

# Short Note: Heritability of Cineole Yield in *Eucalyptus kochii*

By A.F.M. BARTON<sup>1</sup>), P.P. COTTERILL<sup>2</sup>) and M.I.H. BROOKER<sup>3</sup>)

(Received 29th December 1989)

## Summary

Cineole yield (weight/weight fresh foliage) was estimated for 50 individual trees of *Eucalyptus kochii* subspecies *kochii* and *plenissima* Brooker over 24 separate monthly foliage samplings in native stands in Western Australia. Open-pollinated seed was collected from each tree and five seedlings planted in unreplicated family plots in a field trial at Perth, Western Australia. The cineole yield of offspring was determined for seedlings prior to planting, and at one and 2½ years after planting in the field. Family heritabilities were determined from correlations between parent cineole yield and mean offspring yields.

Family heritabilities for cineole yield were 0.34, 0.19 and 0.83 when based on seedling, year-1 and year-2½ offspring means, respectively. Progeny test selection (on year-2½ yields) at an intensity of one parent in every 10 may be expected to increase cineole yield from around 2% in native populations to 2.8% in improved populations.

*Key words:* Cineole yield, heritability, *E. kochii*.

## Introduction

Cineole is the major leaf oil component of commercial pharmaceutical grade eucalyptus oil. Indeed, pharmaceutical oil requires a minimum cineole level of 70%. Although cineole may be extracted from a wide range of plant species, and can be obtained synthetically, eucalyptus oil remains the most important source on world markets.

The production of eucalyptus oil in eastern Australia is based largely on native (almost pure) stands of *Eucalyptus polybractea*, although the harvests of tree crowns include some naturally growing *E. viridis*. Species such as *E. globulus* and *E. radiata* are used to a lesser extent for oil production in eastern Australia. There is also interest in the Western Australian mallees, especially *E. kochii* subspecies *kochii* and *plenissima*, whose leaf oils have high (approximately 90%) levels of cineole (Brooker *et al.*, 1988; Barton *et al.*, 1989). Expansion of oil production from eucalypts in Australia would be assisted by establishing plantations of *E. polybractea*, or of alternative species such as *E. kochii*. The question arises as to whether there would be benefits from using genetically improved plantations.

The objective of this paper is to present heritabilities of cineole yield of *E. kochii*, and estimate genetic gains that may be expected from breeding. The heritabilities were determined from correlations between parent cineole yield and mean offspring yields. There appear to be no other published estimates of heritability of cineole yield for any species of *Eucalyptus*.

<sup>1</sup>) Murdoch University, School of Mathematical and Physical Sciences, South Street, Murdoch, WA 6150, Australia.

<sup>2</sup>) CSIRO, Division of Forestry and Forest Products. Present address: Celulose Beira Industrial (CELBI) SA, Quinta do Furadouro, 2510 Obidos, Portugal.

<sup>3</sup>) CSIRO, Division of Plant Industry, Australian National Herbarium, GPO Box 1600, Canberra, ACT 2601, Australia.

## Materials and Methods

### *Genotypes and Field Design*

Fifty healthy seed-bearing trees were chosen across the native range of *E. kochii*, a mallee species endemic to the northern wheatbelt and adjacent arid lands of Western Australia. The trees sampled included, in approximately equal numbers, representatives of *E. kochii* ssp. *kochii* and *plenissima* (details given in Brooker *et al.*, 1988). Open-pollinated seed was collected from each tree and sown in a glasshouse at CSIRO, Floreat Park, in September 1983. Healthy seedlings were planted out on a 0.1 ha site at Murdoch University, Perth, in May 1984. The field design involved unreplicated row plots of six trees per family.

### *Foliage Sampling and Chemical Analyses*

Leaves were sampled monthly from each of the 50 parent trees over a two-year period ending December 1984 (Brooker *et al.*, 1988). The cineole (1,8-cineole; or 1,3,3-trimethyl-2-oxabicyclo [2.2.2] octane) content of the leaves was determined by gas chromatography on ethanol extracts, as described by Ammon *et al.* (1985). Cineole yield in this paper is expressed as a percentage of the fresh weight of foliage.

