

Identification of yellow stringybark (*Eucalyptus muelleriana*) and silvertop ash (*E. sieberi*) wood is improved by canonical variate analysis of ray anatomy

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Summary

The woods of yellow stringybark (*Eucalyptus muelleriana*) and silvertop ash (*E. sieberi*) are both used for the manufacture of flooring, but yellow stringybark is the preferred species because it contains fewer natural defects and dries faster and with less degrade than silvertop ash. Sometimes companies manufacturing flooring from yellow stringybark find that the wood they process contains higher levels of natural defects than expected, possibly due to the presence of silvertop ash in the wood that they receive from sawmills. It is difficult to prove this, however, because timber from both species looks similar in the rough-sawn condition and is similar anatomically. Hence, a reliable method of identifying the two species is needed. This study used canonical variate analysis to analyse linear combinations of quantitative indicators of ray anatomy in an attempt to clearly separate the wood of the two species. Canonical variate analysis scores derived from the analysis of ray height and cell number, frequency of biseriate rays and frequency of rays per square millimetre were able to completely separate 13 authentic samples of each species. This approach to separating the two species was validated using a further 16 reference specimens provided by industry (eight of each species) and 16 unknown specimens. Analysis of the reference specimens identified one specimen labelled silvertop ash that was clearly yellow stringybark, and one sample of yellow stringybark was identified as silvertop ash. Overall, however, canonical variate analysis of linear combinations of ray features produced fewer misclassifications of the two species than the use of ray parameters on their own. Canonical variate analysis shows promise as a means of more clearly separating yellow stringybark from silvertop ash and may also prove useful in separating those eucalypt wood species whose final identification in taxonomic keys depends on their ray anatomy.

Keywords: identification; separation; wood anatomy; rays; canonical analysis; yellow stringybark; silvertop ash; *Eucalyptus muelleriana*; *Eucalyptus sieberi*

Introduction

The woods of different eucalypt species that look similar can quite often have very different properties. For example, jarrah (*Eucalyptus marginata* Donn ex Smith) and karri (*E. diversicolor* F.Muell.) can look identical, but jarrah is more durable and can be

dried and machined more easily than karri (Cookson 2004; Bootle 2005). These differences may lead to the development of drying or machining defects if the two species are processed together, and reduced service life if karri is used as a substitute for jarrah in end uses requiring natural durability. Hence, both species need to be identified and processed separately to avoid such problems, and the same is true of a number of species in other groups of eucalypts. This need to distinguish superficially similar eucalypt timbers with different properties led to the early development of a range of simple and effective tests to identify individual species within commercially important groups of eucalypt timbers (Dadswell 1931; Dadswell and Burnell 1932). The most successful of these tests is the burning splinter test, which involves burning a match-stick-sized sample of sound heartwood and assessing the colour of the sample and the quantity of ash remaining on it after the test has been completed. This test is capable of separating jarrah which burns to leave a blackened, charred remnant of the sample (charcoal), from karri, which burns to form a full ash, about the same size as the original sample (Dadswell 1931). The same test can also be used to separate three anatomically similar ironbarks, red ironbark (*E. sideroxylon* Cunn. ex Woolls), narrow-leaved red ironbark (*E. crebra* F.Muell.) and grey ironbark (*E. paniculata* Smith), which burn to form a charcoal, partial ash and full ash, respectively (Dadswell and Burnell 1932). Measurement of the alkalinity of ash derived from burning eucalypt wood was also used by Dadswell (1931) to separate tallowwood (*E. microcorys* F.Muell.), whose ash is quite alkaline, from blackbutt (*E. pilularis* Smith) and species of the white mahogany group (*E. acmenoides* Schauer, *E. umbra* subspecies *umbra* R.Baker and *E. umbra* subspecies *carnea* (R.Baker) L.Johnson).

