Discrimination between seedlings of *Eucalyptus globulus*, *E. nitens* and their F_1 hybrid using near-infrared reflectance spectroscopy and foliar oil content

By J. R. Humphreys[1),*)], J. M. O'Reilly-Wapstra[1)], J. L. Harbard[1)], N. W. Davies[2)], A. R. Griffin[1)], G. J. Jordan[1)] and B. M. Potts[1)]

(Received 5th June 2007)

Summary

Identification of plant hybrids produced from closely related species can be difficult using morphological characteristics alone, particularly when identifying young seedlings. In this study, we compared the performance of three calibration models developed to discriminate between seedlings of *Eucalyptus globulus*, *E. nitens* and their first-generation hybrid using either foliar oil chemistry or near-infrared reflectance spectral data from fresh, whole leaves. Both oil and near-infrared reflectance spectroscopy (NIRS) models were developed using partial least-squares discriminant analysis and showed high classification accuracy, all correctly classifying more than 91% of samples in cross-validation. Additionally, we developed a larger, "global" and independently validated NIRS model specifically to discriminate between *E. globulus* and F_1 hybrid seedlings of different ages. This model correctly classified 98.1% of samples in cross-validation and 95.1% of samples from an independent test set. These results show that NIRS analysis of fresh, whole leaves can be used as a rapid and accurate alternative to chemical analysis for the purpose of hybrid identification.

Key words: eucalypt, spectra, 1,8-cineole, NIRS, fresh leaves, discriminant analysis, partial least-squares regression.

Introduction

Eucalypts are renowned for their propensity to hybridise, both in the wild and artificially (Pryor, 1976; Griffin *et al.,* 1988; Potts *et al.,* 2003). There has been considerable interest in using eucalypt hybrids in commercial forestry to extend the geographic range of economically important species and to optimise economic traits (Martin, 1989; Tibbits *et al.,* 1991; Potts and Dungey, 2004). One such case involves *Eucalyptus globulus*, which is an important hardwood plantation tree in many temperate regions throughout the globe, due to its fast growth and excellent pulpwood properties (Potts *et al.,* 2004). The relatively poor frost tolerance of this species restricts its use in some regions, where it is replaced in plantations by more frost tolerant species, such as *E. nitens* (Tibbits *et al.,* 1997). Efforts have been made to produce hybrids that combine the frost tolerant properties of *E. nitens* with the favourable wood properties of *E. globulus* (Tibbits, 1989; Volker, 1995; Potts *et al.,* 2000; Volker, 2002). Such hybrids are currently used commercially in plantations in Chile (Espejo *et al.,* 1995; Griffin *et al.,* 2000).

Previous attempts to produce hybrid crosses between *E. globulus* and *E. nitens* have been constrained, as crosses with *E. nitens* as the mother produce low numbers of seeds (Tibbits, 1989; Gore *et al.,* 1990) and conventional crosses with *E. globulus* as the mother are not possible due to differences in flower size (Gore *et al.,* 1990). A new method of crossing with *E. globulus* as mother has been developed to produce hybrids (J.L. Harbard, unpublished data), although, inevitably a small proportion of pure *E. globulus* contaminants are also produced. Early identification of hybrid seedlings is important for clonal evaluation programs to avoid the propagation of non-target genotypes. Visual classification of *E. globulus* x *E. nitens* hybrid seedlings can be achieved by trained observers, but can be difficult, particularly when seedlings are in the early stages of development (Tibbits, 1988). Alternative approaches to hybrid identification include isozyme (Barbour *et al.,* 2002), DNA (Sale *et al.,* 1996; Delaporte *et al.,* 2001) and chemical (Grayling and Brooker, 1996; Espejo *et al.,* 2004) analysis. However, such approaches can be costly and time-consuming, making large-scale analysis impractical. A rapid, cost-effective and accurate analytical technique for large-scale screening of these hybrids is therefore required.

Near-infrared reflectance spectroscopy (NIRS) is a rapid analytical technique that reduces laboratory time considerably compared to conventional laboratory analysis techniques (Foley *et al.,* 1998). It is non-destructive, requires little sample preparation and can be used for the simultaneous analysis of numerous constituents or attributes (Batten, 1998). For NIRS analysis, samples are irradiated with near-infrared light, which is absorbed selectively, depending on the presence and arrangement of bonds between carbon, hydrogen, oxygen and nitrogen (Givens *et al.,* 1997). The collected spectrum provides a detailed representation of the biochemical profile and certain physical characteristics of a sample (Bokobza, 1998; Ourcival *et al.,* 1999). Spectral data are combined with multivariate analysis techniques such as principal components analysis and partial least-squares regression to enable numerous types of quantitative or qualitative analysis. Near-infrared reflectance spectroscopy is being increasingly used in

1) School of Plant Science and Cooperative Research Centre for Forestry, University of Tasmania, Private Bag 55, Hobart, Tasmania, Australia, 7001.

2) Central Science Laboratory, University of Tasmania, Private Bag 74, Hobart, Tasmania, Australia, 7001.

*) Corresponding author: Telephone: (03) 62262563, Fax: (03) 62262698. E-mail: jrh@utas.edu.au

DOI:10.1515/sg-2008-0040
edited by Thünen Institute of Forest Genetics

forestry and forest ecology. For example, it has been used to quantify wood property (POKE and RAYMOND, 2006) and phytochemical traits (GILLON *et al.,* 1999; STOLTER et al., 2006), classify tree provenances based on leaf or wood-core spectra (SCHIMLECK *et al.,* 1996; LISTER *et al.,* 2000), monitor putative seed sources (TIGABU *et al.,* 2005) and to discriminate between infested and uninfested forest tree seeds (TIGABU *et al.,* 2004). However, to our knowledge, only one study to date has successfully used NIRS to identify hybrids (ATKINSON *et al.,* 1997).

