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Seed germination on filtrates from soil sampled beneath trees of *Eucalyptus globulus*, *E. nitens* and their F_1 hybrid

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Public report

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June 2010

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Contents

Abstract

The effect of filtrates from soil sampled from beneath *E. globulus*, *E. nitens* and their F_1 hybrid trees was tested on the laboratory germination and root (radicle) growth of linseed, *E. globulus* and *E. nitens* seeds. Two Tasmanian field trials were studied: one where *E. globulus*, *E. nitens* and their F_1 hybrids were grown inter-mixed as single-tree family plots; the second where *E. globulus* and *E. nitens* were grown in replicated blocks. The trees had been growing on each site for 10 and 15 years respectively. No significant differences were detected between filtrates from soils beneath *E. nitens* and *E. globulus* for pH, proportion of seeds germinated and root radicle length in either experiment. There was also no difference between the distilled water controls and filtrates from the soils beneath the standing eucalypt trees. There was thus no evidence to suggest a major difference between any of the genetic treatments tested with this plant-based bioassay, although we cannot dismiss the possibility that concentration of the filtrates may reveal differences in bioactivity.

Introduction

Trees are the dominants of many terrestrial ecosystems and the influence of their genetics on dependent biotic communities and ecosystem processes is receiving increasing study world-wide (Schweitzer *et al.* 2004; Whitham *et al.* 2006). Such research linking the fields of genetics and ecology (termed community genetics) commenced early with the Tasmanian eucalypts, initially focusing on the effects of hybridisation between species (e.g. *E. amygdalina* x *E. risdonii*—Whitham *et al.* 1991; Whitham *et al.* 1994; Whitham *et al.* 1999; Dungey *et al.* 2000). More recently this research has extended to the study of the effects of intra-specific genetic variation, using the well-studied *E. globulus* as a model. The studies with *E. globulus* have been based on common-environment field trials established from seed collected from native stands from throughout the geographic range of *E. globulus* (i.e. different native races and multiple families from each race—Dutkowski and Potts 1999). Such studies have shown that genetic-based differences in the geographic race of origin of the trees may have statistically detectable effects on the canopy, trunk and litter community of arthropods and fungi (Barbour *et al.* 2009c; Barbour *et al.* 2009b; Barbour *et al.* 2009a). However, no such genetic effect has as yet been detected on the fungal communities developing on decaying logs of different pedigree (Barbour *et al.* 2009d).

Community genetic studies are just starting to address the more distal effects of tree genetics, such as the effects on the biotic and abiotic characteristics of the soils in which the trees are growing (Schweitzer *et al.* 2008a). In one of the studies with *E. globulus*, Barbour *et al.* (2009a) compared soil samples from immediately beneath canopies of felled trees from two well-differentiated races of the species. Genetic-based race effects were detected from differences in linseed (*Linum usitatissimum*) seed germination and root radicle growth on water filtrates derived from soil samples taken after the eucalypt canopies had decayed on the ground for eight months.

In this technical report, we report results of a preliminary study undertaken in 2005 aimed at investigating whether genetic differences between standing trees resulted in differences in soil characteristics and focused on an inter- rather than an intra-specific comparison. The study focused on comparing *E. globulus* and *E. nitens*. These are the two main plantation eucalypts in Australia, including in Tasmania (Parsons *et al.* 2006). *Eucalyptus globulus* is native to Tasmania, but *E. nitens* is not (Dutkowski and Potts 1999; Hamilton *et al.* 2008). Both species belong to the subgenus *Symphyomyrtus* and, consistent with this subgeneric classification, the major components of the leaf volatile oils of *E. nitens* are qualitatively the same as most of the other Tasmanian native *Symphyomyrtus* species (Potts *et al.* 2010). While the two species are classified in the same taxonomic series, they belong to different subseries (Brooker 2000; McKinnon *et al.* 2008). As with many even closely related *Eucalyptus* (e.g. *E. risdonii* and *E. amygdalina* - Dungey *et al.* 2000), *E. globulus* and *E. nitens* are genetically differentiated in leaf phytochemistry, mainly due to quantitative differences in multiple compounds (Li and Madden 1995; Humphreys *et al.* 2008). For example, *E. nitens* foliage has significantly lower yields of foliar essential oils than *E. globulus* (Li and Madden 1995; Humphreys *et al.* 2008), and there are differences in relative proportions of many components within the oils (Li and Madden 1995; Potts *et al.* 2010). *Eucalyptus globulus* foliage contains sideroxylonals and

