

Variation in frost tolerance of the 1,8-cineole-rich variants of the peppermint eucalypts, *Eucalyptus radiata* and *E. dives*

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Revised manuscript received 29 March 2005

Summary

Frost tolerance is a necessary characteristic for the successful establishment of *Eucalyptus radiata* subsp. *radiata* Sieber ex DC (1,8-cineole variant) for production of essential oil on frost-prone sites. The frost tolerance of seedlings from six commercial oil-producing provenances of this taxon was examined using the electrical conductivity method. One provenance of *E. radiata* subsp. *robertsonii* and two provenances of *E. dives* Schauer were also included for comparison. To verify the validity of this method, frost tolerance was also assessed in two field trials near Armidale on the New England Tableland of New South Wales where plants at one year from planting were scored for physical damage attributed to the effects of frost, viz. leaf fall and stem splitting.

There were consistent and significant differences in modified relative conductivity (RC) between provenances, and between open-pollinated families within provenances, across temperatures. *Eucalyptus radiata* subsp. *radiata* from Isabella River, followed closely by provenances from Reedy Creek and Yowrie, were the most cold-tolerant, while the two *E. dives* provenances and *E. radiata* subsp. *radiata* from Alexandra were the least frost tolerant, based on RC values. Rankings of *E. radiata* subsp. *radiata* provenances based on scores for stem splitting and leaf fall after their first winter in the field corresponded well to the RC rankings, with Reedy Creek the best performing provenance overall. Apart from limiting frost damage through selection of hardy provenances, the significant within-provenance family variation in frost tolerance, and the substantial variation between individuals within families noted in these trials, suggest good potential for selection and breeding amongst hardy individuals of the best-ranked families to improve frost tolerance.

Keywords: genetic variation; frost resistance; provenance trials; essential oil plants; *Eucalyptus radiata*; *Eucalyptus dives*; New South Wales

Introduction

In the most recent classification of the genus *Eucalyptus* L'Her. (Myrtaceae), Brooker (2000) placed *E. radiata* Sieber ex DC (narrow-leaved peppermint) and *E. dives* Schauer (broad-leaved peppermint) in the subgenus *Eucalyptus*, section *Aromatica*, series *Radiatae*. Among other attributes, these species are characterised

by possessing entire juvenile and mature leaves with numerous oil glands containing strongly scented essential oils.

Three subspecies are recognised in *E. radiata*:

- (i) subsp. *radiata* ascribing populations on the tablelands, ranges and foothills of central and southern NSW, north-eastern and southern central Victoria and in the Lemnathyme area in northern Tasmania;
- (ii) subsp. *sejuncta* L.A.S.Johnson & K.Hill for populations on the northern tablelands of NSW with a small extension into southern Queensland; and
- (iii) subsp. *robertsonii* (Blakely) L.A.S.Johnson & Blaxell for populations south from Orange along the western side of the Southern Tablelands of NSW and at higher elevations near Canberra (Brooker and Kleinig 1999; Brooker *et al.* 2002).

Intra-specific variation in foliar oil composition abounds in *E. radiata* and *E. dives*, where up to six and five chemical variants respectively have been described (Johnstone 1984). The 1,8-cineole-rich variants of these species are of commercial interest. Their oils are classed as medicinal *Eucalyptus* oils and are used for many purposes, either neat or in formulations, including inhalants, liniments, ointments, aromatherapy, soaps and as disinfectants and deodorizers (Boland *et al.* 1991; Coppen 1995; Davis 2002). Natural populations of *E. radiata* and *E. dives* that produce a 1,8-cineole-rich oil are limited in number and extent. Commercial production centres based on natural populations include the New South Wales south coast hinterland north of Bega, the NSW Southern Tablelands around Tumbarumba and Rosewood, and, in the past, in the ranges outside Canberra. Production in Australia of oil from 1,8-cineole-rich variants of *E. radiata* and *E. dives* is now relatively small: probably less than 10 t annually.