The aim of this paper is to determine whether this rapid analysis technique can be used to discriminate between leaves of pure *Eucalyptus globulus*, *E. nitens* and their first-generation (F_1) hybrid. Consequently, we specifically aimed to:

1. Determine whether foliar oil and NIRS models can be developed to accurately discriminate between the leaves from these cross types, particularly *E. globulus* and their F_1 hybrid, sampled at the same time.

2. Compare the performance of NIRS and oil models to determine whether NIRS can be used instead of the slower and more expensive analysis of foliar oil composition.

3. Develop and independently validate a "robust" NIRS model to discriminate between *E. globulus* and F_1 hybrid leaves sampled at different times.

Materials and Methods

Genetic Material

The study was conducted on seedlings grown from: 1) intraspecific *E. globulus* poly-crosses from four mother trees; 2) pure open-pollinated *E. nitens* from four mother trees; and 3) F_1 hybrid offspring produced from controlled crosses of a mix of the pollen of the four *E. nitens* trees to each of the four *E. globulus* trees. *Eucalyptus nitens* seed was sown nine months before the planting of the *E. globulus* and F_1 hybrid seed. It is possible that the resulting age differences could have influenced the ability of the oil and NIRS models to discriminate leaves of *E. nitens* from those of *E. globulus* and the F_1 hybrid. However, we considered this effect to be minimal; while the chemical profile of *E. nitens* foliage changes considerably during early development, once seedlings are aged approximately six months to two years, the chemical profile of this species remains relatively stable (LONEY, 2007). All seedlings were grown under glasshouse conditions; *Eucalyptus globulus* and F_1 hybrid seedlings were grown in 18 trays, with 96 pots per tray and *E. nitens* seedlings were planted in four family blocks within one tray. Trays were planted in family blocks and cross-type blocks within family and their position randomised, resulting in the *E. globulus*, *E. nitens* and F_1 hybrid seedlings being effectively intermixed at random within the glasshouse.

To cull out pure *E. globulus* contaminants from the F_1 progeny, two observers experienced with *Eucalyptus* morphology (authors POTTS and GRIFFIN) independently classified the seedlings at two and a half months of age as either F_1 hybrid or *E. globulus*. Because of the varying developmental stages of the seedlings, it was impracticable to use the morphometric approach of TIBBITS (1988). The observers therefore relied on a visual integration of leaf and apical bud shape and glaucousness of the foliage as discriminating traits. Decisions between observers were unanimous in the majority of cases and only this sub-set of the population was retained for further study.

Sampling

Nine F_1 hybrids and 39 *E. globulus* seedlings were sampled at 2.5 and 6 months of age for NIRS and chemical analyses, which constituted sampling time one and two, respectively (Some plants were not harvested at each sampling time if leaf material of sufficient quality was unavailable). At 6.5 months of age (sampling time three), the same plants and an additional 89 *E. globulus* and 84 F_1 hybrid seedlings were sampled for NIRS analysis. Thirty-nine 11.5 month old *E. nitens* seedlings (sown nine months prior to *E. globulus* and F_1 hybrid seedlings) were also sampled for NIRS and chemical analyses at sampling time one. Sampling involved harvesting the youngest fully expanded leaf pair from the main stem of each seedling. One leaf was used for NIRS analysis and the other for oil analysis, where required. To determine if the performance of the *E. globulus*-F_1 hybrid NIRS model could be improved by using spectra from a particular leaf, or the average of three leaves, an additional two leaves were sampled at the fourth and eighth nodes from the 6 month old seedlings. This increased sampling did not improve the model. Hence, these additional leaves were not retained in the model and only one leaf was sampled from the 6.5 month old seedlings. Harvested fresh leaves were kept cool and analysed for NIRS and chemistry on the same day.

Chemical analysis

Analyses of essential oil composition were carried out on all samples collected at the first and second sampling (78 *E. globulus* leaves, 18 F_1 hybrid leaves and 39 *E. nitens* leaves). Essential oils were extracted using dichloromethane with heptadecane as an internal standard (O'REILLY-WAPSTRA et al., 2004). Analyses were carried out using Varian 8400 autosampler on a Varian 3800 gas chromatograph directly coupled to a Varian 1200 triple quadrupole mass spectrometer. Based on prior analyses of pure *E. nitens* and *E. globulus* (J.L. Harbard, unpublished data), 21 volatile compounds were selected as markers for subsequent quantitative analysis and identification of hybrids. These compounds were alpha-pinene, alpha-phellandrene, p-cymene, limonene, 1,8-cineole, gamma-terpinene, isoamyl isovalerate, alpha-terpineol, terpinyl acetate, caryophyllene, aromadendrene, humulene, alloaromadendrene, bicylcogermacrene, ledol, spathulenol, linalyl isovalerate, globulol, viridiflorol, beta-eudesmol and eudesmyl acetate (putative). They were identified based on their electron ionization mass spectra and Kovats' retention indices (ADAMS, 1989; DAVIES, 1990). Reference mass spectra were available from commercial libraries (NIST MS database, 2002) and a specific essential oil mass spectral library developed in-house.

DOI:10.1515/sg-2008-0040
edited by Thünen Institute of Forest Genetics

Compounds were quantified based on their total ion current (TIC) peak areas. Where peaks were not fully resolved the chromatogram of a diagnostic ion for the target compound was generated. Final results were expressed as the ratio of the TIC for each target compound to the TIC for the internal standard.

Collection of NIRS Spectra

Individual whole fresh leaves were scanned with near-infrared (NIR) radiation using a Bruker MPA FT-NIR spectrometer coupled to a fibre-optic probe. NIR spectra (expressed as log (1/R)) were collected from 7690–4350 cm^{-1} (1300–2300 nm) at a resolution of 4 cm^{-1}. The tip and the basal region of the adaxial surface of each leaf were scanned, avoiding the midrib and damaged areas. The resulting two spectra (four scans each) for each leaf were averaged.