Leaves were sampled from five of the six seedlings per open-pollinated family in the glasshouse in March 1984 (immediately before planting out), and again from the same seedlings in March 1985 (one year after planting out) and December 1986 (2½ years).

### *Statistical Analyses*

The mean cineole yield ( $\bar{P}$ ) of each parent was determined by averaging the 24 separate monthly foliage analyses. Mean cineole yield ( $\bar{O}$ ) of the offspring were determined for each measurement of the progeny trial (i.e. at seedling, 1 year and 2½ year stages of growth). Seven parents having fewer than four surviving offspring at the 2½ year measurement were omitted from data analyses (leaving 43 parent-offspring combinations).

Heritability (denoted  $h_f^2$ ) was estimated on a family basis as twice the correlation ( $r_{\bar{P}\bar{O}}$ ) between mean parent cineole yield ( $\bar{P}$ ) and mean offspring yield ( $\bar{O}$ ) at different ages - (1)

$$h_f^2 = 2 r_{\bar{P}\bar{O}}. \quad (1)$$

The parent-offspring correlation is doubled in Equation 1 because the covariance between  $\bar{P}$  and  $\bar{O}$  provides an estimate of only half the additive genetic variance (Turner and Young, 1969; Chapter 8). The heritability in Equation 1 is estimated on a family basis (with offspring means used in the analyses) and is relevant to predicting genetic gains from progeny test selection. It is worth mentioning that heritability can also be estimated from parent-offspring regression but the correlation approach is more appropriate where offspring have been measured at different ages (Turner and Young, 1969). Phenotypic correlations ( $r$ ) were determined among the mean cineole yields of offspring at different ages.

Genetic gains (denoted  $\Delta A$ ) expected from progeny testing to improve cineole yield were determined as - (2)

$$\Delta A = ih_f^2 \sigma \quad (2)$$

where  $i$  is the standardised selection differential and  $\sigma$  the phenotypic standard deviation. In this study we assume selection is at an intensity of one parent in every 10 (giving  $i=1.76$  for an infinite population size).

### Results and Discussion

Mean cineole yield of the parents analysed was 2.0% (standard deviation  $\sigma=0.57\%$ ) weight/weight fresh leaves collected over the 24 separate monthly samplings. The mean cineole yield of offspring increased markedly from 0.7% for seedlings prior to planting, to 1.9% at year-1 and 2.3% at year-2½ after planting (Table 1).

Family heritabilities of cineole yield were relatively low (values of  $h_f^2=0.34$  and 0.19) when estimated from parent-offspring correlations involving seedling and year-1 offspring means (Table 1). However, the family heritability of cineole yield increased to a high  $h_f^2=0.83$  when based on year-2½ measurements of offspring. It seems that the breeding values of mature trees for cineole production may be reliably estimated from progeny trials at around three years, but perhaps not from measurements of younger offspring. This finding is supported by the phenotypic correlations in Table 1 which indicate a poor association ( $r=0.25$ ) between cineole yield at the seedling stage and year-2½, but a stronger association ( $r = 0.70$ ) between year-1 and year-2½ measurements.

It can be shown that cineole production is expected to improve by about 0.8% (w/w fresh foliage) following selection of the best parent in every 10 on the basis of yield of 2½ year old offspring. This expected gain is calculated by solving Equation 2 for  $i=1.76$ ,  $h_f^2=0.83$  and  $\sigma=0.57\%$ . To put this gain calculation in perspective of the present progeny trial, suppose the best five parents (out of the 50 under test) were selected on 2½ year old offspring yields. The intermating of these five parents (by controlled-crossing or open-pollination in clonal orchards) may be expected to produce an improved population of *E. kochii* which would yield around 2.8% cineole, compared with an average yield of around 2% for native stands. It is interesting to note that most offspring in the progeny trial were sexually mature at 2½ years and the opportunity would, therefore, exist to cross best offspring rather than best parents.

