Industrial Crops and Products 102 (2017) 32–44

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Assessment of a non-destructive method to predict oil yield in *Eucalyptus polybractea* (blue mallee)

David Kainer[a,*], David Bush[b], William J. Foley[a], Carsten Külheim[a]

[a] Research School of Biology, The Australian National University, Canberra ACT 2601, Australia
[b] CSIRO Australian Tree Seed Centre, GPO Box 1600, Canberra ACT 2601, Australia

ARTICLE INFO

Article history:
Received 22 July 2016
Received in revised form 18 February 2017
Accepted 10 March 2017

Keywords:
Eucalyptus
Terpenes
Oil yield
Biomass
Tree improvement
Leaf area

ABSTRACT

Eucalyptus oil is produced from a number of "oil mallee" species with high concentration of foliar essential oils, high proportion of 1,8-cineole and the ability to re-sprout with multiple stems from lignotubers after coppicing. *Eucalyptus polybractea* is one of the most planted mallees in Australia, but few efforts have been made to improve crop yields through selection or breeding programs, with the exception of seed collection from trees with high oil and 1,8-cineole concentration. The yield of essential oil from commercial eucalypt plantations is subject to several quantitative traits that interact, both positively and negatively. Using non-destructive methods we assessed traits relating to oil yield (quantitative and qualitative variation of foliar essential oils and biomass-related parameters) for their variability, heritability as well as phenotypic and genetic interactions in an open-pollinated progeny trial with 40 families and 480 individuals of *E. polybractea*. From this we built models to predict family yield performance and compared our predictions to commercial scale harvests of the same trial.

Our models show that relying on oil concentration and 1,8-cineole proportion alone is not ideal for selection of top performing families. Rather a mixture of biomass related traits, foliar oil concentration, 1,8-cineole proportion and leaf architecture contribute to the top performing families in varying ways.

1. Introduction

Essential oils are volatile mixtures of naturally occurring plant terpenes which occur in many plant families, with Myrtaceae being notably diverse (Padovan et al., 2014). Oils found in specialised cavities in the leaves of various Myrtaceae have a range of commercial applications, depending on the terpene composition, or chemotype, present in the oil. Within a genus or species there may be an array of such chemotypes, not all of which are commercially desirable. *Melaleuca alternifolia* (medicinal tea tree), for example, displays up to six chemotypes, with the type rich in terpinen-4-ol being valued for its anti-bacterial and anti-fungal properties (Keszei et al., 2010). *Eucalyptus* oil is valued primarily for the monoterpene 1,8-cineole, and is used in pharmaceuticals, cleaning products and as an environmentally friendly industrial solvent (Barton, 1999). More recently it has been demonstrated that certain terpenes, such as α-pinene and limonene, can be processed into high-energy biofuel (Harvey et al., 2010; Meylemans et al., 2012), while the major monoterpene constituents of tea tree oil can be used as a sustainable precursor in the fabrication of graphene (Jacob et al., 2015), potentially opening up new markets for myrtaceous species with chemotypes of the appropriate oil composition.

Eucalyptus oil is often produced internationally as a by-product in plantations of species such as *E. camaldulensis* and *E. globulus* that are grown primarily for their wood, but also contain useful amounts of 1,8-cineole in their leaves (Coppen, 2002). Annualized oil yield per hectare from such systems is relatively low due to the long time between harvests, which is typically between 7 and 15 years, or due to sub-optimal foliar oil concentration.

For greater yields, dedicated *Eucalyptus* oil producers in Australia primarily grow certain "mallee" species (growth habit of certain eucalypt species that have multiple stems arising from an underground lignotuber, e.g. *E. polybractea, E. kochii, E. loxophleba*). These mallee species have a high ratio of leaf to stem, naturally high foliar oil concentration, very high 1,8-cineole content, and rapid harvest/regrowth cycle via coppicing (Goodger et al., 2007). Coppicing is performed every two or so years by mechanically harvesting all of the above-ground shoots close to the ground, after which the shoots re-emerge from the lignotuber. The harvested mallee is chipped and steam distilled to extract the volatile oils found predominantly in the leafy biomass.

* Corresponding author.
E-mail address: david.kainer@anu.edu.au (D. Kainer).

http://dx.doi.org/10.1016/j.indcrop.2017.03.008
0926-6690/

Smaller oil producers harvest directly from natural stands of mallees, while plantations are stocked with seedlings originating from natural stands or from open-pollinated, first-generation seed orchards, so resulting harvest yields are low or highly inconsistent year to year (King et al., 2004). Since oil mallees may be harvested and re-harvested for over 80 years (Coppen, 2002), planting genotypes that have consistent high yields can make a great difference to long term profitability. Higher oil yields may also reduce barriers to environmental land care in agricultural land beset with dryland salinity or soil erosion by increasing the value of mallee belts planted there to combat these problems (Bartle, 2009; Wildy et al., 2000b; Wu et al., 2008). Furthermore, new commercial uses for terpenes, such as biofuels and graphene production, will require significant increases in oil yield per hectare to be commercially viable.