Oil and NIRS models

Oil and NIRS models were created for pair-wise discrimination of leaves from seedlings of: a) pure *E. globulus* and *E. nitens*; b) *E. globulus* and F_1 hybrids; and c) *E. nitens* and F_1 hybrids. Prior to analysis, a principal component analysis was carried out on both the raw chemical and spectral data to identify any outliers. No spectral or chemical outliers were detected. Oil models were developed using oil chemistry data obtained from the first two sampling times. One set of NIRS models (hereafter referred to as NIRS1) used the same samples as those used for the oil chemistry models. In addition, a larger and independently validated NIRS model (NIRS2) contrasting *E. globulus* and F_1 hybrids was developed using 207 samples randomly selected from the 310 samples (98 from the original model and 212 sampled at age 6.5 months) available in total. The remaining 103 plants were used to validate the resulting model.

Models for both NIRS and oil chemistry data were created using The Unscrambler software (version 9.7). Calibration models to discriminate between each class were derived with partial least-squares discriminant analysis (PLS-DA; see GELADI *et al.,* 1996; NAES *et al.,* 2002). For this two-group analysis, each class (y-vector) was arbitrarily assigned a "dummy" variable of either "0" or "1". These numerical values were then regressed against the spectra matrix. Samples predicted by the model as <0.5 were assigned to group "0", whereas samples predicted to be ≥ 0.5 were assigned to group "1". Segmented cross-validation was used to optimise the number of PLS factors used in each model and to provide a preliminary indication of the models' predictive potential (e.g., COZZOLINO *et al.,* 2003; LAWLER *et al.,* 2000). Cross-validation involves removing one or more samples at a time from the calibration set and predicting its value using a model created from the remaining samples (DAVIES, 1998). In the present case, each validation segment comprised eight samples. The ability of the model to predict these removed samples was then averaged, producing the standard error of cross-validation (SECV), which provides an indication as to the accuracy of the model in predicting samples within the calibration set. In addition, the coefficient of determination (r^2) was calculated, which describes the correlation between the variation in the response variable (class) and the predictor variables (in this case either spectra or oil chemistry). The percentage of correctly classified samples was also calculated (% correct), which provided an additional method for assessing the performance of the model. The model was further refined by carrying out an uncertainty test (DAVIES, 2001). Using this procedure, statistically non-significant predictor variables were removed and the model was recalculated.

In order to achieve NIRS models with the best accuracy, a number of pre-treatments were performed on all spectra, including: 1) no treatment; 2) conversion to first derivative (Norris-Gap with 3 smoothing points); 3) standard normal variate (SNV); and 4) SNV and first derivative conversion. Standard normal variate is used to reduce noise related to physical characteristics of the sample and derivative conversions are used to reduce the effects of baseline shift and to emphasise the presence of subtle spectral features (BARNES *et al.,* 1989; CONZEN, 2003). In the present case, treatment 4 produced models with the greatest accuracy and only the results from these models were analysed.

In addition to the pair-wise approach we also undertook the PLS discrimination analysis using a three-way approach (PLS2 regression) which sequentially discriminated one class from the pool of the other two classes (e.g. WOODCOCK *et al.,* 2007). However, these three-way models were generally not as accurate in classifying the F_1 hybrids. We have thus focused on the pair-wise analyses as in most cases the application of the model will focus on separating F_1 hybrids from contaminant pure species seedlings from the female parent.

Results

Chemical and spectral profiles

Eucalyptus globulus, *E. nitens* and F_1 hybrid seedlings differed in both oil chemistry and NIRS spectra. Total oils occurred in greater concentrations in *E. globulus*, least in *E. nitens* and intermediate concentrations in the F_1 hybrid (*Figure 1*). A similar pattern was observed for concentrations of 1,8-cineole, which was highly correlated with total oils and comprised almost half of the total oil content (data not shown). Kruskal-Wallis tests between the same-aged *E. globulus* and F_1 hybrid seedlings, showed statistically significant differences between these two groups for total oils ($X^2_1 = 5.25$, $P = 0.022$) and 1,8-cineole ($X^2_1 = 8.30$, $P = 0.004$). Highly significant ($P<0.01$) differences also occurred for a number of minor oil components, some of which occurred in greater concentrations in *E. globulus* (terpinyl acetate, caryophyllene, aromadendrene, alloaromadendrene, bicylcogermacrene, ledol, globulol, viridiflorol, beta-eudesmol and eudesmyl acetate) and others which were higher in the F_1 hybrid (linalyl isovalerate, isoamyl isovalerate).

Subtle differences could be seen in several regions of the average NIRS spectrum of *E. globulus*, *E. nitens* and their F_1 hybrid after first derivative and SNV transformations (*Figure 2*). Notable differences occurred between approximately 1900 nm and 2150 nm, which

DOI:10.1515/sg-2008-0040
edited by Thünen Institute of Forest Genetics

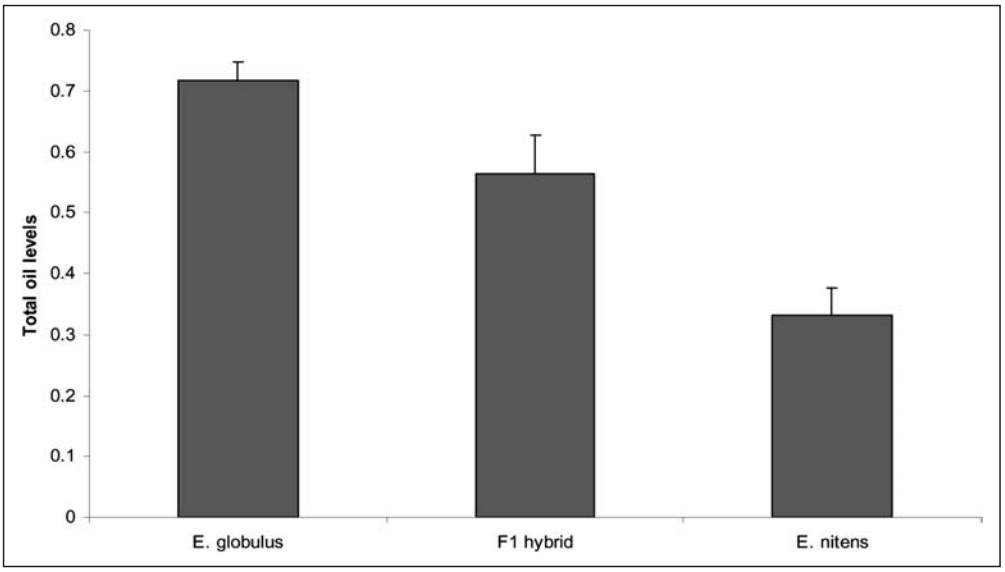

Figure 1. – Differences in total foliar oil concentration as measured by the logarithm of the total ion current (TIC) ratio relative to an internal standard, for seedlings of *E. globulus* (n = 78 leaves), *E. nitens* (n = 39) and F_1 hybrid (n = 18).