Table 1. — Overall means ( $u \pm$  phenotypic standard deviation) of all offspring in the progeny trial, family heritabilities ( $h_f^2$ ) and phenotypic correlations among cineole yields measured on offspring at the seedling stage, and 1 and 2½ years after planting.

Offspring	$u \pm \sigma$ (w/w % fresh)	$h_f^2$	Phenotypic correlations ( $r$ )	
			Year-1	Year-2½
Seedling	0.7 ± 0.21	0.34	0.13	0.25
Year-1	1.9 ± 0.41	0.19		0.70
Year-2½	2.3 ± 0.52	0.83		

In practice, breeders would be interested in improving the gross weight of foliage per tree, as well as improving the percent oil yield of foliage collected. We have no information on heritabilities of foliage biomass of *E. kochii* but there is no reason to believe this trait cannot also be improved by selection. Silvicultural factors such as planting density, fertilizing and irrigation should also have a major influence on foliage biomass.

It is important to stress that the heritabilities and expected genetic gains presented in this paper are based on only one progeny trial and we are unable to predict whether the results will apply for other sites or species. Some aspects of field design of the present trial may tend to bias the parent-offspring correlations and subsequent estimates of genetic gain. For instance, COTTERILL and JAMES (1984) show that the five offspring planted per parent in the present study represent a minimum number of offspring required to reliably evaluate family performance. If the trial had included more offspring per parent the family heritabilities may have been higher. The fact that the present trial involved unreplicated family plots may also have tended to bias parent-offspring correlations. However, the experimental site was small and relatively uniform and the lack of replication should not have seriously affected mean estimates of cineole yield.

### Conclusions

The heritabilities presented in this paper represent the first published estimates for cineole yield of *E. kochii* or any other species of *Eucalyptus*. The heritabilities provide encouraging evidence that substantial genetic progress can be achieved by selecting *E. kochii* for higher yields of cineole per unit weight of leaves. It appears that the generation interval in breeding for oil production can be relatively short because progeny trials may be reliably evaluated by at least three years of age by which time the trees are reproductively mature.

### Acknowledgements

We are grateful to Mr B. A. ROCKEL for collecting seed and raising seedlings, and to staff employed under a Murdoch University Special Research Grant and Australian International Development Assistance Bureau Grant. Assistance at Murdoch University was also provided by Mr D. G. AMMON, Mr D. A. CLARKE, Mr P. G. NICHOLAS and Ms L. A. SIMPSON. It is a pleasure to acknowledge R. D. BARNES, J. DORAN and C. A. RAYMOND for their helpful reviews of this paper.

### Literature Cited

- AMMON, D. G., BARTON, A. F. M., CLARKE, D. A. and TIANDRA, J.: Rapid and accurate chemical determination of terpenes in the leaves of *Eucalyptus* species. *Analyst* **110**, 921–924 (1985). — BARTON, A. F. M., TIANDRA, J. and NICHOLAS, P. G.: Chemical evaluation of volatile oil in eucalypts. *J. Agric. Food Chem.* **37**, 1253–1257 (1989). — BROOKER, M. I. H., BARTON, A. F. M., ROCKEL, B. A. and TIANDRA, J.: The cineole content and taxonomy of *Eucalyptus kochii* MAIDEN and BLAKELY and *E. plenissima* (GARDNER) BROOKER, with an appendix establishing these two taxa as subspecies. *Aust. J. Bot.* **36**, 119–129 (1988). — COTTERILL, P. P. and JAMES, J. W.: Number of offspring and plot sizes required for progeny testing. *Silvae Genet.* **33**, 203–209 (1984). — TURNER, H. N. and YOUNG, S. S. Y.: *Quantitative Genetics in Sheep Breeding*. Macmillan, Melbourne (1969).