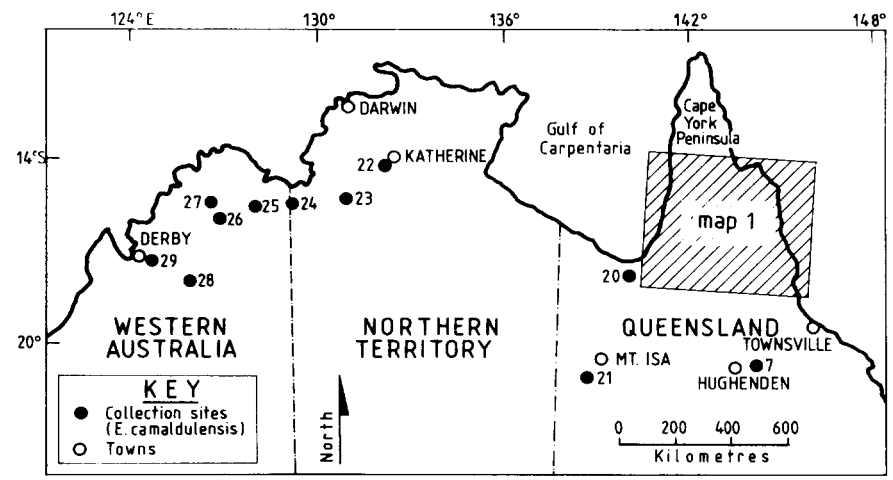


Fig. 1. Location of provenances of *E. camaldulensis* (Maps 1, 2) and *E. tereticornis* (Map 1) sampled in Australia and Zimbabwe for analysis of leaf oils.

Table 1. Origin of provenances of *E. camaldulensis* and *E. tereticornis* sampled in Australia and Zimbabwe for analysis of leaf oils.

Provenance and Map No	Provenance name	Lat (°S)	Long (°E)	Alt (m)	Leaf collection N. Aust.	Zimbabwe
E. camaldulensis (1—29)						
1	Hales Siding	17°22′	145°13′	780	X	
2	Headwaters Emu Creek	17°29′	145°13′	860	X	
3	NW Irvinebank	17°24′	145°09′	710	X	X
4	Eureka Creek	17°11′	145°03′	460	X	
5	Emu Creek, Petford	17°20′	144°57′	490	X	X
6	Flat Rock Pool	17°10′	144°56′	420	X	
7	Bullock Creek	20°49′	144°48′	451		X
8	NW Chillagoe	16°59′	144°18′	240		X
9	Palmerville	16°00′	144°05′	400	X	
10	"Mt Mulgrave"	16°23′	144°02′	160	X	
11	"Wrotham Park"	16°40′	144°02′	152		X
12	Einasleigh River	18°11′	144°01′	390	X	
13	Walsh River	16°33′	143°47′	140	X	
14	Etheridge River	18°16′	143°33′	340	X	
15	Lynd River Junction	16°28′	143°18′	170	X	
16	Gilbert River Bridge	18°12′	142°52′	150		X
17	Healeys Yard	16°01′	142°34′	150	X	
18	Staaten River	16°32′	142°03′	150	X	
19	Gilbert River	17°11′	141°45′	75	X	
20	SW Normanton	18°07′	140°20′	50		X
21	SW Mt Isa	21°26′	139°06′	410		X
22	Katherine	14°29′	132°15′	95	X	X
23	Victoria River	15°35′	131°02′	35		X
24	Cockatoo Creek	15°38′	129°01′	50		X
25	Pentecost River	15°48′	127°43′	60		X
26	Gibb River	16°08′	126°30′	430	X	
27	Drysdale River	15°41′	126°22′	400	X	
28	Fitzroy Crossing	18°06′	125°42′	110		X
29	May River	17°25′	124°07′	45		X
E. tereticornis (30—46)						
30	Morehead River	15°02′	143°40′	50	X	X
31	Kennedy River	15°26′	144°10′	80	X	X
32	Laura River	15°33′	144°27′	100		X
33	Ruth Creek	15°43′	144°37′	130	X	
34	Helenvale	15°47′	145°13′	170		X
35	Palmer River	16°07′	144°47′	400	X	X
36	Holmes Creek	16°32′	145°07′	370	X	X
37	Leichhardt Creek	16°35′	145°12′	370	X	
38	Luster Creek	16°39′	145°14′	390	X	
39	Spear Creek	16°39′	145°19′	370	X	X

Table 1 (Continued)

Provenance and Map No	Provenance name	Lat (°S)	Long (°E)	Alt (m)	Leaf collection N. Aust.	Zimbabwe
40	Walkamin	17°09′	145°26′	630	X	
41	"Springmount"	17°11′	145°20′	540	X	
42	Baldy Mt	17°16′	145°26′	930	X	
43	Stannary Hills	17°19′	145°13′	750	X	
44	Watsonville	17°22′	145°19′	790	X	
45	Archer Creek	17°38′	145°25′	80	X	
46	SW Mt Garnet	18°24′	144°45′	890		X

ml of ethanol were weighed accurately into sample bottles, 5 g of leaf were sampled to represent each provenance (approx. one leaf (1 g) per individual tree) and placed in the same bottles. At least 2 weeks were allowed for full extraction (Ammon et al. 1985); chemical analysis was by gas chromatography (see Table 2).