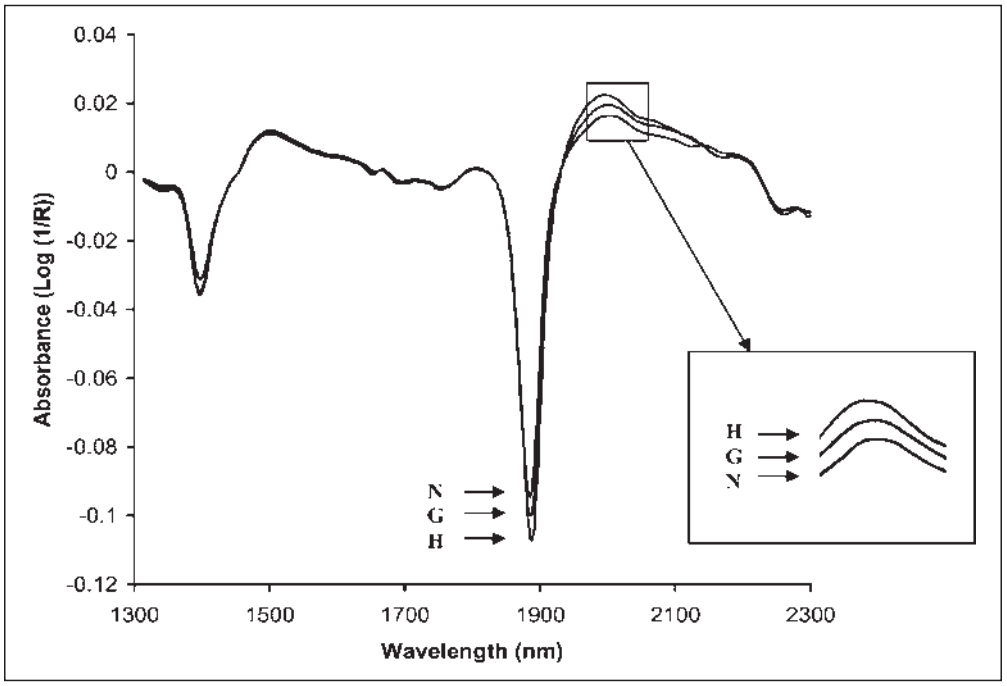

Figure 2. – The average spectra (1300–2300 nm) after first derivative and SNV transformations of *E. globulus* (G; n = 78 leaves), *E. nitens* (N; n = 39) and F_1 hybrid (H; n = 18) seedlings.

included a major water peak at 1900 nm. This region is characterised by N-H and O-H combination bands and C=O stretch first overtones (CONZEN, 2003). Differences were also observed at approximately 1400nm, which is another water absorption region and is associated with C-H combination bands and O-H first overtones (CONZEN, 2003).

Oil and NIRS models

All oil and NIRS models correctly classified more than 91% of samples in the calibration set (*Table 1*). However, the NIRS models showed the same or higher classification accuracy (96.9–100%), higher r^2 and lower SECV values than the corresponding oil models (*Table 1*; *Figure 3*), all of which suggest that the NIRS models have a predictive power at least equivalent to the oil models. Furthermore, the NIRS2 model correctly classified 95.1% of samples in the independent test set. Observation of the NIRS regression coefficients (not presented) showed that similar regions throughout much of the spectral range contributed to the development of each NIRS model. In particular, regions around and above

DOI:10.1515/sg-2008-0040
edited by Thünen Institute of Forest Genetics

Table 1. – Calibration statistics for the oil and NIRS *E. globulus-E. nitens*, *E. nitens*-F_1 and *E. globulus*-F_1 models. NIRS1 refers to models developed from leaves collected fom 2.5 and 6 month old seedlings. NIRS2 refers to the larger *E. globulus*-F_1 NIRS model (heading codes are described in Materials and methods).

Model	Method	Factors	r^2	SECV	% correct	N
E.globulus-nitens	Oil	5	68.9	0.26	96.5	117
	NIRS1	4	90.0	0.14	100	117
E. nitens-F_1 hybrid	Oil	5	61.6	0.29	100	57
	NIRS1	4	82.8	0.19	100	57
E.globulus-F_1 hybrid	Oil	8	54.9	0.26	91.3	96
	NIRS1	6	57.9	0.25	96.9	96
	NIRS2*	6	88.1	0.23	98.1	207

* The NIRS2 model correctly classified 95.1% of samples in the independent test set.

1900 nm were important in developing models, which was not surprising given the notable visual differences in this region between the average spectra of the three groups (*Figure 2*). For all oil models, the full suite of compounds were retained after the uncertainty test and used to derive the calibration models.

Discussion

The results of this study demonstrate that multivariate analyses of both oil chemistry and NIRS spectra of whole fresh leaves are effective methods for discriminating between leaves of *E. globulus*, *E. nitens* and their F_1 hybrid. However, in all three comparisons, the NIRS models were more accurate at discriminating between groups than the oil models. The results from the NIRS models are consistent with other studies using NIRS, where high levels of accuracy are often attained when discriminating between groups or species of plants (LISTER *et al.,* 2000; TIGABU and ODEN, 2003; BERTRAND *et al.,* 2005; COZZOLLINO *et al.,* 2005). In particular, Atkinson's (2004) NIRS model discriminated between two species of *Betula* and their F_1 hybrid with 100% accuracy.