An alternative approach to separating eucalypt species is to quantify differences in the microscopic features of the wood such as numbers and sizes of both pores and rays. Such quantitative indicators are incorporated into all of the major taxonomic keys for the identification of eucalypt woods (Dadswell and Burnell 1932; Dadswell *et al.* 1934; Dadswell 1972; Ilic 1997, 2002). Nevertheless, some eucalypt species are still difficult to separate even when such quantitative indicators and specialised chemical tests are employed. A case in point is the difficulty in separating yellow stringybark (*E. muelleriana* A.Howitt) from silvertop ash (*E. sieberi* L.Johnson). Both species are found growing together in tall open forests in New South Wales and their woods

are similar anatomically. Their wood properties and processing characteristics, however, are quite different. Yellow stringybark contains fewer natural defects (gum veins, pencil streaks and pinhole borer holes and discolouration) than silvertop ash (Bootle 2005). Yellow stringybark also dries readily whereas silvertop ash dries slowly and is much more prone to surface checking and collapse during drying than yellow stringybark (Bootle 2005). Hence, of the two species, yellow stringybark is preferred for the manufacture of flooring, which requires the use of seasoned, defect-free wood, even though it is more expensive than silvertop ash. Companies manufacturing flooring from yellow stringybark sometimes find that the wood they process contains higher levels of natural defects than expected. They have long suspected that this might be due to the presence of silvertop ash in the packs of yellow stringybark wood they receive from sawmills. Accordingly, there is a strong desire by these companies for a reliable method of identifying and separating yellow stringybark from silvertop ash.

Dadswell *et al.* (1934) separated the two species based on their observations that yellow stringybark had more numerous ($>75 \text{ mm}^{-2}$) rays than silvertop ash ($<75 \text{ mm}^{-2}$), and the rays in yellow stringybark were occasionally two cells wide (biseriate, 10–25%), whereas those in silvertop ash were mainly one cell wide (uniseriate). Dadswell *et al.* (1934) also noted that ray cells in silvertop ash were mostly filled with extraneous materials (extractives) whereas in yellow stringybark such deposits, if present, were found in the cells in the centre of rays. This difference in the location of extractives was confirmed by Ilic (2002), and he made the additional observation that extractives found in the central portion of the rays in yellow stringybark were more common in the biseriate part of the rays. Ilic (2002) found considerable overlap in the ray frequency and percentage of biseriate rays in yellow stringybark and silvertop ash. Hence, his scheme for identifying the two species used differences in the abundance and distribution of extractives in rays rather than variation in ray frequency and percentage biseriation of rays. When identifying and separating wood species it is desirable to use more than one character (Jane 1970), and therefore it would be useful to find additional features that could be used to separate yellow stringybark from silvertop ash. In this study we re-examined the possibility of using the anatomy of rays to separate yellow stringybark from silvertop ash. Our approach involved the use of the multivariate statistical technique, canonical variate analysis, to analyse linear combinations of quantitative indicators of ray anatomy that maximised the variation between yellow stringybark and silvertop ash, relative to that occurring within the species. Using this quantitative approach we anticipated that it would be possible to clearly separate yellow stringybark from silvertop ash, possibly allowing companies to verify if the yellow stringybark wood they purchase inadvertently contains silvertop ash.

Materials and methods

Wood samples and scanning electron microscopy

Thirteen authentic wood samples of each species (*E. muelleriana* and *E. sieberi*) were obtained from the wood collections (xylaria) of The Australian National University Department of Forestry (now part of Fenner School of Environment and Society) and the

New South Wales Forestry Commission Laboratories in Pennant Hills, Sydney (now Forest Resources Research, NSW Department of Primary Industries). These xylarium samples were originally obtained from separate trees in southern New South Wales where yellow stringybark and silvertop ash are being harvested and processed into flooring. We also obtained 16 kiln-dried and planed reference samples of yellow stringybark and silvertop ash (eight each) from a company manufacturing flooring from both species. The former were mostly yellow-brown in colour while the silvertop ash specimens were a pinky-brown colour, except for one specimen. The same company also supplied us with sixteen unknown rough sawn wood specimens obtained from packs of yellow stringybark timber that were thought to contain silvertop ash.