Experiment 3: Assessment of progeny test, Zimbabwe

This experiment had goals similar to Experiment 2 but with two important differences:

— it was based on replicated progeny/provenance trials (c.f. natural stands) which allowed detailed statistical analysis of the data;
— the provenance trials were planted in Zimbabwe on a site representative of the areas where the tropical red gums grow best and the trees were of a young age (3.75 years) reflecting more closely the conditions under which these species might be harvested for their oils.

Collection of plant material and extraction and analysis of volatile oils
This experiment involved the sub-sampling of 14 provenances of *E. camaldulensis* and 8 provenances of *E. tereticornis* from two extensive progeny/provenance trials of these species (total area of 15.3 ha) established in January 1985 at Mtao, in Zimbabwe. Mtao (lat. 19°20′S, long. 31°29′E) is about 170 km south of Harare, on deep aeolian Kalahari sands at an elevation of 1480 m and with 690 mm mean annual rainfall (Matheson and Mullin 1987) concentrated in 5 months of the year, November to March.

The trials used seed collected in tropical Australia and local and other African sources as controls. They were established as randomised incom-

Table 2. Summary of the principal methods used in each experiment for analysis of oils.

Exp No.	Method	Machinery /detector	Column	Carrier gas and flow rate	Conditions	Identification and quantification of compounds
1.	glc	Shimadzu GC6AMP /FID	85 m × 0.5 mm SP1000, SCOT	He at 4 ml/min	65—225°C at 3°C/min	Comparison of retention times to those of known compounds and coinjection. Yields obtained by weighing oil following steam distillation of fresh leaf.
	glc/ms	as above, linked to AE1 MS 12			70 eV ionising voltage and accelerating voltage with the ion source at 200 °C	Mass spectra consistent with either those of known compounds or published spectra.
2.	glc	Shimadzu GC9A /FID	4 m × 3.0 mm i.d. glass tube packed with 13% OV 330 coated on 80—100 mesh Chromosorb W-HP	N_2 at 30 ml/min	60—260 °C at 10 °C/min	By retention time and peak area in comparison with standard solution containing the pure compounds in known concentration.
3 & 4	glc	Varian 3400 /FID	20 m × 0.32 mm bonded FSOT coated with Superox FA	N_2 at 1.5 ml/min	80—220 °C at 20 °C/min	By retention time and response coefficients relative to internal standard, n-tetradecane.

glc — gas liquid chromatography
ms — mass spectrometry
FID — flame ionisation detector

plete blocks with individual family row plots of 10 trees grouped by provenance in each block (S. Bleakley, pers. comm.). Four of the *E. tereticornis* provenances were represented by bulked seedlots only. There were five replicates (blocks) in each trial although only two replicates (Blocks 1 and 2) were sampled in this study.

In October 1988, when the trees were 3.75 years from planting, leaves were collected in blocks 1 and 2 of each experiment from 2 trees of each of 3 families in each of 14 provenances of *E. camaldulensis* and 4 provenances of *E. tereticornis*. Families and trees were selected at random and provenances chosen to cover a broad geographic range. Four bulked *E. tereticornis* seedlots were also sampled and included in the analysis. The height and breast height diameter of each tree sampled was recorded. A total of 264 trees were sampled.

About a dozen mature leaves were collected from each tree at approximately two-thirds of tree height. They were placed in plastic bags and refrigerated for about 10 days prior to being airfreighted to Australia. From each tree 3 g of leaf material was carefully weighed and placed in sample bottles containing 50 mls of ethanol.

Chemical analysis was by gas chromatography (see Table 2). An internal standard, n-tetradecane, was added to each sample and quantification of 1,8-cineole, α-pinene, β-pinene, limonene and p-cymene used response coefficients determined for each of these compounds relative to the internal standard. A rough approximation of total yield of monoterpene compounds was obtained by assuming that other peaks in the early part of the chromatogram had a response coefficient of 1 relative to n-tetradecane and adding their mass to the total of the major monoterpenes.

Statistical analysis

The frequency distribution of the oil yield data among trees was highly non-normal and strongly skewed towards the lower values. To cope with this non-normality, a general linear model using a logarithmic link function relating the mean of the given observation to its linear predictor (Baker and Nelder 1978) was employed. The model was $Y_{ijkl} = u + R_i + P_j + F_{jk} + W_{ijkl}$ where u is the overall mean, R_i is the effect of the ith replicate, P_j is the effect of the jth provenance, F_{jk} is the effect of the kth family in the jth provenance and W_{ijkl} is the random error associated with the lth tree in the kth family in the jth provenance and ith replicate.

Experiment 4: Survey, Petford

The objectives of Experiment 4 were:

— to determine the extent and distribution of variation in cineole content throughout the Emu Creek (Petford region) catchment; and
— to identify both high and low oil-yielding genotypes for inclusion in later experiments to determine genetic parameters.

Collection of plant material and extraction and analysis of volatile oils
In the January of 1988 and 1989 and again in May and June of 1989, leaves were collected from 370 individual trees over some 40 km along Emu Creek and its tributaries. This area includes populations commonly referred to as Petford or Irvinebank provenances due to their relative proximity to these townships.

At least two branches were removed from each tree and about equal numbers of mature leaves were plucked from each to make up a sample of about 12 leaves per tree. These were placed in plastic bags and refrigerated for varying periods up to a maximum of about 14 days prior to despatch to the laboratory. The laboratory methodology was identical to that described for Experiment 3.