The yield of foliar essential oil is a complex quantitative trait made-up of several component traits. The best yielding genotypes possess a high concentration of oil per gram of leaf biomass, high total leaf biomass, and oil of the right chemotype (Zhang et al., 2011) (e.g. high proportion of 1,8-cineole for pharmaceutical *Eucalyptus* oil). For oil-producing mallees, vigorous coppice regrowth, the ability to survive adverse environmental conditions and resistance to pathogens are also beneficial traits (Coppen, 2002). Selecting trees from the wild on the basis of their total oil yield, however, is difficult due to the inability to assess the trait directly in the field. Selecting candidate trees for oil yield from within plantations is also problematic since several of the underlying traits cannot be observed until after the initial harvest, when coppice regrowth has occurred and oil yields have been assessed on the second crop. As a consequence, breeding programs select mostly for trees with high foliar oil concentration rather than yield (Doran et al., 1998). This is a sensible approach since many studies in mallees have shown huge natural variation in oil concentration and composition (Goodger et al., 2007; King et al., 2006a,b) with moderate to high heritability (Barton et al., 1991; Doran et al., 2002; Grant, 1997), indicating that much of the variation is genetic rather than environmental. This is further supported by genetic marker studies that have found QTLs explaining significant amounts of the variation in oil traits (Külheim et al., 2011; Webb et al., 2014). The prospective improvements through selection for elite oil concentration can be appreciated from the successful Australian tea tree (*Melaleuca alternifolia*) breeding program, which since 1993 has seen annual yields of tea tree oil double from 150 kg ha^{-1} to 300 kg ha^{-1} (Baker et al., 2014). It has also been speculated that plantations of elite *E. polybractea* clones could potentially produce over 560 kg ha^{-1} yr^{-1} of 1,8-cineole (Goodger et al., 2007).

Focusing solely on oil concentration may not lead to the best yielding genotypes if other traits that affect yield are disregarded (Milthorpe et al., 1998), yet relatively few studies have investigated the combined effect of multiple traits on oil yield. Rather only certain traits have been studied, often on relatively small populations. Grant (1997), for example, measured oil concentration, leaf area, leafy biomass, and predicted oil yield in a population of 61 *E. polybractea* individuals. Doran et al. (1998) examined oil and growth traits and estimated oil yield in 32 families of *E. radiata*. Wildy et al. (2000b) assessed oil yields across several mallee species in Western Australia and found more than 10-fold variation in yield, with much of the variation driven by biomass. Goodger et al. (2007) noted that few trends with respect to oil yield had been established in coppiced oil mallees, and so examined biomass and oil traits and their impact on oil yield across harvests, but in only twenty individual trees. The authors of these studies recognised that profitability hinged not only on selecting genotypes producing leaf with high oil concentration, but also accumulating high biomass, and that non-destructive assessment is necessary for larger scale evaluation of oil yield. Additionally, variation in leaf area and LMA (leaf mass per unit area) have been shown to negatively correlate with oil concentration and plant growth rate respectively (Grant, 1997; Poorter et al., 2009) and so may potentially affect oil yield.

In this study we explore the components of oil yield in a commercially active progeny trial of *E. polybractea* using non-destructive estimation of biomass, oil concentration and composition. The progeny trial, located near West Wyalong, NSW, Australia, consists of over 60,000 individual trees from 40 maternal open-pollinated families, and is harvested through coppicing on a typical rotation of 24 months. Experimental-scale sampling of oil, leaf and biomass traits were used to predict oil yield in a subset of trees from each of the families in the trial, at two time points a year apart. Predicted biomass and oil yield per family were then contrasted with family rankings determined from the industrial-scale harvest of all 60,000 trees. We present the variation in oil, biomass and leaf traits within and between open-pollinated families, and report on the ability to predict family oil yield performance from just a small sample of each family. Finally, we present genetic parameters (heritability and genetic correlations) at both time points, estimated from variance components of mixed effect models.

2. Materials and methods

To predict oil yield per tree through non-destructive measures, we measured a range of phenotypes in a population of *E. polybractea*. We compared the predicted performance to subsequent harvest performance to assess the accuracy of prediction.

2.1. Field site

All measurements and samples were gathered from an active commercial plantation on the property of *Eucalyptus* oil grower GR Davis Pty Ltd. This property, near West Wyalong, NSW, is located at Lat. 33°58′S Long. 147°03′E, is at an elevation of 300 m. The region is one of three small disjunct areas that form the natural geographic range of *E. polybractea*. The region receives an annual average of 465 mm of rainfall, though rainfall varies widely from year to year. In July 2009 GR Davis Pty Ltd propagated open-pollinated families from seed of 40 phenotypically selected *E. polybractea* mother trees. All 40 originate from within about 20 km of the trial site, with 26 sourced from the CSIRO Australian Tree Seed Centre, 11 from natural stands around GR Davis, and 3 from a first generation clonal seed orchard at GR Davis. The seedlings were used to establish two plantings: i) a seedling seed orchard (SSO) with 1008 trees and ii) a family yield trial (FYT) with 83,000 trees. The purpose of the FYT was to assess family performance in order to inform the progressive thinning of the SSO as well as to provide a source of forward selections for the next generation. The 40 mother trees were chosen for their relatively high foliar oil concentration and high 1,8-cineole proportion. The progeny are the result of open-pollination, and as such there is no pedigree beyond the known mother trees.

The FYT site occupies a ~23 ha (630 m × 375 m) area of gently undulating terrain that we categorised into valley, slope or rise classes. The trial is an incomplete randomized block design consisting of three blocks. Within blocks, each plot consists of approximately 800 open-pollinated trees from a single family, planted in two parallel columns separated by a 2 m corridor. A 3 m gap separates each family plot. Within a block each family is represented by at most one plot, with Block 1, Block 2 and Block 3 containing 40, 38 and 26 plots, respectively. Of the 40 families in the FYT, 26 families are represented by a plot in all 3 blocks, 12 families are in 2 blocks and 2 are in only one block In total, approximately 63,500 trees survived initial planting (counted in 2011), resulting in a density of about 2640 trees ha^{-1}.