Wood samples were reduced in size to match-stick sized specimens (4 mm radial \times 4 mm tangential \times 10 mm longitudinal) using a small bandsaw, and these specimens were then soaked in distilled water at 20°C for three days to soften them. These water-saturated specimens were individually clamped in a small vice located beneath the stage of a low power binocular microscope. Each specimen was viewed at low ($\times 10$) magnification and a sharp single-edged razor blade was used to manually slice thin (20–30 μm) sections from the tangential longitudinal face of the specimen until a clean, undamaged surface was obtained. Prepared specimens were then soaked in sodium hypochlorite (12%) for five minutes and cleaned in distilled water in a high-frequency sonic cleaner. They were then washed in several changes of distilled water. These cleaning procedures removed tyloses and extractives from the specimens and made it easier to observe the anatomy of the rays on prepared tangential surfaces. Specimens were dried at atmospheric pressure over silica gel for two days, and then under vacuum for eight hours. They were then cross-cut using a razor blade to make them shorter and attached to separate aluminium stubs using Nylon nail polish. These mounted specimens were then sputter-coated with a 10 nm layer of gold to make them electrically conductive. A Cambridge Instruments S360 scanning electron microscope (SEM) fitted with a high brightness lanthanum hexaboride electron source was used to obtain images of the tangential surfaces of specimens at a magnification of $\times 100$. The surface area that was imaged for each specimen varied from 1.7 to 4.5 mm² and the total number of rays within the sampled areas varied from 114 to 288. The areas that were selected were free of surface debris and contained few vessels. Digital images of surfaces were obtained and stored as TIFF files. These were subsequently viewed and analysed on a Macintosh computer using the public domain image analysis program NIH Image (V. 1.62) available at <http://rsbinfo.nih.gov/nih-image>. The following information was obtained for each wood specimen: (1) number of rays per square millimetre; (2) frequency of rays that were fully and partially biseriate; (3) mean ray height (μm); (4) mean number of cells in rays. When the measurement of an individual ray within an image was completed it was marked to ensure that it would not be recounted. Rays that were truncated by the field of view were not measured, but the ones found at the top of each image were included in the count of ray number. To balance the inclusion of these partial rays in the estimate of number of rays per square millimetre, partial rays found at the bottom of each image were not counted. Information on the anatomy of rays within each image was recorded by hand and subsequently transferred to a spreadsheet.

Canonical variate analysis

Prior to statistical analysis of data it was necessary to find appropriate summaries of ray anatomy from the measurements obtained from the different yellow stringybark and silvertop ash specimens. Exploratory analysis including detailed examination of the statistical properties of data led to the identification of five quantitative measures of ray anatomy: (1) logit of the proportion of fully biseriata rays (logit FBR); (2) logit of the proportion of fully and partially biseriata rays; (3) natural logarithm of the number of rays per square millimetre (log RN); (4) natural logarithm of ray height (log RHt); (5) mean number of cells per ray (NRC). Data for individual rays were aggregated to the specimen level and analysis of variance was used initially to examine differences in the separate features of the anatomy of rays in both yellow stringybark and silvertop ash. Canonical variate analysis was then used to formally examine in a multivariate way the differences in ray anatomy between yellow stringybark and silvertop ash (Blackith and Reymont 1971). This analysis identified linear combinations of the five anatomical features of rays that best discriminated between the two species. Combinations of variables were sought which maximised the variation between species relative to the variation within species. An important assumption which underpins the use of significance tests in canonical variate analysis is the homogeneity of co-variance matrices (Phillips *et al.* 1973). In our analyses various diagnostic methods indicated that this assumption was not violated. All statistical computation was performed using Genstat 5 (Genstat 2000).