macrocarpals (Freeman *et al.* 2008) which are formylated phloroglucinol compounds (FPCs) well known for their defensive role in eucalypts. Only the sideroxylonals have been reported in significant amounts in *E. nitens* (e.g. McArthur *et al.* 2010).

As with even closely related Tasmanian native eucalypts (e.g. Dungey *et al.* 2000), the genetic-based differences in phenotype between the species appears to impact to some extent on the organisms which are dependent upon these tree species for food or habitat. While many of the same generalist species are found on the leaves of both *E. globulus* and *E. nitens* (Lawrence 1998; de Little *et al.* 2008), there is evidence that some dependent animals and fungi exhibit preference for one or other eucalypt species as a host. For example, while no difference in feeding preference of the adult leaf-eating beetle *Chrysophtharta agricola* (now re-assigned to the genus *Paropsisterna* [see de Little *et al.* 2008]) was evident on trees from a trial at Tyenna (see Methods), laboratory feeding trials revealed a significant preference for *E. nitens* (Lawrence *et al.* 2003).

The present study was undertaken in two common environment field trials in southern Tasmania and compared seed germination responses on filtrates from soil sampled from beneath standing *E. globulus*, *E. nitens* and their first generation (F_1) hybrid. At one site (Tyenna), families of *E. globulus*, *E. nitens* and their F_1 hybrids were grown intermixed as single-tree family plots. At another site, *E. globulus* and *E. nitens* were grown in relatively large replicated blocks more akin to the way the species would be deployed in plantations.

Methods

Tyenna trial—tree scale

Study site

The first field trial studied was near Tyenna in south-eastern Tasmania, and comprised control pollinated families of *E. globulus, E. nitens,* F_1, F_2 and backcross trees of common parentage (Potts *et al.* 2003). Most families were derived from crossing amongst the *E. globulus*, *E. nitens* and F_1 hybrids in the crossing program detailed in Volker *et al.* (2008). The *E. globulus* parents were inter- or intra-provenance crosses involving pollen from unselected trees in native populations from King Island and Taranna (southern Tasmania) and selected female trees from these provenances which were growing in a seed orchard in north-western Tasmania. The *E. nitens* parents were from crosses amongst selected trees in families grown in north-western Tasmania from seed collected from native stands in the Toorongo region of Victoria. The Tyenna trial was established in October 1995 with 960 trees planted in rows, spaced 2.5 m within the rows and 3 m between rows. This spacing was typical of eucalypt plantations in the area. The trial comprised seven replicates with each replicate normally containing one individual of each family. Experimental trees were selected so as to represent most of the *E. globulus*, *E. nitens* and F_1 families represented in the trial (14, 17 and 11 families respectively). For the current study, 17 new field replicates were established throughout the trial consisting of one individual of *E. nitens, E. globulus* and an F_1 hybrid growing in close proximity.

Field sampling

From each of the 51 experimental trees, a soil sample was collected from beneath the litter within ½ metre of the tree trunk in late July 2005, when the trees were 10 years old. All soil samples were collected on one day and by replicate. A soil core of diameter 65 mm and depth 95 mm was taken at the base of each tree under the highest accumulation of leaf litter. Soil samples were labelled according to tree identifier and the field replicate (1-17) and placed in sealed plastic bags. On return to the laboratory, samples were stored at 4 ˚C.