We were concerned that oil content might depend on season (month) of sampling (viz. Experiment 2) and we also wished to test if aspect had any control over oil content. Hence two follow-up harvests of leaves were made from a set of 10 trees, selected from the January 1988 analysis to cover a range of oil patterns found in the population. The first was in July 1988 when, using the normal method, one bulked leaf sample per tree was obtained and the second in December 1988 when samples were obtained from three points around the crown (N, SE, SW). Chemical analysis was by gas chromatography (see Table 2).

Results and discussion

Experiment 1: Survey, northeastern Queensland

The summary of results in Table 3 indicates very minor qualitative differences between the populations chosen to represent core *E. camaldulensis* and *E. tereticornis* and one reputedly of intermediate character in northern Queensland. Under more intensive sampling these minor differences may disappear altogether. There appears to be little scope, therefore, to use oil fingerprinting as a tool in elucidating taxonomic problems between these species throughout this region or as a basis for inferences about the possibility of introgression of *E. tereticornis* in the Petford population. Clearly quantitative variation in the major oil components is of greater interest. Although there is considerable overlap between species there is

very substantial quantitative variation within and between provenances especially in yields of cineole.

This experiment shows that, amongst the Petford and Einasleigh River populations of *E. camaldulensis*, there are individual trees that give an excellent 1,8-cineole-rich oil. In addition, the crude extract from Petford may qualify directly as a medicinal oil as it is high in cineole, up to 84%, with good odour and negligible phellandrene. These trees yield reasonable quantities of oil, about 1—2% (fresh weight). It is important to note, however, that oil yields obtained under the cohobation conditions used in these analyses may be about one-third greater than those obtained under field conditions. Oil yields of this magnitude are below the threshold for economic production in Australia but may be acceptable when oil is harvested as a secondary product to wood in countries where labour is inexpensive.

This experiment led to the recognition of a distinct chemotype in the population at Petford (see Table 3), characterised by low cineole content (about 10%) and high proportions of sesquiterpenoids. This chemical form is present in the population at a frequency of about 1 in 10. Assuming it is a genetically stable form, it has potential to adversely affect oil quality. This cineole-poor form should be avoided in any selection program aimed at improving the quality of *E. camaldulensis* oils for medicinal purposes.

Most previous assessments of the oils of *E. camaldulensis* have labelled them of little or no value for pharmaceutical purposes due to low cineole and high α-phellandrene contents and low yields (e.g. Penfold and Willis 1961; Rao et al. 1970; Senanayake et al. 1983; Ndou et al. 1986). The identification in this study of cineole-rich populations of *E. camaldulensis* with commercial potential is in stark contrast to the earlier studies. It highlights the importance of chemical forms in this genus and the need for extensive sampling before categorising a whole species as to its oil type(s).

Experiment 2: Survey, northern Australia

The results of the analyses and quantification of α-pinene, β-pinene, limonene and 1,8-cineole in the bulk ethanol extracts of 18 *E. camaldulensis* and 14 *E. tereticornis* provenances are summarised in Figs. 2 and 3. To assist in interpretation, the *E. camaldulensis* provenances are plotted according to longitude in an east to west direction, starting at the headwaters of the Emu Creek system in Queensland and concluding at the Drysdale River in the northwest of Western Australia. *E. tereticornis* is graphed in order of increasing latitude in Queensland from Morehead River in the north to Archer Creek, southwest of Ravenshoe.

Table 3. Total yield of oil, the main components and their range in composition in the oil amongst trees and between provenances of *E. camaldulensis* and *E. tereticornis* from northern Queensland, Australia. Compounds are listed in order of their increasing retention time on a polar glc column (FFAP). The oil analysis of the low-cineole, high-sesquiterpene chemotype amongst the Petford provenance is given for comparison with regular oil types of the same provenance.

Compound	Type[1]	*E. camaldulensis* Einasleigh River	*E. camaldulensis* Petford		*E. tereticornis* Walkamin
			% in the oil		
		% in the oil	Regular	Chemotype	% in the oil
α-pinene	m h	2.1 –23.4	1.6 – 6.8	2.2 – 4.07	0.78–27.0
β-pinene	m h	0.0 –13.0	0.05– 6.4	1.72–18.7	0.04–18.0
α-phellandrene	m h	0.0 – 7.9	0.0 – 0.10	0.01– 0.05	0.0 – 0.94
Limonene	m h	2.0 –10.1	3.6 –12.6	1.72– 2.27	3.5 –18.7
1,8-cineole	m	19.3 –84.0	38.4 –83.8	4.7 –15.63	0.11–32.9
γ-terpinene	m h	0.05– 6.2	0.02–17.8	0.04– 0.33	0.06– 1.5
p-cymene	m h	0.39–33.8	0.13– 5.7	0.03– 0.51	0.40– 5.7
Terpinolene	m h	0.01– 0.28	0.32– 1.6	0.05– 0.90	0.04– 0.44
Campholenic aldehyde	m al				0.0 – 1.9
Pinocarvone	m k	0.0 – 0.76	0.0 – 0.04	0.0 – 1.71	0.0 – 0.22
Terpinen-4-ol	m a	0.10– 2.5	0.13– 2.4	0.09– 0.13	0.12– 0.73
β-caryophyllene	s h	0.0 – 0.10	0.0 – 0.03	0.01– 0.20	0.02– 1.9
Aromadendrene	s h	0.72– 8.0	0.79– 3.5	0.20– 1.38	0.23– 7.9
Trans-isopinocarveol	m a	0.0 – 4.9	0.0 – 0.35		0.0 – 1.9