Results

Figure 1 shows low-power scanning electron photomicrographs of tangential surfaces of yellow stringybark (Fig. 1a) and silvertop ash (Fig. 1b). The most noticeable difference is that biseriata rays are more common in yellow stringybark than in silvertop ash. Accordingly, analysis of variance of individual ray parameters in the 26 authentic xylarium specimens indicated that there were highly significant differences ($P < 0.001$) in the frequency of biseriata rays in the two species. There were also highly significant differences ($P < 0.001$) in ray number per square millimetre and in ray height between yellow stringybark and silvertop ash, but there was no significant difference ($P = 0.188$) in the number of cells in the rays of the two species. These observations confirm previous findings that yellow stringybark has shorter rays with fewer cells, a greater number of rays per square millimetre and greater levels of biseriata than silvertop ash (Dadswell *et al.* 1934; Ilic 2002).

Figure 2 compares the individual features of the rays in yellow stringybark and silvertop ash. The degree of separation of the two species was greatest for the logit of the proportion of fully biseriata rays and log of ray height, but there was overlap in the data between species. In contrast, canonical variate analysis of a linear combination of ray parameters was able to completely separate the 26 authentic xylarium specimens into their respective species. The function for discriminating between the two species is

$$\text{CVA score} = -68.4 - 0.858 \times \text{logit FBR} - 2.22 \times \text{NRC} + 16.346 \times \text{log RHt} - 0.202 \times \text{log RN.} \quad (1)$$

This equation simply discriminates between two groups and in such cases the fraction of variance explained is always 100%.

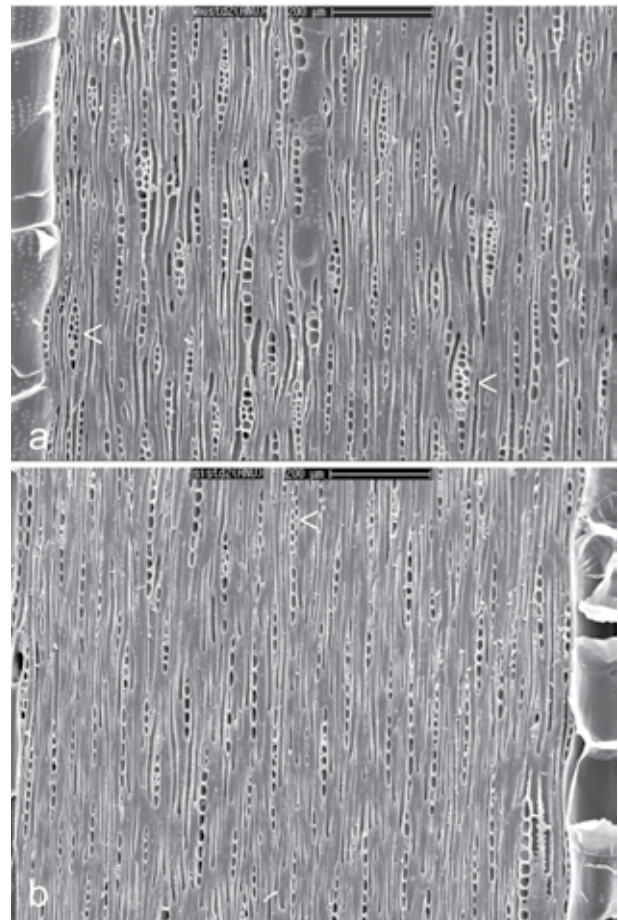


Figure 1. Scanning electron photomicrographs of tangential longitudinal surfaces of (a) yellow stringybark: note the fully biseriata ray (arrowed, left) and the partially biseriata ray (arrowed, right) and (b) silvertop ash: note the biseriata ray (arrowed, top)

A negative value for the canonical variate analysis (CVA) score indicates that the specimen is yellow stringybark, whereas a positive value indicates that the specimen is silvertop ash. The larger the absolute values the more certain the classification.