Analysis of the regression coefficients for the NIRS models showed that a number of spectral regions were used to develop these models, including regions reported to be associated with absorption of oil components. For example, EBBERS *et al.* (2002) found that pure 1,8-cineole absorbed strongly in the 2250–2300 nm region, which is a region that contributed to the development of the NIRS models in the current study. However, it is difficult to attribute regions of the NIR spectrum to specific chemical compounds, as the broad absorption peaks typical of NIR spectra can represent more than one functional group (FOLEY et al., 1998). The fact that important information occurred throughout much of the NIR spectrum rather than any distinct regions, suggests that there may be other chemical differences between these species, besides foliar oil content. For example, *E. globulus* seedlings contain much higher levels of a formylated phloroglucinol compound, the macrocarpals, compared to *E. nitens* seedlings (N.E. Glancy, Personal Communication). Nevertheless, the broad biochemical profile of a sample provided by NIRS can enable the development of useful models, regardless of whether the chemical differences that occur between species or groups are known (RICHARDSON et al., 2003; MOLLER, 2004).

The present study differs from the majority of other studies that have used NIRS, in that the NIRS models were developed using spectra from whole, fresh leaves.

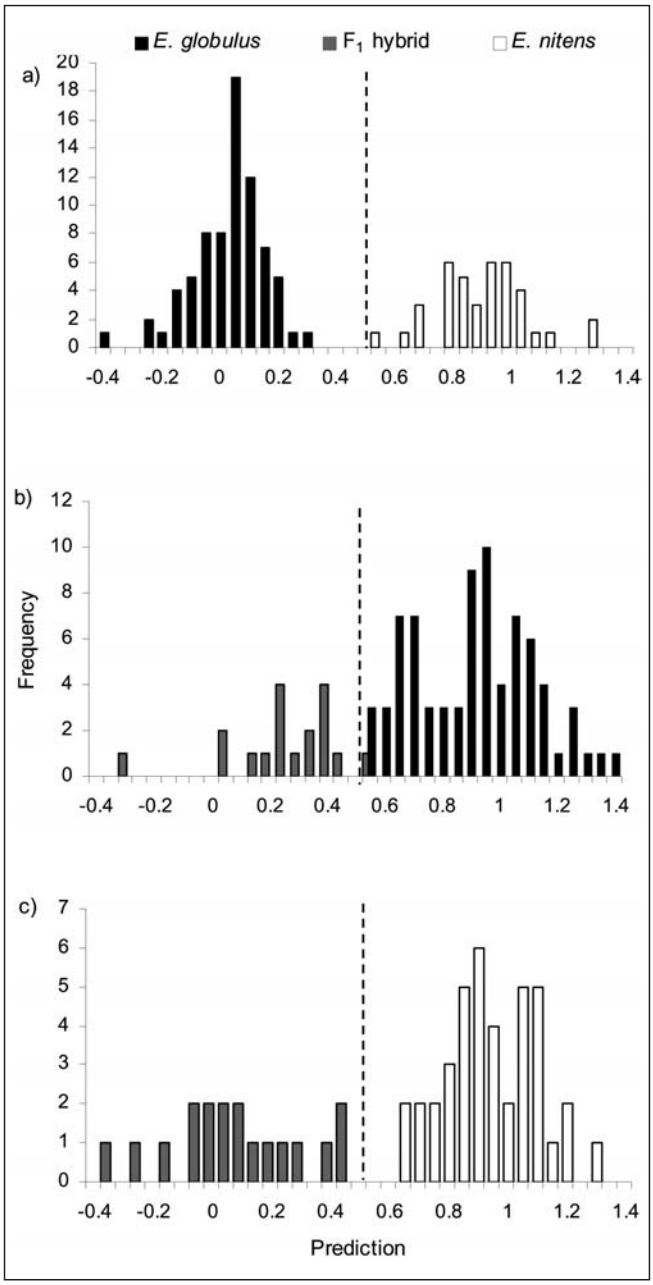

Figure 3. – Predictions using NIRS1 models for: (a) *E. globulus-E. nitens* (b) *E. globulus*-F_1 and (c) *E. nitens*-F_1 models. Samples predicted by the model as <0.5 are assigned to class "0", whereas samples predicted to be ≥0.5 are assigned to class "1". Vertical dotted lines indicate the boundary between class "0" (left of line) and class "1" (right of line).

DOI:10.1515/sg-2008-0040
edited by Thünen Institute of Forest Genetics

The conventional method of preparation for NIRS analysis is to freeze-dry and grind samples prior to scanning. The former procedure reduces the potential for the hydrogen bonds in water to mask the presence of compounds whose absorption peaks overlap those of water (VILJOEN *et al.,* 2007), while the latter makes the sample homogeneous, thus minimising the effects of light scattering (GIVENS *et al.,* 1997). However, a limited number of other studies have demonstrated that successful NIRS models can be developed using fresh leaves. For example, leaf chemical traits in animal silages and wheat have been quantified using fresh leaf material (PARK *et al.,* 1998; MORON *et al.,* 2007). In an ecological study, EBBERS *et al.* (2002) developed successful quantitative NIRS models for 1,8-cineole, nitrogen and sideroxylonal in whole, fresh, eucalypt leaves, even though the absorption of pure sideroxylonal was shown to overlap with water absorption regions. Hence, there is great potential for analysis of fresh, intact leaves using NIRS.

Chemical analysis techniques have been used to identify or validate hybrids in a range of plant species. However, while the oil models developed in this study showed high accuracy, these models performed less well than NIRS models and the chemical analyses were time-consuming. With an average direct labour requirement of approximately 25 minutes per sample (including sample preparation and analysis of GC-MS results) plus approximately twenty minutes of machine time to run each sample, this method would be impractical for large-scale studies. In contrast, collection of NIRS spectra took only one minute per sample, representing a considerable reduction in laboratory time.