Recognition that these species produce highly marketable medicinal oil has led to interest in establishing plantations of these species for oil production in Australia. More than 200 ha of *E. radiata* subsp. *radiata* oil plantation have been established near Bendemeer on the New England Tableland of NSW since 1998 (M. Weber¹, *pers. comm.* 2002). Other plantings have taken place,

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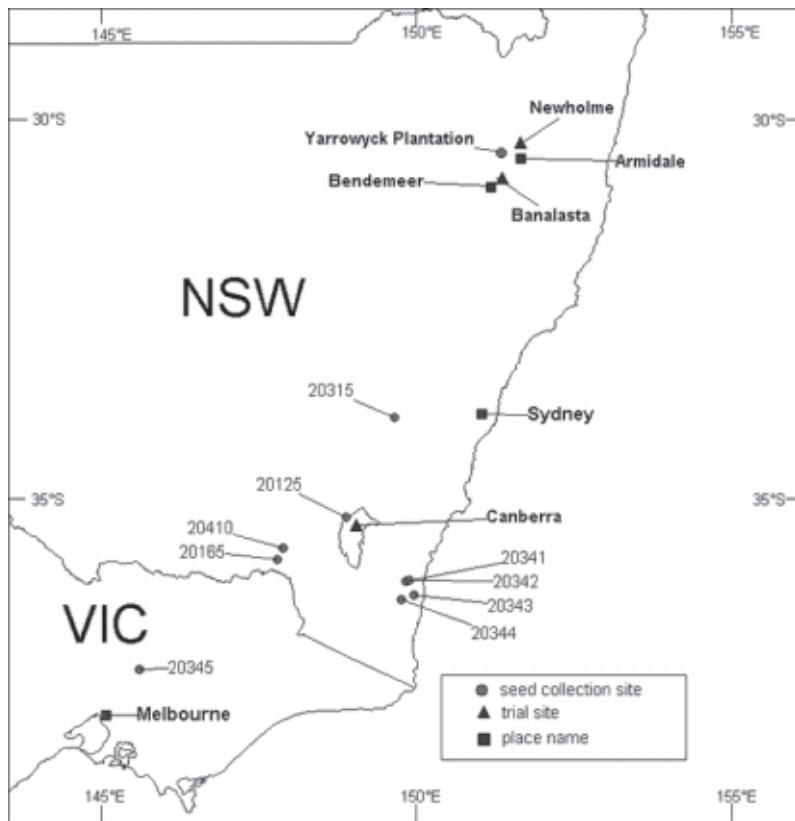


Figure 1. Map of provenance location and trial sites

usually in a farm forestry setting, on several properties along the Great Dividing Range in NSW and Victoria in the past 10 y.

Young *E. radiata* subsp. *radiata* trees at Bendemeer suffer frost damage each winter in the form of stem splitting, leaf burn and even death.

The purpose of this study was to screen most of the known 1,8-cineole variant populations of the peppermint species for frost tolerance as young seedlings to aid in provenance selection for oil plantations on frosty sites and to gain an understanding of variation in seedling frost tolerance in this group of species. A secondary aim was to compare two methods of determining frost tolerance, namely a laboratory-based method (conductivity of leaf leachates) and subjective scoring of actual frost damage in the field.

Materials and methods

Two methods were used to estimate frost damage. The first employed the electrical conductivity method on 6-mo-old seedlings growing in the open in a nursery. This method was developed by Raymond *et al.* (1986, 1992a) and Owen and Raymond (1987). It has been shown to yield the same rankings of seedling frost tolerance as those obtained from whole-plant studies for *E. delegatensis* and *E. regnans*, and it was expected that there would be a similar trend for the peppermint eucalypts which are in the same subgenus, *Monocalyptus*.

The second method was a subjective assessment of leaf fall and stem splitting among 12-mo-old seedlings in two field trials on the New England Tableland of northern New South Wales. The main trial site was at 'Banalasta' (30°462 S, 151°142 E, 900 m a.s.l.) near Bendemeer, with a secondary planting at Newholme (30°312 S, 151°402 E, 1000 m a.s.l.) near Armidale (Fig. 1).