Figure 3 plots the CVA scores for the authentic yellow stringybark and silvertop ash xylarium specimens. In one case there was a misclassification of a silvertop ash sample, which had a small negative CVA score of -0.04 (arrowed in Fig. 3). This sample was classified correctly if the variable logit of the proportion of fully and partially biseriata rays was used instead of the logit of the proportion of fully biseriata rays in equation (1). In general, however, the latter variable maximised variation between yellow stringybark and silvertop ash, relative to that occurring within species, and was therefore used in preference to the logit of the proportion of fully and partially biseriata rays for the separation of the two species.

The additional 16 industry reference samples that were examined in this study were then classified using the canonical variate scores derived from the xylarium samples of known identity. The method of assignment involved calculating, from the CVA scores, the Mahalanobis-squared distances between each sample and the group means for the yellow stringybark and silvertop ash xylarium samples. Each sample was then allocated to the group

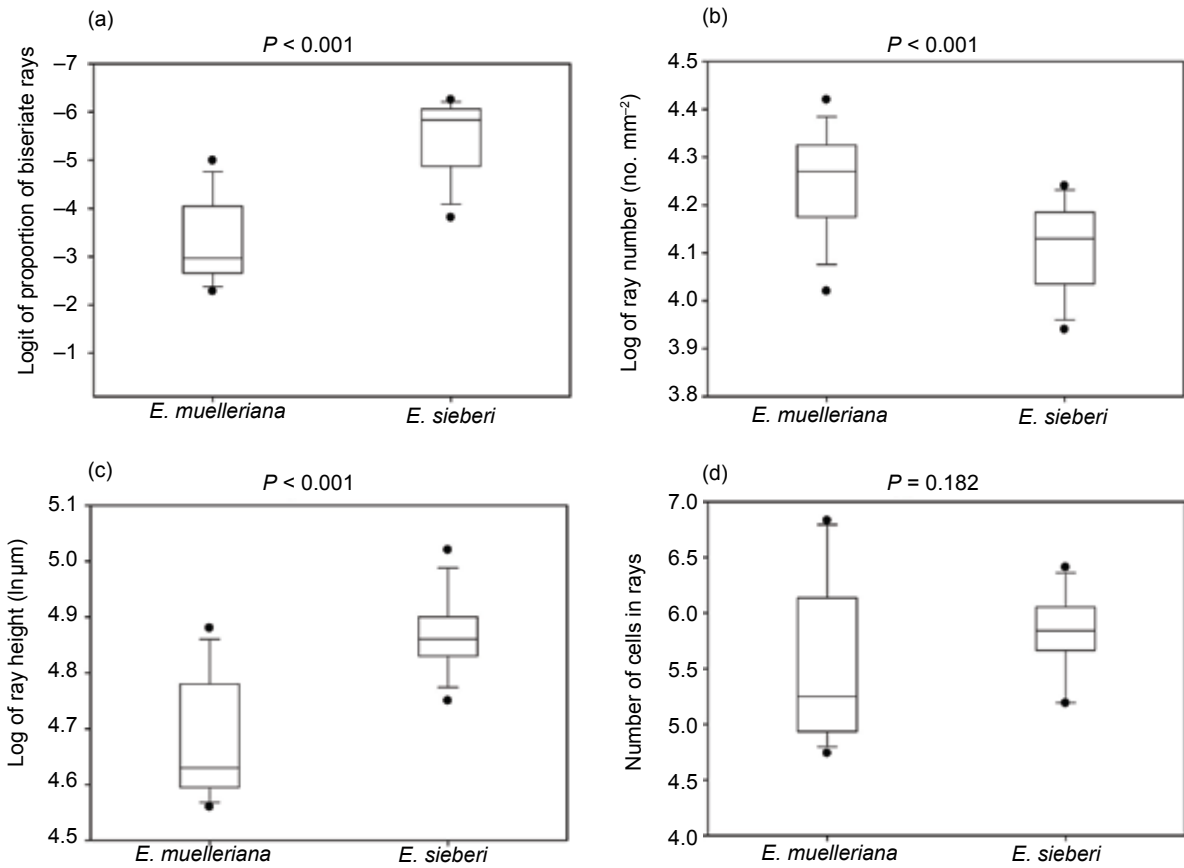


Figure 2. Comparison of individual ray parameters in yellow stringybark (*E. muelleriana*) and silvertop ash (*E. sieberi*); (a) logit of the proportion of fully biseriata rays; (b) natural logarithm of number of rays per square millimetre; (c) natural logarithm of ray height; (d) number of cells per ray

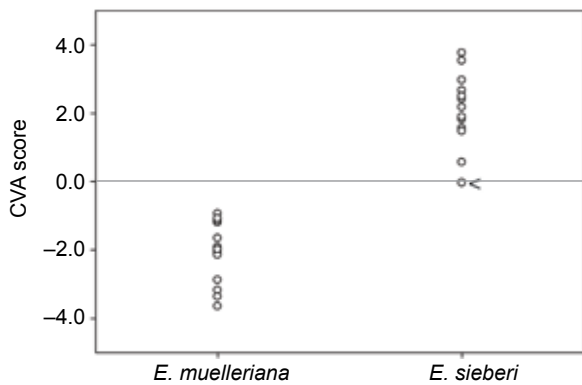