The main practical objective of this study was to develop a "robust" model to discriminate between *E. globulus* and F_1 hybrid leaves sampled at different times. The high level of accuracy attained from this larger NIRS2 model, both in cross-validation and independent validation, demonstrates the potential for developing models that can be applied to seedlings of different ages. To further increase the effectiveness of this model for future use, the model could be made more robust by including leaf material from seedlings of other ages, families, seasons and grown under different environmental conditions. There is also the potential to expand the model to enable analysis of seedlings younger than two and a half months, which may be beneficial in cases where it is important to determine whether seedlings are hybrids or pure species earlier in their development. Furthermore, the successful analysis of fresh leaves opens the possibility for using portable NIR instruments to carry out rapid, non-destructive sampling of seedlings in field or nursery situations.

Acknowledgments

Thanks go to CLARE BROOKER, SUSAN FOSTER, CHRISTIANE SMETHURST, STEVE PATERSON and NATASHA WIGGINS for technical assistance. We also thank THOMAS RODEMANN at the Central Science Laboratory for technical advice on NIRS analysis and Naomi Glancy for comments on the manuscript.

References

ADAMS, R. P. (1989): Identification of Essential Oils by Ion Trap Mass Spectroscopy. Academic Press Inc, San Diego.

ATKINSON, M. D., A. P. JERVIS and R. S. SANGHA (1997): Discrimination between *Betula pendula*, *Betula pubescens*, and their hybrids using near-infrared reflectance spectroscopy. Canadian Journal of Forest Research **27**, 1896–1900.

BARBOUR, R., B. M. POTTS, R. E. VAILLANCOURT, W. N. TIBBITS and R. J. E. WILTSHIRE (2002): Gene flow between introduced and native *Eucalyptus* species. New Forests **23**, 177–191.

BARNES, R. J., M. S. DHANOA and S. J. LISTER (1989): Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. Applied Spectroscopy **43**, 772–777.

BATTEN, G. D. (1998): Plant analysis using near infrared reflectance spectroscopy: the potential and the limitations. Australian Journal of Experimental Agriculture **38**, 697–706.

BERTRAND, B., H. ETIENNE, P. LASHERMES, B. GUYOT and F. DAVRIEUX (2005): Can near-infrared reflectance of green coffee be used to detect introgression in *Coffea arabica* cultivars? Journal of the Science of Food and Agriculture **85**, 955–962.

BOKOBZA, L. (1998): Near infrared spectroscopy. Journal of Near Infrared Spectroscopy 6, 3-17.

CONZEN, J. P. (2003): Multivariate Calibration: A practical guide for developing methods in the quantitative analytical chemistry. Bruker Optik GmbH, Germany.

COZZOLINO, D., H. E. SMYTH, W. CYNKAR, R. G. DAMBERGS and M. GISHEN (2005): Usefulness of chemometrics and mass spectrometry-based electronic nose to classify Australian white wines by their varietal origin. Talanta **68**, 382–387.

COZZOLINO, D., H. E. SMYTH and M. GISHEN (2003): Feasibility study on the use of visible and near-infrared spectroscopy together with chemometrics to discriminate between commercial white wines of different varietal origins. Journal of Agricultural and Food Chemistry **51**, 7703–7708.

DAVIES, A. M. C. (1998): Cross validation: do we love it too much? Spectroscopy Europe **10**, 24–25.

DAVIES, A. M. C. (2001): Uncertainty testing in PLS regression. Spectroscopy Europe **13**, 16–19.

DAVIES, N. W. (1990): Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. Journal of Chromatography **503**, 1–24.

DELAPORTE, K. L., G. G. COLLINS, J. G. CONRAN and M. SEDGLEY (2001): Molecular analysis of an interspecific hybrid ornamental eucalypt for parental identification. Euphytica **122**, 165–170.

EBBERS, M., I. R. WALLIS, S. DURY, R. B. FLOYD and W. J. FOLEY (2002): Spectrometric prediction of secondary metabolites and nitrogen in fresh *Eucalyptus* foliage: towards remote sensing of the nutritional quality of foliage for leaf-eating marsupials. Australian Journal of Botany **50**, 761–768.

ESPEJO, J., V. HERNANDEZ, V. SIERRA, J. BECERRA and R. LOPEZ (2004): Determination of terpenes through gas chromatography-mass spectrometry (GC-MS) – A new tool in the early determination of hybrid progenies of Eucalyptus nitens x E. globulus. *In: Eucalyptus* in a changing world. Proc. of IUFRO conf., Aveiro, Portugal. 11–15 October.

DOI:10.1515/sg-2008-0040
edited by Thünen Institute of Forest Genetics

ESPEJO, J. E., N. F. ENGLAND and A. R. GRIFFIN (1995): Results of a crossing program with *Eucalyptus nitens* and *E. globulus* in Chile. pp. 239–240. *In:* Eucalypt Plantations: Improving Fibre Yield and Quality. Proc. of CRCTHF-IUFRO conf., Hobart, Tasmania. 19–24 February.

FOLEY, W. J., A. MCILWEE, I. LAWLER, L. ARAGONES, A. P. WOOLNOUGH and N. BERDING (1998): Ecological applications of near infrared reflectance spectroscopy – a tool for rapid, cost-effective prediction of the composition of plant and animal tissues and aspects of animal performance. Oecologia 116, 293-305.

FRITZ, R. S. (2001): Plant Hybrids. Encyclopedia of Biodiversity **4**, 659–675.

GELADI, P., H. MARTENS, L. HADJIISKI and P. HOPKE (1996): A calibration tutorial for spectral data. Part 2. Partial least squares regression using Matlab and some neural network results. Journal of Near Infrared Spectroscopy **4**, 243–255.