Species and provenances included

Six provenances of *E. radiata* subsp. *radiata*, one provenance of *E. radiata* subsp. *robertsonii* and two provenances of *E. dives* were studied in the electrical conductivity experiment and the same six provenances of *E. radiata* subsp. *radiata* in the field trials (Table 1, Fig. 1). The material included in these studies originated from seed collected from natural populations. Most provenances were known to produce 1,8-cineole-rich essential oils. The one exception was the population of *E. dives* from Tumarumba which is a piperitone-rich form ('type' oil). It was included in the electrical conductivity experiment for comparison. The field trials also included four seedlots of *E. radiata* subsp. *radiata* from a planted stand on the New England Tableland of unknown provenance (H. Harris², *pers. comm.* 2001).

Raising, hardening and planting of seedlings

Canberra conductivity experiment

Species, provenances and families included in this experiment are described in Table 1. *Eucalyptus radiata* families were sown into plastic punnets containing a moist sowing mix of 1:1:1 of peat, perlite and washed sand during November 2000. These were sealed in plastic bags and placed under cold-moist stratification in a refrigerator at 3–5°C for two weeks. They were then transferred to a temperature-controlled glasshouse (25/18°C) for germination under mist sprays.

In the first week of January 2001, seedlings had reached the 1–2 leaf-pair stage. They were then dibbled into forestry tubes (5 cm × 5 cm × 12 cm) containing a sterilised potting mix of pine bark compost, sand, vermiculite and peat in the ratio of 5:2:2:1, and a mix of fertilisers consisting of Osmocote® 18-2.6-10 (1.6 g L⁻¹), Osmocote® 19-2.6-10 (0.6 g L⁻¹), dolomite (1.1 g L⁻¹), Micromaz® nutrients (1 g L⁻¹) and lime (0.3 g L⁻¹) as well as Saturade® (1.5 g L⁻¹). The families of *E. dives* were raised in like fashion except that washed sand was used as the sowing mix and the seed in the punnets was cold-moist stratified for four weeks.

The seedlings were raised in a glasshouse for 3 mo. In April 2001, 10 of the most vigorous seedlings from each family were transferred to a raised bench outside. They were arranged in a latinised row-column design generated by the computer software package Alpha + (Williams and Talbot 1993) with 8 rows and 10 columns per replicate. Pots were spaced 6 cm apart from one another. Survival was excellent; seedling mortality was less than 2% at the time of sampling in July 2001.

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Table 1. Origin of provenances (CSIRO seedlot no.) of the peppermint eucalypts in the trials at CSIRO Canberra (C) and in the field trials at Banalasta (B) and Newholme (N) on the New England Tableland of New South Wales. The climatic data for each site were estimated using the ESOCIM module in the ANUCLIM program (Houlder *et al.* 2000).

CSIRO Seedlot No.	Provenance	State	Latitude (°S)	Longitude (°E)	Altitude (m)	Rainfall (mm)	MAT (°C)	MTCM (°C)	AMT (°C)	No. of families per trial		
										C	B	N
<i>Eucalyptus radiata</i> subsp. <i>robertsonii</i>												
20125	Uriarra	ACT	35.25	148.87	840	1051	11.5	0.3	-10.0	9	—	—
<i>E. radiata</i> subsp. <i>radiata</i>												
20315	Isabella R.	NSW	33.93	149.65	1050	1410	10.0	-0.5	-9.0	8	4	4
20341	Big Belimbla	NSW	36.1	149.82	200	976	14.1	2.1	-5.0	10	13	12
20342	Gulph Ck	NSW	36.08	149.88	80	980	14.7	2.8	-3.0	10	10	10
20343	Reedy Ck	NSW	36.28	149.95	40	967	14.9	3.5	-2.0	10	10	10
20344	Yowrie	NSW	36.33	149.75	180	962	14.0	1.7	-5.0	9	10	9
20345	Alexandra	Vic	37.25	145.61	400	1059	11.7	2.0	-4.0	10	9	9
	Yarrowyck Plantation	NSW	30.44	151.35	837	715	13.8	0.4	-9.0	—	4	4
<i>E. dives</i>												
20410	Rosewood	NSW	35.65	147.87	640	1000	11.6	0.1	-8.0	1	—	—
20165	Tumbarumba	NSW	35.8	147.78	850	1157	10.6	-0.3	-8.0	3	—	—
Trial sites												
	Newholme	NSW	30.33	151.65	1020	734	12.7	0.1	-9.0			
	Banalasta	NSW	30.76	151.25	920	755	13.0	-0.5	-9.0			
	Canberra	ACT	35.3	149.1	580	654	12.9	0.6	-9.0			