Figure 3. Canonical variate analysis scores for authentic xylarium specimens of yellow stringybark (*E. muelleriana*) and silvertop ash (*E. sieberi*). See text for comment on the point arrowed.

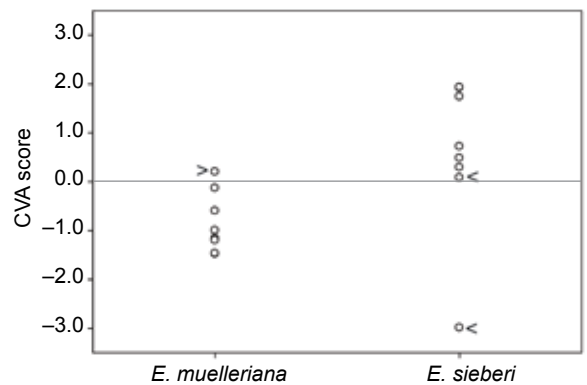


Figure 4. Canonical variate analysis scores for a reference set of yellow stringybark (*E. muelleriana*) and silvertop ash (*E. sieberi*) specimens provided by industry. See text for comment on the points arrowed.

that had the smallest Mahalanobis-squared distance to the group mean. As there are only two groups in this case, the allocation rule reduces to assignment based on the sign of the score (negative or positive) as described above. Figure 4 plots the CVA scores for the reference set of samples that were obtained from industry and believed to contain eight samples of each species.

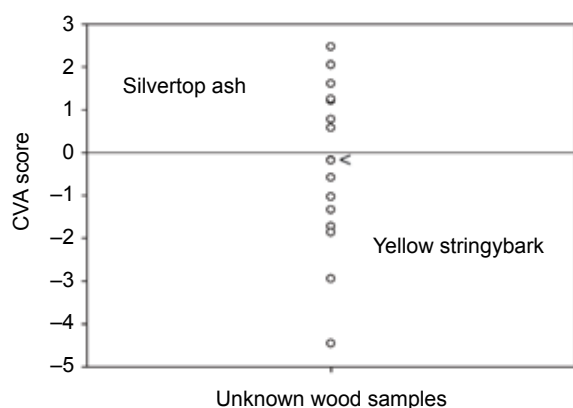
One sample of silvertop ash from the industry reference set was clearly misclassified as it had a large negative CVA score of -2.99 and it was not the same pinky-brown colour as the other silvertop

ash samples. One sample of yellow stringybark had a positive CVA score of 0.2 and therefore was identified as silvertop ash (arrowed in Fig. 4), and one sample of silvertop ash had a very low CVA score of 0.08 (arrowed in Fig. 4). Table 1 shows the false identifications produced by using CVA scores to identify yellow stringybark and silvertop ash compared to those produced by using individual features of the rays to identify the two species. The results exclude the silvertop ash sample with the very large negative CVA score that was clearly misclassified, and includes