Bioassay

Soil samples were processed as quickly as possible after collection. All glassware was washed, rinsed with fresh water, and then double rinsed with distilled water. Samples were randomly assigned to batches of eight samples, with batches assayed across four days. A 20 g sample of soil was weighed into a beaker and 100 mLs of distilled water added and the sample mixed to a slurry. This was allowed to stand for 15 minutes. pH was then measured using a pH meter, WTW pH315i, calibrated with pH 7 and pH 4.01 technical buffers, and then dipped in the soil–water mixture. The soil–water mixture was filtered using 110 mm Whatman No.1 cellulose filter paper (folded concertina style), to exclude major soil particles. The linseed (*Linum sp.*) seed was sterilised by placing in 4% sodium hypochlorite for two minutes, then in 70% ethanol for one minute. It was then rinsed with distilled water. Two 85 mm Whatman No.1 filter papers were placed in a sterile petri dish, followed by two mLs of the soil/distilled water filtrate. Twelve linseed seeds were placed onto the filter paper, ensuring even spacing. The petri dish was sealed with Parafilm®. For each soil sample these final steps were repeated

using *E. globulus* and *E. nitens* seed in place of linseed. The three petri dishes from a soil sample, containing different seed types, were kept together and grouped with other groups of three from the same batch. These were placed together in a darkened oven set to 20 ˚C. This process was repeated for all soil samples, randomly sampled in batches of eight. A control comprising one petri dish with distilled water replacing the filtrate was included for each seed lot in each batch. The petri dishes with linseed were scored for germination proportion and radicle length after 4 days, and eucalypt seed after seven days at 20 ˚C. The radical length was measured on all germinated seed, and the petri dish average used for analysis. The *E. globulus* seed was a commercially available, multi-seedlot mix from the Heath seed orchard of seedEnergy Pty Ltd. The *E. nitens* seed was also a commercially available, multi-seedlot mix from the Bream Creek seed orchard of Derford Nitens Pty Ltd (Hamilton *et al.* 2008). The soil filtrate preparation and linseed bioassay methodology used was similar to that of Harvest *et al.* (2008) and Barbour *et al.* (2009a), except our concentration of soil in the slurry from which the filtrate was produced was 50% less.

Data analysis

One tree sampled as an F_1 was removed from the analysis due to its atypical capsule morphology. The data was analysed using a mixed model REML analysis undertaken with Proc MIXED of SAS (Version 9.1). Various linear mixed models were fitted to the data. For soil pH, the model fitted included the fixed effect of cross type of each tree (*E. nitens*, F_1, *E. globulus*) and the random effect of field replicate and laboratory batch. For the germination proportion and radicle length the model included the fixed effects of cross type, assay seed type (linseed, *E. globulus* and *E. nitens*), and their interaction as well as the random effects of field replicate, laboratory batch, as well as the interactions amongst field replicate and cross type. The random field replicate and cross type interaction was used to test the cross type effect as well as the specific contrast between *E. nitens* and *E. globulus* cross types. The proportion data was angular transformed and the radicle length log10 transformed for this analysis. Cross type least square means and standard errors were calculated using the same model and untransformed data. Difference between the control and the filtrate treatments was tested in a separate analysis based on cross type means for each laboratory batch and fitting treatment (cross type plus control), seed type and their interaction as fixed effects and batch and treatment by batch interaction as random effects. The control was compared with the pooled filtrate effect in a specific contrast within this analysis.