MAT = mean annual temperature, MTCM = mean daily minimum temperature of the coldest month, AMT = absolute minimum temperature

Outdoor temperatures during the Canberra winter are sufficiently low for cold hardening of eucalypt seedlings, as the minimum temperature of most nights is well below the threshold level of 5°C (Harwood 1980). A period of 28 consecutive days with minimum temperatures below 5°C is accepted as the requirement for plants to be considered fully hardened, and this was achieved in the lead up to the samples being taken for determination of frost tolerance by the electrical conductivity method.

Banalasta and Newholme field trials

The Banalasta and Newholme sites share similar poor soils (grey-brown podzols) and a climate characterised by warm, wet summers and cold, dry winters. Only *E. radiata* subsp. *radiata* families were included in these trials (see Table 1 for details). A commercial nursery propagated family-identified seedlings and they were hardened at Banalasta for two weeks prior to planting out. The two trials were established on 15–16 November 2000 using row-column designs, with 60 treatments (open pollinated families) at Banalasta and 58 treatments at Newholme, in 5-tree row plots along prepared mounds 80 cm wide and 30 cm high. A single-row buffer consisting of seedlings of mixed provenance origin was established around each trial.

At Banalasta, the trial comprised eight replications and spacing was 3 m × 1.2 m. Mound soil was mixed with sulphate of potash (145 kg ha⁻¹), chicken manure (2.4 t ha⁻¹), Grow Mag® (Ca 32%, MgO 8% at 3 t ha⁻¹) and Zeolite® (about 300 kg ha⁻¹). The ground was sprayed with Goal® (3 L ha⁻¹) and Trifluralin® (1 L ha⁻¹)

before planting. Additional fertiliser applied at planting consisted of Osmocote (1–2 g per plant), worm compost liquid (20 L ha⁻¹) and Maxicrop® (4 L ha⁻¹). Irrigation through T-tape was applied as needed during the growing season.

The Newholme trial comprised four replications. A wider tree spacing of 3 m × 3 m was employed as the site was not irrigated. Before planting, the mounds were sprayed with glyphosate (5 L ha⁻¹); post-planting weed control was with Fusilade® (2 L ha⁻¹). Fertiliser (N:P 18:20) at 100 g per plant was applied 2 mo after planting.

Temperatures 1.1 m above ground at Banalasta during the first winter (2001) were recorded by Tinitag® data loggers located adjacent to the trial site. Frosts were frequent from April to September, with temperatures <-5°C common. Mean daily temperature of the coldest month (MTCM) was -0.6°C, with an absolute minimum temperature of -8.2°C.

Determination of frost tolerance

Conductivity method

All plants in the trial were assessed for height growth immediately before the frost measurements commenced. Frost tolerance was determined over a 10-day period. The capacity of the frosting equipment was sufficient to assess all of the seedlings in one replicate in one day, consequently the ten replicates were assessed, one replicate per day, on 10 successive days.

Leaf discs, 8 mm in diameter, were excised using a purpose-designed punch. Six discs were taken from the uppermost fully expanded leaf pair of each seedling and six from the leaf pair immediately below. Discs were taken only from healthy leaves. Samples of the same leaf area were also collected by cutting sections of leaves where the leaf width was too narrow for a complete disc to be removed. Each disc was placed in a separate 12 mm × 75 mm test tube. Sets of four discs per plant (two from each leaf pair) were exposed to one of three test temperatures. Tubes were immersed in liquid baths at 2°C. Bath temperatures were lowered to -2°C over 1 h, after which 0.2 g of finely crushed ice was added to each test tube to ensure ice formation in the leaf discs and to prevent supercooling. Bath temperatures were then lowered at a rate of 4°C h⁻¹ to the three test temperatures of -5.1°C, -7.2°C and -9.3°C which were maintained for 1 h, then temperatures were raised at 4°C h⁻¹ to 4°C, which was maintained while damage symptoms developed over 24 h. Two millilitres of deionised water was then added to each sample and left at room temperature for a further 24 h. The electrical conductivity of the leachate (*ct*) was then measured. Tubes were then placed in a water bath at 80°C for 10 minutes and left for 24 h at room temperature before a final conductivity (*ck*) was measured.