GILLON, D., C. HOUSSARD and R. JOFFRE (1999): Using near-infrared reflectance spectroscopy to predict carbon, nitrogen and phosphorus content in heterogeneous plant material. Oecologia **118**, 173–182.

GIVENS, D. I., J. L. DE BOEVER and E. R. DEAVILLE (1997): The principles, practices and some future applications of near infrared spectroscopy for predicting the nutritive value of foods for animals and humans. Nutrition Research Reviews **10**, 83–114.

GORE, P. L., B. M. POTTS, P. W. VOLKER and J. MEGALOS (1990): Unilateral cross-incompatibility in *Eucalyptus*: the case of hybridisation between *E. globulus* and *E. nitens*. Australian Journal of Botany **38**, 383–394.

GRAYLING, P. M. and M. I. H. BROOKER (1996): Evidence for the identity of the hybrid *Eucalyptus 'brachyphylla'* (Myrtaceae) from morphology and essential-oil composition. Australian Journal of Botany **44**, 1–13.

GRIFFIN, A. R., I. P. BURGESS and L. WOLF (1988): Patterns of natural and manipulated hybridization in the genus *Eucalyptus* L'Herit.: a review. Australian Journal of Botany **36**, 41–66.

GRIFFIN, A. R., J. L. HARBARD, C. CENTURION and P. SANTINI (2000): Breeding *Eucalyptus grandis* x *globulus* and other inter-specific hybrids with high inviability – problem analysis and experience with Shell Forestry projects in Uruguay and Chile. pp. 1–13. *In:* Hybrid Breeding and Genetics of Forest Trees. Proc. of QFRI/CRC-SPF Symposium, 9–14th April, Queensland, Australia.

LAWLER, I. R., W. J. FOLEY and B. M. ESCHLER (2000): Foliar concentration of a single toxin creates habitat patchiness for a marsupial folivore. Ecology **81**, 1327–1338.

LISTER, S. J., M. S. DHANOA, J. L. STEWART and M. GILL (2000): Classification and comparison of *Gliricidia* provenances using near infrared reflectance spectroscopy. Animal Feed Science and Technology **86**, 221–238.

LONEY, P. E. (2007): Links between ontogeny, chemical and physical characteristics of foliage and mammalian herbivory in *Eucalyptus nitens*. Unpublished PhD Thesis, University of Tasmania.

MARTIN, B. (1989): The benefits of hybridization. How do you breed for them? pp. 79–92. *In:* Population structure and genetic improvement strategies in clonal and seedling forestry. Proc. of the IUFRO conf. on breeding tropical trees, Pattaya, Thailand. November 1988.

MOLLER, B. (2004): Near infrared transmission spectra of barley of malting grade represent a physical-chemical fingerprint of the sample that is able to predict germinative vigour in a multivariate data evaluation model. Journal of the Institute of Brewing **110**, 18–33.

MORON, A., A. GARCIA, J. SAWCHIK and D. COZZOLINO (2007): Preliminary study on the use of near-infrared reflectance spectroscopy to assess nitrogen content of undried wheat plants. Journal of the Science of Food and Agriculture **87**, 147–152.

NAES, T., T. ISAKSSON, T. FEARN and T. DAVIES (2002): A user friendly guide to multivariate calibration and classification. NIR Publications, Chichester, UK.

NIST/EPA/NIH Mass Spectral Data Base, Version 2.0a. U.S. Department of Commerce (2002).

O'REILLY-WAPSTRA, J. M., C. MCARTHUR and B. M. POTTS (2004): Linking plant genotype, plant defensive chemistry and mammal browsing in a *Eucalyptus* species. Functional Ecology **18**, 677–684.

OURCIVAL, J. M., R. JOFFRE and S. RAMBAL (1999): Exploring the relationships between reflectance and anatomical and biochemical properties in *Quercus ilex* leaves. New Phytologist **143**, 351–364.

PARK, R. S., R. E. AGNEW, F. J. GORDON and R. W. J. STEEN (1998): The use of near infrared reflectance spectroscopy (NIRS) on undried samples of grass silage to predict chemical composition and digestibility parameters. Animal Feed Science and Technology **72**, 155–167.

POKE, F. S. and C. A. RAYMOND (2006): Predicting extractives, lignin, and cellulose contents using near infrared spectroscopy on solid wood in *Eucalyptus globulus*. J. Wood Chem. Technol. **26**, 187–199.

POTTS, B. M., R. C. BARBOUR, A. HINGSTON and R. E. VAILLANCOURT (2003): Turner Review No. 6: Genetic pollution of native eucalypt gene pools – identifying the risks. Australian Journal of Botany **51**, 1–25.

POTTS, B. M. and H. S. DUNGEY (2004): Interspecific hybridization of *Eucalyptus*: key issues for breeders and geneticists. New Forests **27**, 115–138.

POTTS, B. M., R. E. VAILLANCOURT, G. J. JORDAN, G. W. DUTKOWSKI, J. COSTA E. SILVA, G. E. MCKINNON, D. A. STEANE, P. W. VOLKER, G. A. LOPEZ, L. A. APIOLAZA, Y. LI, C. MARQUES and N. M. G. BORRALHO (2004): Exploration of the *Eucalyptus globulus* gene pool. pp. 46–61. *In: Eucalyptus* in a changing world. Proc. of IUFRO conf., Aveiro, Portugal. 11–15 October.

POTTS, B. M., P. W. VOLKER, P. A. TILYARD and K. JOYCE (2000): The genetics of hybridisation in the temperate *Eucalyptus*. pp. 200–211. *In:* Hybrid Breeding and Genetics of Forest Trees. Proc. of QFRI/CRC-SPF Symposium, 9–14th April, Queensland, Australia.

POTTS, B. M. and R. J. E. WILTSHIRE (1997): Eucalypt genetics and genecology. In 'Eucalypt Ecology: Individuals to Ecosystems'. (Ed. J. W. a. J. WOINARSKI.) Cambridge University Press, Cambridge.