Lewisham trial—patch scale

Study site

An experimental trial established by the CSIRO, at Lewisham (southern Tasmania) in August 1990, was used to assess whether *E. globulus* and *E. nitens* have an effect on the soil at a patch scale. This trial had originally been an irrigation trial (White *et al.* 1996), but had also been subject to other experimental treatments. Six field replicates were present at this site, each consisted of four blocks, two of *E. globulus*, and two of *E. nitens*. Only three of these blocks were used in this experiment as the fourth block consisted of only one row of trees. Thus from each field replicate two larger blocks (209 trees arranged as 11x19 block) and one smaller block (57 trees arranged as 3x19 block) were sampled. The blocks within each replicate

comprised an *E. globulus* provenance (derived from King Island) and two provenances of *E. nitens* (Barrington Tops [native stand] and a Forest Resources seed orchard bulk). The provenance order within a field replicate was random.

Field sampling

For the two larger blocks, nine sub-samples were taken from the spaces between the central nine (3x3) trees. For the 3 x 19 block, sampling occurred between the central nine trees along the central row in early September 2005. Each sub-sample consisted of 60 mL (¼ metric cup) of soil taken from beneath the leaf litter, sampling close to the surface to maximise any effect leaf litter decomposition was having on the soil chemistry. The nine sub-samples from each block were pooled, placed in sealed bags and labelled. This sampling was repeated for each field replicate.

Bioassay

The bioassays essentially followed the methods described previously, except only linseed seed was used and two technical replicates (petri dishes) were used for the soil sample from each block. Bioassays were completed in two batches (each containing nine samples). The first batch included field replicates 1–3; the second included field replicates 4–6. Both batches were assayed on the same day. A distilled water control was included with each replicate. Within each batch, petri dishes (including the two technical replicates) were randomised in the incubation oven. The germination proportion and radicle length were scored after four days at 20 ˚C. The petri dish average for radicle length was used for analysis. For pH, one value was collected for each block by provenance combination.

Data analysis

As with the previous experiment, a linear mixed model was fitted to the data. The model fitted for pH included the fixed effect of provenance (King Island *E. globulus*, seed orchard and Barrington Tops *E. nitens*) and the random effect of field replicate (1–6). For the germination proportion and mean radicle length, the model also included the random field replicate by provenance interaction which was used to test the significance of the provenance effect. Provenance least square means and standard errors were derived from these analyses and specific contrasts made comparing the *E. globulus* provenance to the combined *E. nitens* (native plus seed orchard). The controls were compared to the filtrate treatments using replicate means for each of the four treatments and fitting a model with treatment (three provenances and the control) fixed and field replicate random. The control was compare with the pooled filtrate effect in a specific contrast within this analysis. The proportion data was angular transformed but the radicle length did not require transformation.

Results

Tyenna trial—tree scale

The least square means and standard errors for the Tyenna trial are shown in Table 1. There was no significant difference between the different tree types in the proportion of seed germinated ($F_{2,26}$= 0.7, P= 0.505) nor the radicle length of seeds which did germinate ($F_{2,26}$= 0.60, P= 0.558). The interaction between seed type and tree type was insignificant for both traits ($F_{4,94}$= 1.14, P= 0.343 and $F_{4,94}$= 0.02, P= 0.999 respectively). Tree type had no significant effect on the pH of the filtrate ($F_{2,26}$= 0.7, P= 0.495). No statistical difference was detected between distilled water controls and the pooled soil filtrates for either the proportion of seeds germinated ($F_{1,18}$= 0.06, P= 0.816) or radicle length ($F_{1,18}$= 0.18, P= 0.678).

Table 1. Bioassay results for the Tyenna trial

The table shows the least square means and standard errors (SE) for pH, proportion of seeds germinated and radicle length (mm) for three seed types grown on the filtrate from soil from beneath *E. globulus*, *E. nitens*, and F_1 hybrid trees, as well as for the distilled water controls. The arithmetic means are shown for the distilled water controls.