Banalasta and Newholme field trials

Plants in these trials were assessed for height growth and survival and subjectively scored for leaf fall (none = 0, 1–25% = 1, 26–50% = 2, 51–75% = 3, >75% = 4) and stem splitting (present = 1, absent = 0) in November 2001 at 12 mo after field planting.

Data analysis

Conductivity method

Modified relative conductivity values,

$$RC = \sqrt{\left(\frac{ck - ct}{ck}\right)}$$

(where *ct* is conductivity after exposure to frost temperature and *ck* is conductivity of same tissue after killing) (Raymond *et al.* 1986) were calculated for each leaf disc. For each test temperature, individual disc *RC* values were subjected to analysis of variance using the statistical package GenStat (2000). Replicates and trees were used as blocking factors. Preliminary analysis indicated that the row and column incomplete blocks used in the spatial layout of the seedlings during growth and hardening did little to reduce the residual mean squares, and differences attributed to leaf pairs also had little effect, so these factors were not included in the model. Day of sampling was fully confounded with replicate, so did not need to be accounted for separately. The treatment structure was specified as species, species.provenance and species.provenance.family. All these treatments were specified as fixed effects including family as heritabilities were not being calculated (Williams *et al.* 2002, ch. 8).

There were a few missing values (200 missing out of 9600). Data from a total of 30 discs were omitted from the final analysis because they were identified as clear outliers from the plots of

residual versus fitted values obtained from the initial analysis of variance. Most (18) of the outlying values were characterised by the absence of any damage at one of the two lower test temperatures, which indicated that the ice added at -2°C had not touched the disc, resulting in abnormal leachate conductivity readings.

Banalasta and Newholme field trials

The software package DataPlus Version 3 (Williams *et al.* 2000) was used to pre-process the data and screen for outlying values. Selected data for several trees that appeared abnormal for the particular attribute were deleted from the data sets. Plot means for the four traits (stem splitting, leaf fall and height and survival at 1 y) were then calculated using DataPlus. Plot variances were then analysed using the ANOVA (Banalasta) and Fit (Newholme, with many missing values) procedures in GENSTAT to check for homogeneity of variances. This revealed plot variances to be homogeneous and transformations of the original data were considered unnecessary.

Analyses of trial data were based on the following linear model:

$$Y_{ijklm} = \mu + R_i + X_{j(i)} + Y_{k(i)} + G_l + F_{m(l)} + e_{ijklm},$$

where Y_{ijklm} is the plot mean of the *m*th family within the *l*th provenance group in the *k*th column-within-replicate *i* and the *j*th row-within-replicate *i* in the *i*th replicate; represents the overall mean; R_i represents the effect of the *i*th replicate; $X_{j(i)}$ represents the effect of the *j*th row within replicate *i*; $Y_{k(i)}$ represents the effect of the *k*th column within replicate *i*; G represents the effect of the *l*th provenance group; $F_{m(l)}$ represents the effect of the *m*th family which is nested within the *l*th provenance group; and e_{ijklm} represents the residual error with a mean of zero.

Computation of family means and analyses of variance (ANOVA) were carried out in two stages. The first stage involved mixed model analyses of the plot means using the REML procedure in GENSTAT, for which families and replicates were treated as fixed effects while both rows-within-replicates and columns-within-replicates were treated as random. For the second stage, family means estimated from this first stage were then analysed, using the ANOVA procedure in GENSTAT, using a nested treatment structure (families nested with provenance). The outputs from the two stages were then combined to produce composite analysis of variance tables for testing the significance of provenance and family effects, following procedures described by Williams *et al.* (2002, ch. 8).