PRYOR, L. D. (1976): The Biology of *Eucalypts*. Studies in Biology. Edward Arnold, London.

PRYOR , L. D. and L. A. S. JOHNSON (1981): *Eucalyptus*, the universal Australian. In 'Ecological Biogeography of Australia'. (Ed. A. KEAST.) pp. 499–536. W. Junk, The Hague.

RICHARDSON, A. D., J. B. REEVES and T. G. GREGOIRE (2003): Multivariate analyses of visible/near infrared (VIS/NIR) absorbance spectra reveal underlying spectral differences among dried, ground conifer needle samples from different growth environments. New Phytologist **161**, 291–301.

DOI:10.1515/sg-2008-0040
edited by Thünen Institute of Forest Genetics

SALE, M., B. M. POTTS, A. WEST and J. REID (1996): Molecular differentiation within and between *Eucalyptus risdonii*, *E. amygdalina* and their hybrids using RAPD markers. Australian Journal of Botany **44**, 559–569.

SCHIMLECK, L. R., A. J. MICHELL and P. VINDEN (1996): Eucalypt wood classification by NIR spectroscopy and principal components analysis. Appita Journal **49**, 319–324.

STOLTER, C., R. JULKUNEN-TIITTO and J. U. GANZHORN (2006): Application of near infrared reflectance spectroscopy (NIRS) to assess some properties of a sub-arctic ecosystem. Basic and Applied Ecology **7**, 167–187.

TIBBITS, W. N. (1988): Germination and morphology of progeny from controlled pollinations of *Eucalyptus nitens* (Deane and Maiden) Maiden. Australian journal of botany **36**, 677–691.

TIBBITS, W. N. (1989): Controlled pollination studies with shining gum (*Eucalyptus nitens* (Deane and Maiden) Maiden). Forestry **62**, 111–126.

TIBBITS, W. N., D. B. BOOMSMA and, S. JARVI (1997): Distribution, biology, genetics, and improvement programs for *Eucalyptus globulus* and *E. nitens* around the world. pp. 1–15. In: Proc. of the 24th Biennial Southern Tree Improvement Conf., June 9–12, Orlando, Florida.

TIBBITS, W. N., B. M. POTTS and M. H. SAVVA (1991): Inheritance of freezing resistance in interspecific F_1 hybrids of *Eucalyptus*. Theoretical and Applied Genetics **83**, 126–135.

TIGABU, M. and P. C. ODEN (2003): Discrimination of viable and empty seeds of *Pinus patula* Schiede & Deppe with near-infrared spectroscopy. New Forests **25**, 163–176.

TIGABU, M., P. C. ODEN and T. Y. SHEN (2004): Application of near-infrared spectroscopy for the detection of internal insect infestation in *Picea abies* seed lots. Canadian Journal of Forest Research **34**, 76–84.

TIGABU, M., P. C. ODEN and D. LINDGREN (2005): Identification of seed sources and parents of *Pinus sylvestris* L. using visible-near infrared reflectance spectra and multivariate analysis. Trees – Structure and Function **19**, 468–476.

VILJOEN, M., L.C. HOFFMAN and T. S. Brand (2007): Prediction of the chemical composition of mutton with near infrared reflectance spectroscopy. Small Ruminant Research **69**, 88–94.

VOLKER, P. (2002): Quantitative genetics of *Eucalyptus globulus*, *E. nitens* and their F_1 hybrid. Unpublished PhD Thesis, Univeristy of Tasmania.

VOLKER, P. W. (1995): Evaluation of *Eucalyptus nitens* x *globulus* for commercial forestry. pp. 222-225. In: Proc. of the CRCTHF-IUFRO Conf., Hobart, Tasmania. 19–24 February.

WOODCOCK, T., G. DOWNEY, J. D. KELLY, C. O'DONNELL (2007): Geographical classification of honey samples by near-infrared spectroscopy: A feasibility study. Journal of Agricultural and Food Chemistry **55**, 9128–9134.

Genetic Parameters of Somatic Clones of Coastal Douglas-fir at $5^1/_2$-Years across Washington and Oregon, USA

By C. A. DEAN[1),*)]

(Received 17th June 2007)

Abstract

Five genetic tests involving 70 somatic clones of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) were planted March-April 1999 in Weyerhaeuser plantations across western Washington and Oregon states, USA. Four of the tests are in Longview and Twin Harbors regions of Washington, and one test is in Springfield, Oregon. Each test is based on single-tree plots with 12 randomized complete-blocks. The 70 coastal Douglas-fir clones were propagated by somatic embryogenesis from two full-sib families that had the same female parent. Results are reported for survival, height, diameter at breast-height (DBH) and volume growth at $5^1/_2$-years.

These tests provide evidence of acceptable growth and survival of somatic trees of coastal Douglas-fir across a range of site conditions. Height had a clonal heritability of 0.25 ± 0.01, DBH 0.21 ± 0.01 and volume 0.20 ± 0.01. The growth traits were all strongly genetically associated with clonal correlations of 0.92 to 0.99.

Clonal performance for growth proved quite stable across tests with an overall between-test correlation of 0.84 ± 0.04. There was little variance due to clone x test interactions.

Key words: Coastal Douglas-fir, somatic embryogenesis, adaptability, clonal heritabilities, clonal stability.

Introduction

Coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) is one of two varieties of *P. menziesii*; the other being Rocky Mountain Douglas-fir (*P. menziesii* var. *glauca*). The natural range of coastal Douglas-fir extends south from central British Columbia, Canada, along the Pacific Coast Ranges of northwest USA and into California and Mexico (HERMANN and LAVENDER, 1999). Coastal Douglas-fir is among the most important commercial forest tree species in North America and,

1) Present address: Weyerhaeuser Company, PO Box 9777, Federal Way, WA 98063-9777, USA.

*) Corresponding author: CHRISTINE A. DEAN.
E-Mail: Christine.Dean@Weyerhaeuser.com

DOI:10.1515/sg-2008-0041
edited by Thünen Institute of Forest Genetics