Alloaromadendrene	s h	0.0 — 0.83	0.27— 0.79	0.0 — 0.85	0.30— 1.3
α-terpineol	m a	0.81— 2.1	1.3 — 2.6	0.72— 1.43	0.30— 5.8
Viridiflorene	s h	0.01— 0.08	0.07— 0.56	0.12— 1.71	0.03— 0.86
Germacrene	s h			0.0 — 1.0	
Bicyclogermacrene	s h			0.40—14.3	0.0 —16.9
Myrtenol	m a	0.0 — 0.43			0.32— 1.0
Sabinol	m a	0.0 — 1.1			
Globulol	s a	2.6 —12.9	3.1 — 6.7	2.0 — 9.7	3.2 —15.5
Viridiflorol	s a	0.16— 1.1	0.29— 0.70	1.0 — 4.8	0.86— 8.4
Spathulenol	s a	0.0 — 0.21		0.0 —25.93	0.0 —12.9
γ-eudesmol	s a	0.0 — 1.3			0.0 — 8.6
α-eudesmol	s a	0.0 — 2.1			0.0 — 9.8
β-eudesmol	s a	0.0 — 3.0			0.0 —14.6
Other C_{10} compounds in trace amounts	m	26	17	5	25
Other C_{15} compounds in trace amounts	s	18	15	34	20
Oil yield (g/100 g of fresh leaf) Mean ± SD	1.07 ± 0.35		1.65 ± 0.37		1.17 ± 0.25

[t] m is monoterpene (C_{10}); *s* is sesquiterpene (C_{15}); *a* is alcohol; *al* is aldehyde; *h* is hydrocarbon; *k* is ketone.

The sampling method, using bulked rather than individual tree analysis, and the combining of results of different field trips in the absence of a detailed knowledge of the effects of sampling time on oil patterns, constrains the inferences that can be drawn from the data. However, a number of trends are apparent. There are substantial differences in oil contents between provenances within species. Greater oil contents are found in the eastern part of the occurrence of *E. camaldulensis* in northern Australia while provenances located towards the Gulf of Carpentaria, Katherine in the Northern Territory and provenances in northwestern Western Australia have little oil. Provenances with a cineole content of 1% or more of the weight of fresh leaf were centered on the Petford region, along Emu Creek (prov. 2 & 5) and a tributary, Gibbs Creek (prov. 3) and on the Walsh River at Flat Rock Pool (prov. 6). Only the Morehead River provenance of *E. tereticornis* (prov. 30) had a cineole content that was greater than 1% of the weight of fresh leaf.

These data at first suggested to us that the relationship between yields of cineole and β-pinene might assist in seeking taxonomic affinities of anomalous provenances. Subsequent work, however, showed great heterogeneity in oil composition even within core populations, thus negating the value of this relationship for elucidating taxonomic problems in the region.

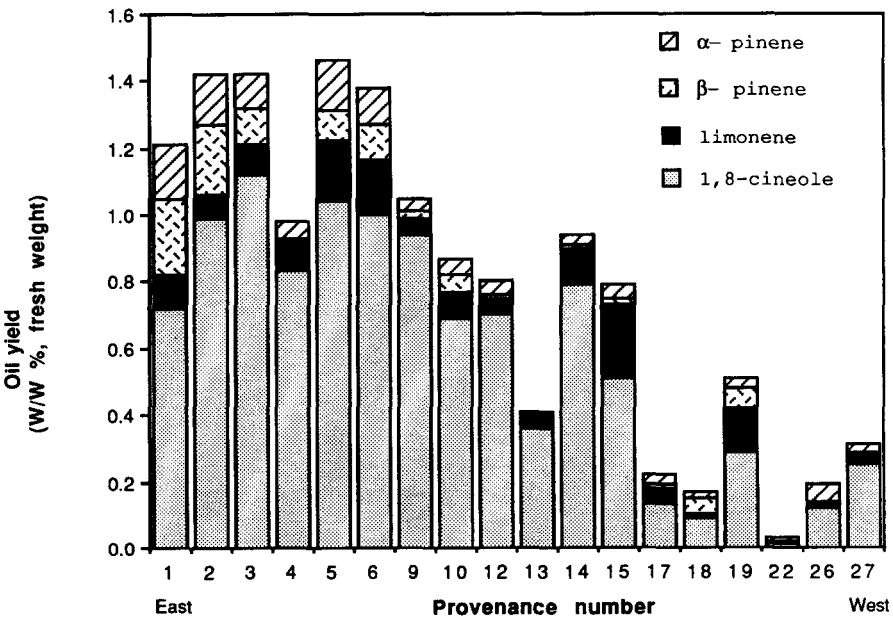


Fig. 2. Yield of 1,8-cineole, α-pinene, β-pinene and limonene in 18 *E. camaldulensis* populations as sampled in the wild in northern Australia.