Results

Differences in *RC* between taxa, provenance within taxa, and family within provenance were all highly significant ($P < 0.001$) at the three test temperatures, with species and provenance differences accounting for substantially more of the variance than families within provenance.

Mean provenance *RC* values for the three test temperatures, and heights of seedlings at time of sampling are given in Table 2. *RC* values can be used to rank provenances in order of notional frost tolerance. A leaf *RC* value of <0.8 corresponded to leaf damage

Table 2. Provenance mean relative conductivity (RC) values at -5.1°C , -7.2°C and -9.3°C , rankings based on RC values, and mean height of progeny.

Seedlot	Provenance	-5.1°C		-7.2°C		-9.3°C		Height	
		RC	Rank	RC	Rank	RC	Rank	mm	Rank
<i>Eucalyptus radiata</i> subsp. <i>robertsonii</i>									
20125	Uriarra	0.876	4	0.727	4	0.446	4	355.8	6
<i>E. radiata</i> subsp. <i>radiata</i>									
20315	Isabella River	0.916	1	0.870	1	0.744	1	314.6	8
20341	Big Belimbla	0.862	6	0.695	6	0.390	6	384.3	3
20342	Gulph Creek	0.864	5	0.706	5	0.407	5	365.5	5
20343	Reedy Creek	0.886	3	0.830	2	0.601	2	379.5	4
20344	Yowrie	0.891	2	0.811	3	0.575	3	337.3	7
20345	Alexandra	0.861	7	0.573	9	0.323	9	395.3	1
<i>E. dives</i>									
20410	Rosewood	0.819	8	0.622	7	0.324	8	387.6	2
20165	Tumbarumba	0.811	9	0.602	8	0.358	7	273.6	9
LSD		0.04		0.11		0.14		37.3	

LSD = least significant difference at 5% level

of greater than 50% for *E. delegatensis* and *E. regnans* (Raymond *et al.* 1986) and also *E. nitens* (Raymond *et al.* 1992b). It seems reasonable to assume a similar association for *E. radiata* subsp. *radiata*, although no such correlation has been attempted with this species. The results at -7.2°C gave the clearest discrimination between frost-tolerant and frost-sensitive material, although there was good discrimination at all test temperatures, and provenance rankings based on RC values were consistent across the temperature range (Table 2).

Eucalyptus radiata subsp. *radiata* families from Isabella River, followed closely by Reedy Creek and Yowrie, were the most cold-tolerant progeny across all three test temperatures, based on RC values. Thirty-one percent of families overall had RC values >0.8 at -7.2°C , but for Isabella River, Reedy Creek and Yowrie 85% of families averaged RCs >0.8 . Uriarra *E. radiata* subsp. *robertsonii* had two families (22%) with RC values >0.8 at -7.2°C , while the two *E. dives* provenances and *E. radiata* subsp. *radiata* from Alexandra were consistent in giving relatively low RC values across all temperatures, indicating relative susceptibility to frost damage. They had no families with RC values >0.8 at -7.2°C .

There was significant ($P < 0.001$) provenance and family-within-provenance variation in characteristics attributed to frost damage (stem splitting and leaf fall) in the field trial of *E. radiata* subsp. *radiata* provenances at Banalasta at 12 mo after planting and following the first winter. At Newholme the differences among plants in stem splitting were not significant, but differences in leaf fall among provenances and families within provenances were significant ($P < 0.001$).

The assessment of frost tolerance in the field trials based on extent of leaf fall matched well with the results obtained for RC values, while the pattern for stem splitting at Banalasta was similar with the notable exception of the provenance from Alexandra (Fig. 2). Of the natural provenances, Isabella River had least frost damage in the field trials, followed closely by Reedy Creek and Yowrie. Alexandra was the most severely defoliated of the natural

provenances, but Big Belimbla and Gulph Creek were significantly more prone to stem splitting than Alexandra at Banalasta. The families from Yarrowyck Plantation were not included in the Canberra conductivity trial but proved to be the sources most resistant to stem splitting and leaf fall in both field trials, suggesting local selection for frost tolerance. They were generally relatively slow growing in comparison with the provenances from south-eastern NSW.