Table 1. Misclassifications of wood samples resulting from the use of canonical variate analysis (CVA) scores or individual ray parameters

| Wood samples | Parameter | | | |
|-------------------------|-----------|--------------------------|------------|---------------------------------------|
| | CVA score | Frequency of biseriation | Ray height | Ray frequency (no. mm ⁻²) |
| Yellow stringybark (13) | 0 | 1 | 3 | 4 |
| Yellow stringybark (8) | 1 | 3 | 2 | 3 |
| Silvertop ash (13) | 0 | 1 | 1 | 3 |
| Silvertop ash (7) | 0 | 0 | 1 | 0 |
| Total* | 1 (2.4) | 4 (9.7) | 7 (17.1) | 10 (24.4) |

*Percentage in brackets

**Figure 5.** Canonical variate analysis scores for 16 unknown yellow stringybark and silvertop ash specimens provided by industry. See text for comment on the point arrowed.

the correct identification of one of the 13 xylarium samples of silvertop ash based on the logit of the proportion of fully and partially biseriate rays rather than the logit of the proportion of fully biseriate rays.

Clearly, the use of CVA scores derived from canonical variate analysis of linear combinations of ray features produced fewer misclassifications of the two species than the use of individual ray parameters. The use of CVA scores to identify the two species produced one misclassification whereas when frequency of biseriation, ray height and ray density were used on their own to identify the species there were four, seven and ten misclassifications, respectively.

CVA scores were also calculated for the 16 unknown samples supplied by a company manufacturing yellow stringybark flooring. These scores are plotted in Figure 5. Nine of these unknown samples had negative CVA scores and were therefore classified as yellow stringybark and the remaining samples had positive CVA scores indicating that they were silvertop ash. Two of the samples classified as yellow stringybark had low negative CVA scores of -0.19 and -0.18 (arrowed in Fig. 5) and their classification is less certain than that of the other samples with negative CVA scores.

Discussion

Canonical variate analysis scores derived from the analysis of a linear combination of ray height and cell number, frequency of fully biseriate rays and number of rays per square millimetre were clearly better at separating yellow stringybark from silvertop ash than the use of any of the ray parameters on their own. Canonical variate analysis of vessel, ray and parenchyma features has been used previously to separate wood species in the Brazilian genus *Swartzia* into groups (Angyalossy-Alfonso and Miller 2002), but this is the first time that it has been used to identify and separate eucalypt woods. There are a number of other eucalypt woods whose final separation in the identification keys developed by Dadswell and co-workers depends on quantitative features of ray anatomy (Dadswell and Burnell 1932; Dadswell *et al.* 1934). For example, messmate stringybark (*E. obliqua* L'Heritier) is separated with difficulty from alpine ash (*E. delegatensis* R.T.Baker) and mountain ash (*E. regnans* F.Muell.) by means of the number of rays per square millimetre, ray height and frequency of bi- and triseriate rays (Dadswell *et al.* 1934). Messmate stringybark is less prone to collapse and is more durable than either alpine or mountain ash, and the figure produced by growth rings on the back-sawn (tangential) faces of sawn boards is more pronounced in alpine ash than in the other two species (Ilic 1997). Accordingly, there has been interest in developing a more reliable method for the identification and separation of messmate stringybark, alpine ash and mountain ash (Ilic 1997). Our results suggest that the use of canonical variate analysis of quantitative features of their ray anatomy would improve the identification and separation of these ash type eucalypts and possibly other anatomically similar eucalypt woods. It should be noted, however, that such an approach would be suitable only for the identification and separation of wood obtained from mature trees. As pointed out by Ilic (1997), 'overlap of the quantitative cell dimensions is wider in young wood', which makes it difficult to use quantitative anatomical features to identify the juvenile wood of different eucalypt species.