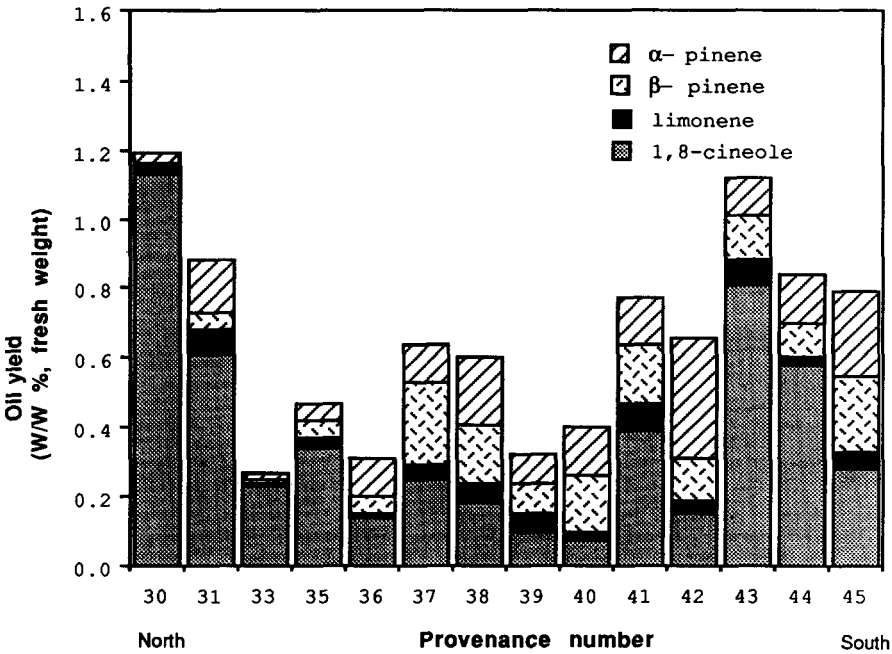


Fig. 3. Yield of 1,8-cineole, α-pinene, β-pinene and limonene in 14 *E. tereticornis* populations as sampled in the wild in northern Queensland.

This survey indicated that the rapidly-growing provenances of *E. camaldulensis* from the Petford region offered most scope as a source of commercial quantities of oil. There was still a need, however, to confirm this result using young progeny growing on a site representative of those where Petford provenance is utilised on a large scale.

Experiment 3: Assessment of progeny test, Zimbabwe

An outstanding and consistent feature of the Zimbabwe data was the very substantial within and between family heterogeneity in yield of the major monoterpenes. This observation indicates that the populations are highly variable in terms of their leaf oils. Despite great within-provenance variability, analysis of variance showed highly significant differences between populations in all oil traits assessed in *E. camaldulensis* and significant differences for most of them, including cineole, in *E. tereticornis*. Provenance means for yields of cineole and total monoterpenes and the standard error of the differences are given in Figs. 4 and 5.

Analysis of variance showed that there were significant differences for both height and diameter amongst the *E. camaldulensis* provenances but

in *E. tereticornis* only the height was significantly different. Provenance means for height are given in Fig. 6.

Provenances giving the best combination of high growth rate and yields

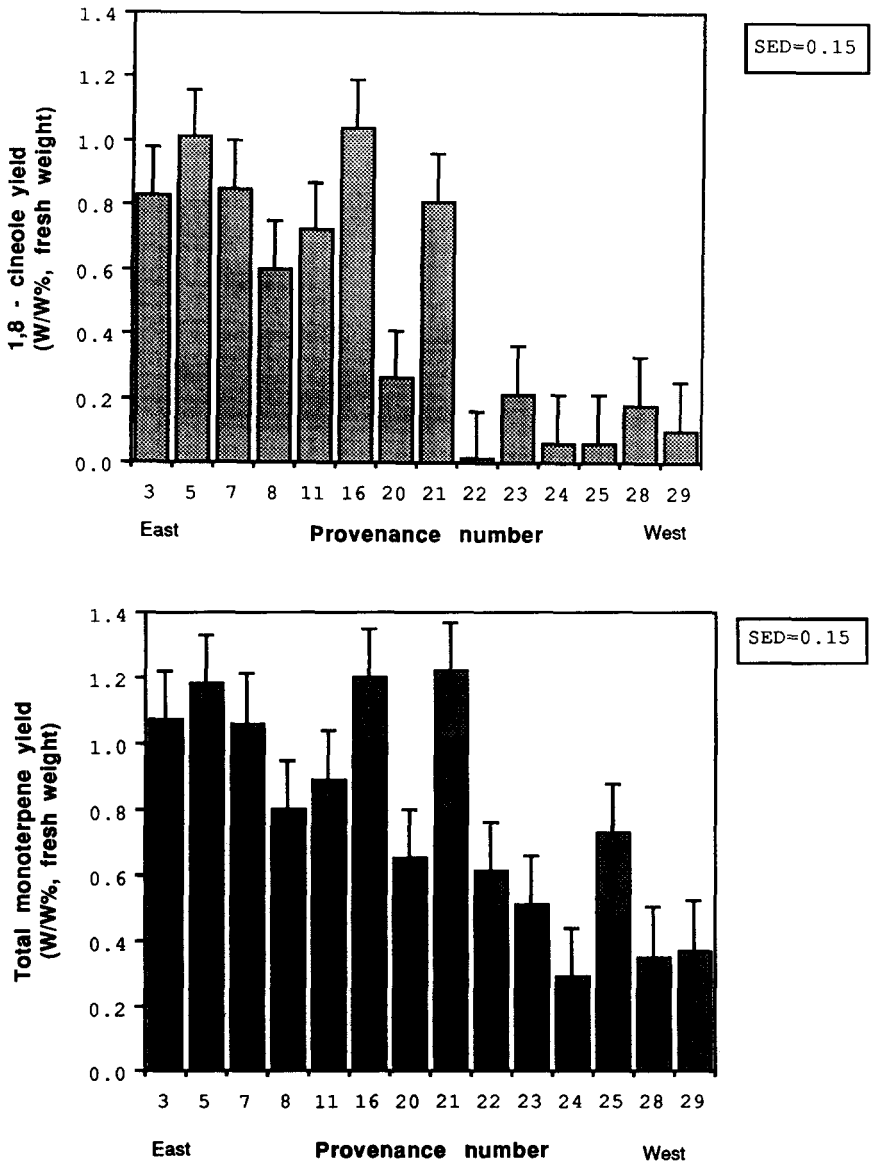


Fig. 4. Average yield of 1,8-cineole and estimate of total monoterpene content of the leaf oils of 14 provenances of *E. camaldulensis* from northern Australia growing in a trial in Zimbabwe. Trees were 3.75 yr when sampled.