There was significant within-provenance family variation in frost tolerance and substantial variation between individuals within families that could benefit selection and breeding programs. For example, in the field trial at Banalasta five families had $>80\%$ of progeny with less than 50% leaf fall — the families were from Yarrowyck Plantation (1 family), Big Belimbla (1 family), Gulph Creek (2 families) and Reedy Creek (1 family).

Isabella River was the slowest-growing natural provenance in the field trials, but ranked amongst the highest for frost tolerance (Fig. 2). Reedy Creek was the fastest-growing provenance in the field trials, sustained the least loss ($<1\%$) of seedlings during March to November 2001 (data not given here), and combined this with high levels of frost tolerance by all the measures used in this set of trials. Survival rates among provenances (data not presented) were not regarded as useful indicators of frost tolerance in these trials because they were most likely influenced by other factors such as deaths due to waterlogging, weed competition and drought.

Discussion

The relatively low frost tolerance recorded for the *E. dives* seedlings in the Canberra conductivity experiment was unexpected. Observations on local site conditions and microclimate amongst the *E. dives* provenances, supported by the ESOCCLIM data (Table 1), suggest that these populations would be subjected to frequent frost events and should be highly frost

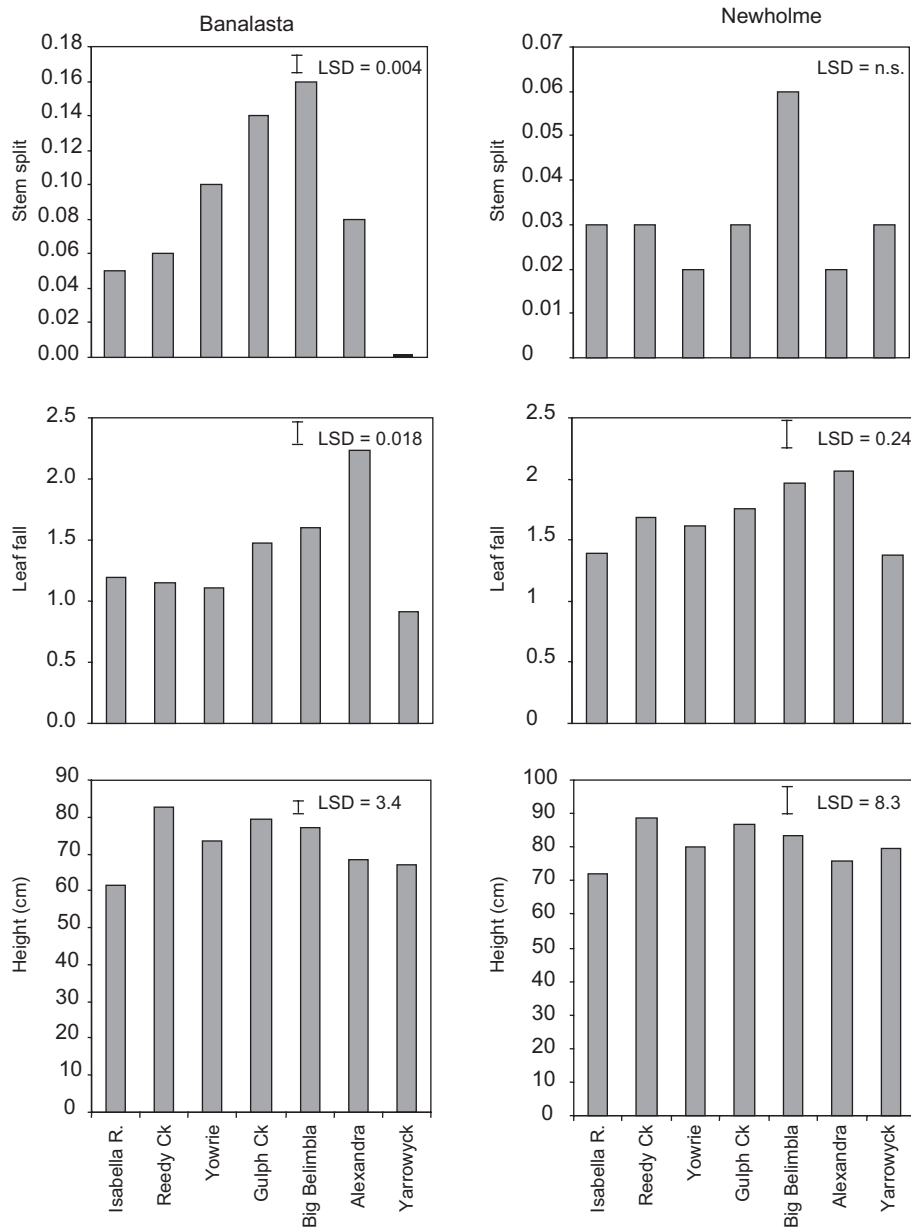


Figure 2. *Eucalyptus radiata* subsp. *radiata* provenance means for stem splitting and leaf fall scores and height growth at 12 months in the field trials at Banalasta and Newholme. The natural provenances are given in rank order (left to right) of their RC values at -7.2°C (Table 2). Yarrowyck plantation was not represented in the Canberra trials but is included for comparison. LSD = least significant differences at 5% level.

tolerant. The experimental data, however, is supported by observations on the two species in plantations in South Africa (J. Owen³, *pers. comm.* 2002) and at Banalasta (M. Weber, *pers. comm.* 2002) that, in the first year of establishment, seedlings of *E. dives* were much more frost sensitive than those of *E. radiata* subsp. *radiata*. This suggests that *E. dives* might require a particular environment for hardening if it is to reach its full frost tolerance potential.

Eucalyptus radiata has a very wide distribution across the south-east of Australia, occurring from near sea level to >1000 m altitude and with a latitudinal range of about $30\text{--}42^{\circ}\text{S}$. It is important to