				Proportion germination						Radicle Length (mm)					
		pH		Linseed		*E. globulus*		*E. nitens*		Linseed		*E. globulus*		*E. nitens*	
Sample	**n**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
E. globulus	17	5.27	0.11	0.85	0.02	0.96	0.02	0.76	0.02	25.3	0.7	19.6	0.7	11.1	0.7
E. nitens	17	5.13	0.11	0.88	0.02	0.95	0.02	0.77	0.02	25.9	0.7	19.9	0.7	11.3	0.7
F_1	16	5.13	0.11	0.88	0.02	0.92	0.02	0.75	0.02	25.1	0.7	19.3	0.7	10.9	0.7
Control	8			0.82	0.03	0.95	0.03	0.77	0.05	23.2	1.5	20.2	1.2	11.2	0.5

Lewisham trial—patch scale

The least square means and standard errors for the Lewisham trial are shown in Table 2. Analysis of the data showed no significant effects of tree provenance on pH ($F_{2,10}$= 0.18, P= 0.835), germination proportion ($F_{2,10}$ = 1.92, P= 0.196) or radical length ($F_{2,10}$ = 0.38, P= 0.696) of the linseed seed. There was also no significant difference when the two provenances of *E. nitens* were pooled and compared to the *E. globulus* provenance (germination proportion $F_{1,10}$ = 3.84, P= 0.08; radicle length $F_{1,10}$ = 0.72, P= 0.418). No statistical difference between the distilled water control and the pooled filtrate treatments was detected for either germination proportion ($F_{1,15}$= 1.17, P= 0.296) or radicle length ($F_{1,15}$= 3.07, P= 0.100).

Table 2. Bioassay results for the Lewisham trial

The table shows the least square means and standard errors (SE) for pH, proportion of seeds germinated (n=12) and radicle length (mm) for linseed seed grown on the filtrate from soil collected from beneath trees of *E. globulus* (King Island provenance) and *E. nitens* (native and seed orchard provenances).

Species	Provenance	pH		Proportion germination		Radicle length (mm)	
		Mean	SE	Mean	SE	Mean	SE
E. globulus	King Island	6.78	0.17	0.91	0.03	17.9	1.6
E. nitens	Barrington Tops (native)	6.87	0.17	0.85	0.03	19.1	1.6
E. nitens	Seed orchard bulk	6.85	0.17	0.85	0.03	19.5	1.6
Control				0.90	0.05	21.5	1.3

Discussion

With demonstrated genetic-based differences in foliar phytochemistry and other traits between *E. globulus* and *E. nitens*, we were interested to test whether there was a readily detectable bioactive effect of the different species on the soil directly beneath the standing tree. Such an effect may arise, for example, from water flow through the canopy or trunk, leaf decomposition on the soil surface, or even indirectly from associated organisms (e.g. insect frass—Jung 2008). With the experimental protocols adopted here we were unable to detect a difference between aqueous filtrates from soils collected from beneath *E. nitens* and *E. globulus* trees, nor between seed orchard and the native stand provenances of *E. nitens*. While there was no evidence to suggest a marked difference between *E. nitens* and *E. globulus* in these plant-based bioassays, we cannot dismiss the possibility that concentration of the filtrates may reveal differences in bioactivity.

There are numerous factors such as season, extraction and bioassay methodology, as well as direct chemical or other assays, and even molecular technologies, which could be explored to assess the flow-on effects of standing trees on soils. Exploration of the extended genetic-based effects of standing trees on soil systems is of interest given that signals have been detected in other tree systems (Schweitzer *et al.* 2004; Schweitzer *et al.* 2008b). However, a key to detecting and estimating the relative importance of genetic-based effects of standing trees on the soils will be the use of good field trials where the treatments being tested (e.g. *E. nitens* and other species such as *E. globulus*; different genotypes or provenances) are grown together in well-replicated experimental designs. Such designs are essential for unravelling genetic and environmental/silvicultural effects.

Acknowledgements

This exploratory work was undertaken as part of a third-year undergraduate research project (CB) in the School of Plant Science, University of Tasmania. We thank Robert Wiltshire for his comments and discussion, Tammy Harvest for technical advice, Peter Gore for provision of eucalypt seed, and Paul Tilyard for providing technical assistance.

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