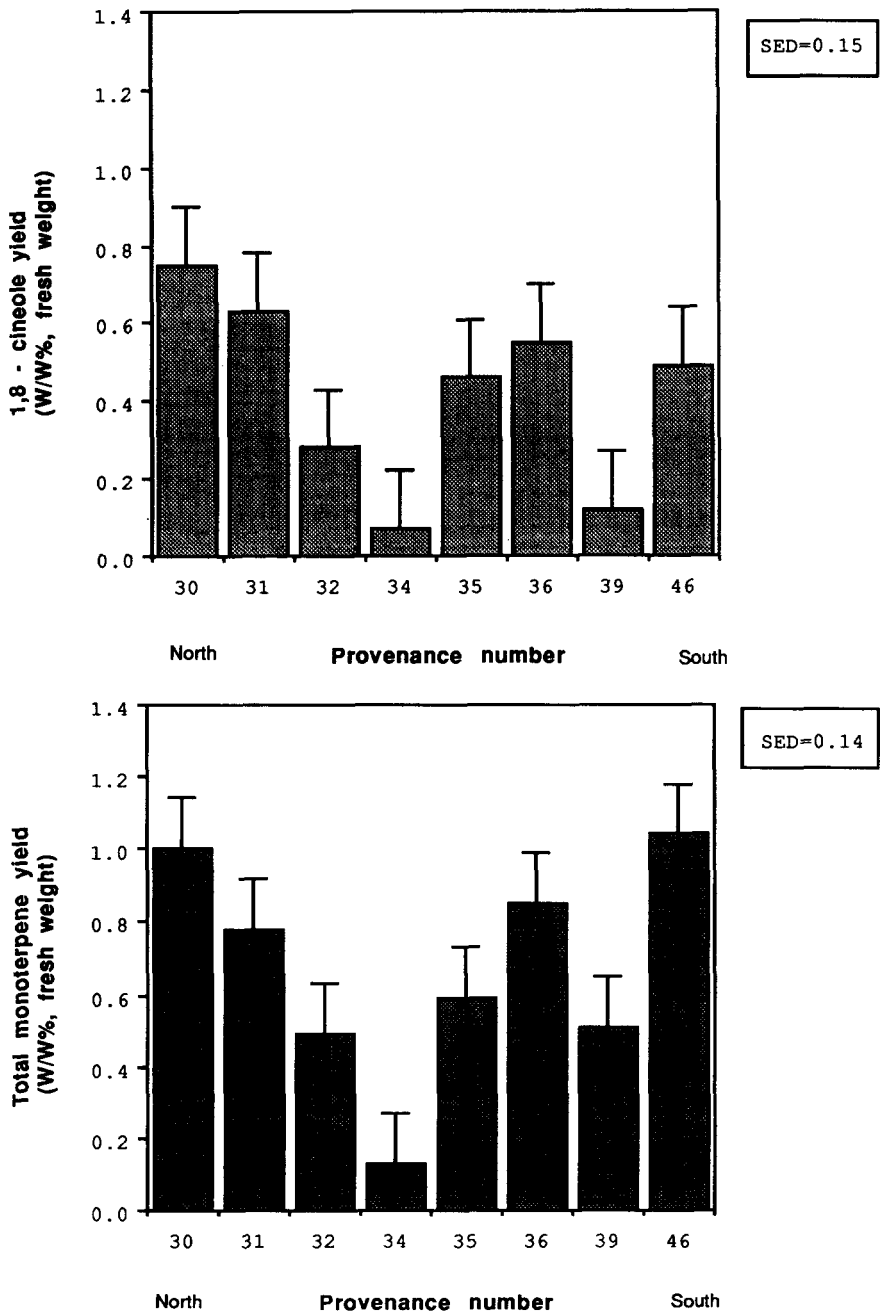


Fig. 5. Average yield of 1,8-cineole and estimate of total monoterpene content of the leaf oils of 8 provenances of *E. tereticornis* from northern Queensland growing in a trial in Zimbabwe. Trees were 3.75 yr when sampled.