note that the studies reported here did not set out to determine range-wide variation in frost tolerance within this species, but rather the relative frost tolerance of a small subset of provenances. These provenances represent most of the populations known to have foliar oils rich in 1,8-cineole and to be suitable for medicinal eucalyptus oil production (Doran 2002).

The field and electrical conductivity results largely corresponded for *E. radiata* subsp. *radiata*. The highest altitude seed source in the trial, Isabella River, was the most cold tolerant, while the most southerly source, Alexandra, was the most frost sensitive. Big Belimbla, a provenance popular for plantation development in southern New South Wales because of its fast growth and high oil yields (Doran *et al.* 1998; Harris 2002), was one of the poorest ranking provenances for frost tolerance in these trials.

³Lion Rock Oils, Durban, South Africa

Foliar oil concentration and leaf biomass production combine to determine oil yield per hectare, a crucial determinant of the economics of oil production. Vigorous height growth, as an indicator of high leaf biomass production potential, is an important selection criterion. Reedy Creek provenance appeared to combine fast growth with high levels of frost tolerance which, if these trends continue, would make it a preferred seed source when frost tolerance is essential for good survival and growth on the New England Tableland of New South Wales.

Variation in frost tolerance and growth rates between families within provenance, and between individuals within families, was apparent in all experiments, indicating that some selection for frost tolerance within most provenances should be possible. To produce better adapted germplasm of *E. radiata* subsp. *radiata* for planting on frosty sites, the Banalasta field trial was to be heavily thinned at about three years of age to convert it to a seed orchard, using an individual-tree selection index combining superior biomass production, frost tolerance and oil characteristics. However, before this could happen, record frosts at Banalasta during the winter of 2002 (as low as -14°C , M. Weber, *pers. comm.* 2002) killed all but a few trees in the experiment.

Acknowledgements

We are grateful to Chris Harwood for analysing the electrical conductivity data and Kron Aken for the analyses of the field measures. Kron also prepared Fig. 1. We thank John Owen for his advice during the conductivity experiment, Ian McLeod for assisting with trial establishment and Tom Jovanovic for providing the climatic data in Table 1. Markus Weber provided the trial site and climatic data for the winter of 2001 at Banalasta. We are grateful to Maurice McDonald, Roger Arnold, Chris Harwood and Lex Thomson for helpful comments on drafts of this manuscript.

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