Canonical variate analysis of rays in yellow stringybark and silvertop ash, however, was not completely effective at separating the two species because it produced one misclassification of a reference specimen of yellow stringybark supplied by industry.

Furthermore, correct identification of one of the xylarium samples of silvertop ash was only possible by changing one of the parameters used to calculate canonical variate analysis scores. Hence, we did not fully achieve our aim of developing a method of separating the two species that was completely effective. Nevertheless, the approach was able to identify 16 unknown wood specimens as either yellow stringybark or silvertop ash based on the sign (negative or positive) of their CVA scores. Better separation of the two species might be achieved by including an additional parameter that has the desired property of maximising variation between yellow stringybark and silvertop ash, relative to that occurring within the species. One parameter worthy of further examination is the proportion of ray cells within rays that are filled with extractives, because previous studies have noted that rays in yellow stringybark contain fewer cells that are filled with extraneous materials than cells in the rays of silvertop ash (Dadswell *et al.* 1934; Ilic 2002). We were unable to use this parameter here because our method of preparing specimens for scanning electron microscopy removed extraneous materials from the rays to make it easier to see them under the microscope. Future research on the use of ray parameters to separate yellow stringybark from silvertop ash should therefore use a method of preparing wood specimens prior to microscopy that does not remove extraneous deposits from rays. The use of sections cut by hand from water-soaked specimens would fulfil this criterion. These would be suitable for examination using light microscopy, which would be a more practical method of obtaining information on ray anatomy than scanning electron microscopy.

The method of separating yellow stringybark from silvertop ash described here could be used by companies processing yellow stringybark to perform occasional spot-checks to determine that the wood they purchase from sawmills does not contain silvertop ash. Our approach, however, would be too time-consuming to be used routinely to separate the two species at the mill gate. A more rapid method of separating the two species would need to be developed to do this. Ideally such a method would also be able to separate yellow stringybark from other stringybark species such as white stringybark (*E. eugenioides* Sieb. ex Spreng.) and blue stringybark (*E. agglomerata* Maiden) that grow in association with yellow stringybark in the forests of New South Wales, and are sometimes included in the packs of yellow stringybark wood delivered to flooring and furniture companies. The quality of the wood of these stringybark species is not as good as that of yellow stringybark. For example, white stringybark has interlocked grain and is reported to be more prone to collapse during drying than yellow stringybark (Bootle 2005). Furthermore, both white and blue stringybark are light brown whereas yellow stringybark is yellow-brown (Bootle 2005). Hence, it would be desirable to develop a means of separating these stringybark species from yellow stringybark. Previous attempts to separate yellow and white stringybark using wood anatomical features have been unsuccessful (Dadswell *et al.* 1934; Ilic 2002) and therefore it may be better to separate the two species using differences in their chemical composition. One technology that shows promise in rapidly separating wood species using differences in their chemical composition is near infrared spectroscopy (Brunner *et al.* 1996; Tsuchikawa 2007). This technique in combination with principal components analysis has been used to separate southern

blue gum (*E. globulus* Labill.), shining gum (*E. nitens* Maiden) and rose gum (*E. grandis* W.Hill ex Maiden) (Schimleck *et al.* 1996), and it is possible that it might be able to rapidly segregate yellow stringybark and silvertop ash and possibly separate yellow stringybark from white and blue stringybark. Further research would be needed to confirm this, and also to examine more generally the ability of multivariate statistical techniques to improve the identification and separation of anatomically similar eucalypts.

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