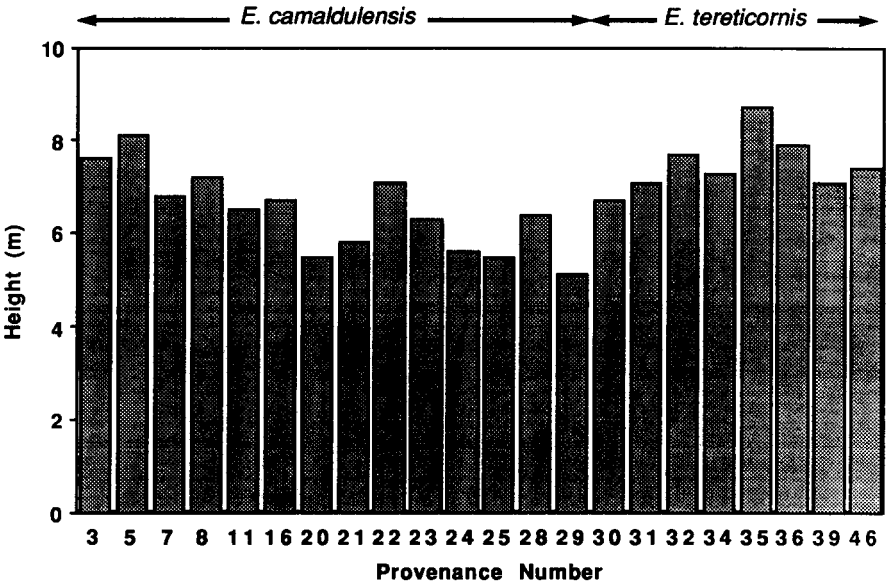


Fig. 6. Variation in mean height (m) by provenance of trees sampled for oil analysis in progeny trials of *E. camaldulensis* and *E. tereticornis* in Zimbabwe aged 3.75 years.

of 1,8-cineole that averaged greater than 1% of the weight of fresh leaf were Petford and Gilbert River Bridge. In addition, individual trees in a limited number of other provenances gave oil of a quality worthy of inclusion in a selection and breeding program should the tree or provenance in question grow well. Provenances showing potential in this category include Irvinebank and Wrotham Park *E. camaldulensis* and Morehead River and Kennedy River *E. tereticornis*. Within these higher-yielding provenances, no correlation was apparent between tree size and oil yield (w/w %, fresh weight). Thus there may be an opportunity to increase per hectare production by selecting and propagating provenances and individuals with both fast growth and high yield of oil.

As in Experiment 2, the easterly sources of *E. camaldulensis* and the northerly sources of *E. tereticornis* reveal most potential for utilisation and improvement of their oils. Provenances from the Gulf of Carpentaria (Normanton), Northern Territory and the northwest of Western Australia are generally low in overall yields. They also usually contain undesirable amounts of α-phellandrene, although this was not quantified in this study.

The similarity of results in Experiments 2 and 3 is notable given the contrast between the trees sampled (aged, slow-growing versus young, fast-growing) and their growing locality (natural versus exotic). The

reproducibility of the results indicates that natural populations might be reliably screened for their oil potential in fast-growing plantations and, as mature oil patterns appear to be exhibited at a young age (3.75 years or earlier), individual tree selection for superior oil characteristics might take place at an equally early age. This latter point is in agreement with the findings of Barton et al. (in press) who concluded that, for the mallee *E. kochii*, selection for oil production can be reliably carried out at three years of age.

Experiment 4: Survey, Petford

Oil composition varies considerably amongst the 370 trees screened in this experiment. The average yield (w/w % fresh weight) of cineole was 0.87% (S.D. ± 0.38%) with a range of 0 to 1.8%, and the average total yield of the major monoterpenes was 1.04% (S.D. ± 0.42%). Thirty-four trees, 9% of the sample, were of the low-cineole, high-sesquiterpene chemotype described in Experiment 1. In one instance, seven trees of the chemotype were grouped in the one small area suggesting that this may be a spatial clumping of relatives and that this distinctive oil type may be strongly inherited.

The 20 best trees for oil thus far identified (selection intensity of 1 in 19) contain an average of 85% cineole and yield (w/w) on average 1.81% (S.D. ± 0.19) total monoterpenes and 1.57% (S.D. ± 0.12) cineole on a fresh weight basis. The best tree produces more than twice the yield of cineole of the average for the population and this differential is likely to broaden as more trees are screened. It is clear that the selected genotypes provide oil not only in increased quantity relative to 'average' trees but also of enhanced quality. If these gains can be captured by selection and breeding amongst elite trees the economics of oil production from the tropical red gums would be significantly improved.

Ten trees included in the January 1988 sampling in Experiment 4 were sampled again in July and December of the same year to test if month of collection or position, i.e. aspect, in the crown had any significant effect on yield of the compounds of interest. Neither month of collection nor position in the crown had any consistent effect on oil production. The stability of estimates of oil yields and individual tree rankings confirms the adequacy of the method for work of this type (Brooker et al. 1988).

Conclusions

There is very substantial inter- and intra-specific quantitative variation in

yield of oil from *E. camaldulensis* and *E. tereticornis* in northern Australia. Qualitative differences are also present, as for example the frequency of high levels of α-phellandrene in some populations and its relative absence from others. However, the significance of these differences was judged to be minor in the context of this paper and they were not pursued beyond the first experiment. There were no clear associations between the amounts of the major monoterpenes and taxonomic affinities.

Oil yields of even the best populations (1—2%) are generally below the economic threshold for viable oil production in Australia. However, these levels should be adequate to support production in countries where labour is less expensive and where oil production could be a by-product of wood harvesting. The economics of oil production from the red gums will be enhanced by the large quantities of leaf generated by the need to thin the prolific coppice that follows clearfalling in the normal plantation silviculture of these species.

Provenances of *E. camaldulensis* from the Petford region combine rapid growth and desirable oil characteristics and offer the best scope for production of 1,8-cineole-rich oil in countries in the wet/dry tropics where this species is grown. Other widely-planted provenances with promise for cineole production in the tropics include *E. camaldulensis* from Gilbert River Bridge and, after some genetic improvement, *E. tereticornis* from Morehead and Kennedy Rivers.

Individual trees amongst promising provenances produce significantly more oil of higher quality than the average trees of the population. There is potential for significant improvement in the economics of oil production from the tropical red gums by means of selection and breeding for these traits as oil characters in *Eucalyptus* appear to be strongly inherited (Pryor and Bryant 1958; Barton et al. 1990). Work is presently being undertaken to determine the extent of the gains which might be expected by employing various tree breeding strategies.

Neither the month of collection nor position on the crown resulted in any significant change in oil content in natural stands, and there appears to be a strong relationship between average oil yields in natural populations and fast-growing progeny of the same sources established in plantation in Zimbabwe. This stability augers well for the validity of combining results from further similar work in Australia and elsewhere.

The identification of cineole-rich variants in *E. camaldulensis* contrasts with earlier reports of this species having no value as a source of medicinal-grade oil and highlights the importance of intra-specific genetic variation in oil content in this genus. The need for extensive sampling before categorising a species as to its oil type(s) is indicated.

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References

Abbott, P. S. 1977. The *Eucalyptus* oils industry. Proc. of Australian Forestry Development Institute. conf., Traralgon, Vic., Oct. 26—30, 1977: 36—43.

——. 1986. Commercial production of oil from eucalypt foliage. Proc. For. Prod. Res. Conf., Melbourne, Vic., 11 pp.

Ammon, D. G., Barton, A. F. M. and Clarke, D. A. 1986. *Eucalyptus* oil as a component of petrol-ethanol fuel blends. Search 17: 92—5.

Ammon D. G., Barton, A. F. M., Clarke, D. A. and Tjandra, J. 1985. Rapid and accurate chemical determination of terpenes in the leaves of *Eucalyptus* species. Analyst 110: 921—4.

Anon. 1988. Eucalyptus oil. In: British Pharmacopoeia, Vol. 1. (Her Majesty's Stationery Office:London) 239 pp.

Baker, R. J. and Nelder, J. A. 1978. Generalised Linear Interactive Modelling (GLIM) Manual. Section 3.3. Numerical Algorithms Group, Oxford.

Barton, A. F. M., Cotterill, P. P. and Brooker, M. I. H. 1990. Heritability of cineole yield in *Eucalyptus kochii*, a mallee species endemic to Western Australia. Silvae Genet (in press).

Brooker, M. I. H., Barton, A. F. M., Rockel, B. A. and Tjandra, J. 1988. The cineole content and taxonomy of *Eucalyptus kochii* Maiden and Blakely and *E. plenissima* (Gardner)

Brooker, with an appendix establishing these two as subspecies. Aust. J. Bot. 36: 119—29.

Lassak, E. V. 1979. The volatile leaf oils of three species of *Melaleuca*. J. Proc. Roy. Soc. NSW 112: 143—5.

——. 1988. The Australian *Eucalyptus* oil industry, past and present. Chemistry in Aust. 55: 396—406.

Matheson, A. C. and Mullin, L. J. 1987. Variation among neighbouring and distant provenances of *Eucalyptus grandis* and *E. tereticornis* in Zimbabwean field trials. Aust. For. Res. 17: 233—50.

Midgley, S. J., Eldridge, K. G. and Doran, J. C. 1990. Genetic resources of *Eucalyptus camaldulensis*. Commonw. For. Rev. 64: 295—308.

Ndou, T. T. and von Wandruszka, R. M. A. 1986. Essential oils of South African *Eucalyptus* species (Myrtaceae). S. Afr. J. Chem. 39: 95—100.

Penfold, A. R. and Willis, J. L. 1961. The Eucalypts. World Crop Series: Leonard Hill: London, and Interscience Publishers: New York, 550 pp.

Pryor, L. D. and Bryant, L. H. 1958. Inheritance of oil characters in *Eucalyptus*. Proc. Linn. Soc. NSW 83: 55—64.

Rao, H. S., Shiva, M. P. and Jain, P. P. 1970. *Eucalyptus* oil potential from large-scale plantations. Indian For. 96: 135—9.

Senanayake, U. M., Udakandage, S. J., Jayewardene, A. L. and de Silva, K. T. D. 1983. Studies on volatile oil of *Eucalyptus camaldulensis*. Paper to Ninth International Congress of Essential Oils, Singapore, 13—17 March 1983, Book 3: 93—6.

Turnbull, J. W. 1973. The ecology and variation of *Eucalyptus camaldulensis* Dehnh. Forest Genetic Resources Information 2, FAO, Rome: 32—7.

——. 1975. Seed collection of eucalypts. In Report on the FAO/DANIDA Training Course on Forest Seed Collection and Handling. Vol. 2, FOR:TS-RAS 11(DEN), FAO, Rome: 337—46.

Turnbull, J. W. and Griffin, A. R. 1986. The concept of provenance and its relationship to infraspecific classification in forest trees, 157—89. In: Styles, B. T. (Ed) Infraspecific Classification of Wild and Cultivated Plants, Vol. 29, The Systematics Association Special. Clarendon Press.

White, K. J. 1986. Tree farming practices in the Bhabar Terai of Central Nepal. Manual No. 2, Sargarnath Forest Development Project, Ministry of Forests. Kathmandu, Nepal, 191 pp.